Immune Responses Post Haematopoietic Stem Cell Transplant: Clinical Studies of Vaccination and Autoimmunity

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MBBS MRCP

A Thesis Submitted to the Cancer Institute,

University College London (UCL), for the Degree of

Doctor of Medicine (Research)

Author's Declaration

I, Paul Miller, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Dr Paul Miller

23 May 2018

Abstract

For months to years after haematopoietic stem cell transplant (HSCT), recipients may experience immune complications including graft versus host disease (GvHD), infections, and autoimmune phenomenon. These manifestations of impaired and dysregulated immunity contribute substantially to the burden of morbidity and mortality amongst HSCT recipients. In the general population, vaccination is an effective strategy for prevention of a number of infectious diseases. In HSCT recipients, the same immune impairment that renders them susceptible to infections may also blunt the adaptive immune response essential for effective immunisation. The optimum approach to vaccination of HSCT recipients is largely unclear. The first study in this thesis explores how current evidence and recommendations for vaccination of HSCT recipients translate into clinical care. A national survey of vaccination practice at allogeneic HSCT programmes was conducted on behalf of the British Society of Blood and Marrow Transplantation(BSBMT). We identified marked heterogeneity in all aspects of practice. The second study uses two serological assays to investigate the immunogenicity of the seasonal inactivated influenza vaccine administered to recipients of reduced intensity conditioning allogeneic HSCT in the first-year post-transplant. Immunogenicity was universally poor by both assays. The third study in this thesis identifies specific patient demographics and patterns of health belief that are associated with low vaccination intent amongst HSCT recipients. These findings may help to develop targeted patient education programmes. The final study in this thesis moves away from vaccination and explores the immune-mediated complication

autoimmune cytopenia (AIC) following transplant for acquired aplastic anaemia (aAA).

This study was supported by the European Society for Blood and Marrow Transplantation (EBMT). Cumulative incidence of AIC at 10 years post HSCT was 5.1%. Peripheral blood stem cell (PBSC) source, was associated with a higher incidence of AIC and myeloablative conditioning with a lower incidence in a multivariable Cox analysis.

Impact Statement

Through three related studies this thesis explores prophylactic vaccination of stem cell transplant recipients against infectious diseases. The first study in this thesis highlights the marked heterogeneity in vaccination practice across UK allogeneic HSCT programmes. These findings have been disseminated to the international transplant community through publication in *Bone Marrow Transplantation*. The study highlights the need for UK practice recommendations that incorporate the expertise of international guidelines with considerations specific to the UK. Discussions around recommendation development are underway with interested parties. The study provides a benchmark against which practice can be assessed in the future after development of such recommendations. However, it is important to acknowledge that the evidence base for optimum vaccination of HSCT recipients is limited. The second study contributes to this evidence base and explores vaccination of HSCT recipients receiving reduced intensity conditioning regimens using a standard haemagglutination inhibition assay (HAI), and a potentially more sensitive viral microneutralization (VMN) assay. Our findings demonstrate that administration of a single dose of the seasonal inactivated influenza vaccine (SIIV) is poorly immunogenic within the first-year post HSCT. This pathes the way for future studies exploring novel vaccination schedules, possibly incorporating pre-HSCT vaccination, or novel vaccine formulations. The VMN is shown to be more sensitive than the HAI in this patient group, and future studies may usefully correlate immunogenicity with vaccine efficacy data. In the third study in this thesis, focus is turned to the sociodemographic factors and patient health beliefs that

influence influenza vaccine intent amongst HSCT recipients. A modified Health Belief Model was a good predictor of vaccine intent, and adults aged over 65 who had not previously received the influenza vaccine were likely to have lower vaccine intent. The importance of vaccine promotion by both general practitioners and stem cell transplant specialists is highlighted. The findings may be used as a basis to develop targeted interventions to promote vaccine uptake amongst this high-risk group. These findings will be disseminated to the bone marrow transplant and broader healthcare community through publication in *BMJ Open*.

The final study in this thesis was conducted with support from the severe aplastic anaemia working party (SAAWP) and autoimmune disease working party (ADWP) of the European Group for Blood and Marrow Transplantation (EBMT). The study is the first to explore the incidence, risk factors and treatment of autoimmune cytopenias (AIC) following allogeneic HSCT for a single disease indication. This clinically-focussed study highlights patient subgroups that may be at higher risk of AIC and demonstrates the challenges of treating this relatively resistant complication. Our findings contribute to existing evidence that that peripheral blood and bone marrow stem cells have different immunological properties, and future immune reconstitution studies may demonstrate how this relates to post-HSCT autoimmune complications.

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Acknowledgements

I would like to thank the following for their contribution to this thesis:

Dr Bronwen Shaw, for encouraging me to undertake this MD(Res), supporting my application to Anthony Nolan, offering support and advice along the way, and for recommending great literature when I needed a diversion.

Professor John Snowden, to whom I extend much gratitude for being an inspiring supervisor and offering direction, encouragement, and opportunity in equal measure over the last two years.

Professor Alejandro Madrigal, for his infectious enthusiasm, bringing his vast experience to bear on the evaluation of my study proposals, and for helping to secure funding for my laboratory work.

Professor Karl Peggs, for offering valuable feedback and practical advice regarding the challenges of managing the clinical studies in this thesis.

Dr Thushan de Silva, for his guidance, collaboration on all the projects in this thesis, and also for his prompt and thoughtful feedback on my work, which has improved greatly my academic writing.

Dr Maria Gilleece and Professor Rod Skinner for their expert input into the development of the survey in Chapter 1, and for their invaluable comments on the resulting abstract and manuscript.

Professor Diana Greenfield for her support and feedback on the work in chapters 2 and 4.

Dr Alice Forster to whom I am enormously grateful for agreeing without hesitation to collaborate on the work in Chapter 4, and for helping me to focus my ideas for this project and generously offering her time and expertise at all stages.

Dr Chloe Anthias, for both her good humour and good sense whenever I sought her advice either as a friend, or as my boss at Anthony Nolan.

I would especially like to thank Hayley Leonard at the Royal Marsden Hospital, Dr Kathryn Goddard at the Royal Hallamshire Hospital, Susan Paskar at the Freeman Hospital, and Michaela Mayhew at St George's Hospital for recruiting patients to the studies in chapters 3 and 4. Without the help of these colleagues I could not have taken these studies forward, and I am truly grateful for this.

At Public Health England, Professor Maria Zambon and Dr Katja Hoschler for allowing me to carry out my bench work in their laboratory. Also, Janice Beldavarona, Monali Patel, Christine Carr and Max Pitcher for patiently passing on their expertise in serological techniques.

At the EBMT Statistical Unit, Dr Simona Iacobelli for her advice in developing the study protocol for chapter 5, and Dirk-Jan Eikema for his work and patience analysing the data and answering my many questions. At the EBMT Leiden Data Office, Cora Knol-Bout, Sofie Terwel and Paul Bosman for their work collecting and managing data from 41 participating transplant centres.

At Anthony Nolan, Henny Braund, Chief Executive, for employing me as a Medical Officer, and for providing the funding for this fantastic opportunity.

And last but very far from least, my wonderful wife Lukxmi

Publications and Presentation

Published Papers

Miller P D E, de Silva T I, Skinner R, Gilleece M, Peniket A, Hamblin A, Greenfield D, Anthias C, Peggs K, Madrigal A, Snowden J A on behalf of the BSBMT Clinical Trial Committee. Routine Vaccination Practice after Adult and Paediatric Allogeneic Haematopoietic Stem Cell Transplant: A Survey of UK NHS Programmes. Bone Marrow Transplantation advance online publication 23 January 2017.

Manuscripts Submitted or in Preparation

Miller P D E et al. Sociodemographic and psychological determinants of influenza vaccine intention amongst recipients of autologous and allogeneic haematopoietic stem cell transplant: a cross-sectional survey of UK transplant recipients using a modified health belief model. Accepted for publication by BMJ Open

Miller P D E et al. A comparison of viral microneutralization and haemagglutination inhibition assays as measures of seasonal inactivated influenza vaccine immunogenicity in the first year after allogeneic haematopoietic stem cell transplant. In preparation.

Miller P D E et al. Autoimmune cytopenias (AIC) following allogeneic haematopoietic stem cell transplant for acquired aplastic anaemia: A joint study of the Autoimmune

Diseases and Severe Aplastic Anaemia Working Parties (ADWP/SAAWP) of the European Society for Blood and Marrow Transplantation (EBMT). In preparation.

Peer-Reviewed Presentations

April 2016. EBMT Annual Meeting, Valencia, Spain. Poster Presentation

Miller P D E, de Silva T I, Skinner R, Gilleece M, Peniket A, Hamblin A, Greenfield D, Anthias C, Peggs K, Madrigal A, Snowden J A on behalf of the BSBMT Clinical Trial Committee. Routine Vaccination Practice after Adult and Paediatric Allogeneic Haematopoietic Stem Cell Transplant: A Survey of UK NHS Programmes

April 2017. EBMT Annual Meeting, Marseille, France. Poster Presentation

Miller P D E, de Silva I, Leonard H, Goddard K, Anthias C, Hoschler K, Peggs K, Madrigal A, Snowden J A. Viral Microneutralization Assay to Determine Seasonal Influenza Vaccine Immunogenicity in the First-Year Post Reduced Intensity Conditioning Allogeneic Haematopoietic Stem Cell Transplant.

December 2017. ASH Annual Meeting and Exposition. Poster Presentation

Miller P D E, Snowden J A, Madrigal A, Iacobelli S, Eikema D-J, Knol C, Marsh J C W, Rice C, Koh M, Fagioli F, Chaganti S, Finke J, Duarte R, Bader P, Passeg J, Peffault De Latour R, Dufour C. Autoimmune cytopenias (AIC) following allogeneic haematopoietic stem cell

transplant for acquired aplastic anaemia: A joint study of the Autoimmune Diseases and Severe Aplastic Anaemia Working Parties (ADWP/SAAWP) of the European Society for Blood and Marrow Transplantation (EBMT).

Invited Speaker Presentations

October 2015. BSBMT Educational Day, London, England.

Miller P D E. The Evidence for Vaccination post Haematopoietic Stem Cell Transplant

November 2015. Federation of Infection Societies Annual Conference, Glasgow, Scotland.

Miller P D E. Vaccination after Haematopoietic Stem Cell Transplant – The Current State of Play in the UK

List of Abbreviations

aAA: Acquired aplastic anaemia

Ab: Antibody

aGvHD: Acute graft versus host disease

A(H1N1)pdm09: Influenza A/California/7/2009(H1N1)pdm09

AIC: Autoimmune cytopenia

AIHA: Autoimmune haemolytic anaemia

AIN: Autoimmune neutropenia

ASBMT: American Society for Blood and Marrow Transplantation

Anti HA Ab: Anti-Haemagglutinin Antibody

APC: Antigen presenting cell

ASBMT: American Society for Blood and Marrow Transplantation

ATG: Anti thymocyte globulin

AutoHSCT: Autologous haematopoietic stem cell transplant

AlloHSCT: Allogeneic haematopoietic stem cell transplant

BAFF: B cell activating factor

B cells: B lymphocytes

BMH: Bone marrow harvest

BSBMT: British Society of Blood and Marrow Transplantation

CCLH: Children's Cancer and Leukaemia Group

CD: Cluster of differentiation

CHMP: Committee for Medical Products for Human Use

cGvHD: Chronic graft versus host disease

COP: Correlate of protection

CPcGVHD: Clinical Consensus Conference on Clinical Practice in cGvHD

CPE: Cytopathic effect

CTLA4: Cytotoxic T lymphocyte antigen 4

DAT: Direct antiglobulin test

DOH: Department of Health

DAMP: Damage-associated molecular patterns

DLI: Donor lymphocyte infusion

E-HBM: Extended Health Belief Model

ELISA: Enzyme-linked immunosorbent assay

EMA: European Medicines Agency

FACT: Foundation for the Accreditation of Cellular Therapy

G-CSF: Granulocyte-colony stimulating factor

GMT: Geometric mean titre

GP: General Practitioner

GMR: Geometric mean ratio

GvHD: Graft versus host disease

GvL: Graft versus leukaemia

HA protein: Haemagglutinin protein

HA assay: Haemagglutination assay

HAI: Haemagglutination Inhibition

hATG: horse anti thymocyte globulin

HBM: Health belief model

HEPA filter: High-efficiency particulate air filter

HepB: Hepatitis B

Hib: Haemophilus influenza type B

HLA: Human leucocyte antigen

HPV: Human papillomavirus

HRA: Health Research Authority

HSC: Haematopoietic stem cells

IDSA: Infectious Diseases Society of America

IGI: Immunisation Guidelines for Ireland

IIV: Inactivated Influenza Vaccine

IL7: interleukin 7

IST: Immunosuppressive therapy

ITP: Immune thrombocytopenia

IVIg: Intravenous Immunoglobulin

JACIE: Joint Accreditation Committee ISCT EBMT

M1: Matrix 1 protein

M2: Matrix 2 protein

MAC: Myeloablative conditioning

MDCK: Madin-Darby canine kidney

MenACWY: Meningitis ACWY

MenB: Meningitis B

MenC: Meningitis C

MMR: Measles-Mumps-Rubella

MIIV: Monovalent inactivated influenza vaccine

MUD: Matched unrelated donor

NA: Neuraminidase

NHS: National Health Service

NK Cells: Natural killer cells

NMA: Non-myeloablative conditioning

NP: Nucleoprotein

PAMP: Pathogen-associated molecular patterns

PBSC: Peripheral blood stem cells

PCV: Pneumococcal conjugate vaccine

PHE: Public Health England

PIIV: Paediatric intranasal influenza vaccine

PIS: Participant Information Sheet

PPSV: Pneumococcal polysaccharide vaccine

PRR: Pattern recognition receptors

rATG: rabbit anti thymocyte globulin

RBC: Red blood cells

RCPCH: Royal College of Paediatrics and Child Health

RDE: Receptor Destroying Enzyme

RIC: Reduced intensity conditioning

RNP: Ribonucleoprotein

RPM: Revolutions per minute

RVP: Routine vaccination programme

SIAT1: Human 2,6-sialyltransferase

SIIV: Seasonal inactivated influenza vaccine

SOP: Standard Operating Procedure

SOS: Sinusoidal obstructive syndrome

TB: Tuberculosis

TBI: Total body irradiation

T cells: T lymphocytes

TIIV: Trivalent inactivated influenza vaccine

TPB: Theory of Planned Behaviour

T reg: Regulatory T lymphocyte

UCB: Umbilical cord blood

VAR: Varicella

VGM : Virus growth medium

VMN : Viral microneutralization

VPD : Vaccine preventable diseases

VUD : Volunteer unrelated donor

WHO: World Health Organization

1 Introduction

1.1 Overview of the Human Immune System

The essential function of the human immune system is to protect from the multitude microorganisms that surround us. Despite continually encountering pathogenic viruses, bacteria, fungi and parasites, it is only relatively rarely that humans succumb to serious infection. The human immune system achieves this through three key functions: Recognition of infection, eradication of infection, and development and maintenance of long-term immune memory. A fourth crucial function is immune-regulation which serves to limit allergic and autoimmune reactions(1).

All the cells of the blood and the immune system are the progeny of the self-renewing pluripotent haematopoietic stem cells (HSC) of the bone marrow (2). HSCs give rise to myeloid and lymphoid progenitor cells. Myeloid progenitors differentiate into erythrocytes, megakaryocytes, and cells of the immune system, namely macrophages, mast cells, dendritic cells, and the three granulocytes (neutrophils, eosinophils and basophils). These are collectively referred to as the cells of the 'innate' immune system. Lymphoid progenitors differentiate into three classes of lymphocytes: natural killer (NK) cells which are also part of the innate immune system, and B and T-Lymphocytes (B and T cells) which comprise the 'adaptive' immune system (*Figure 1*).

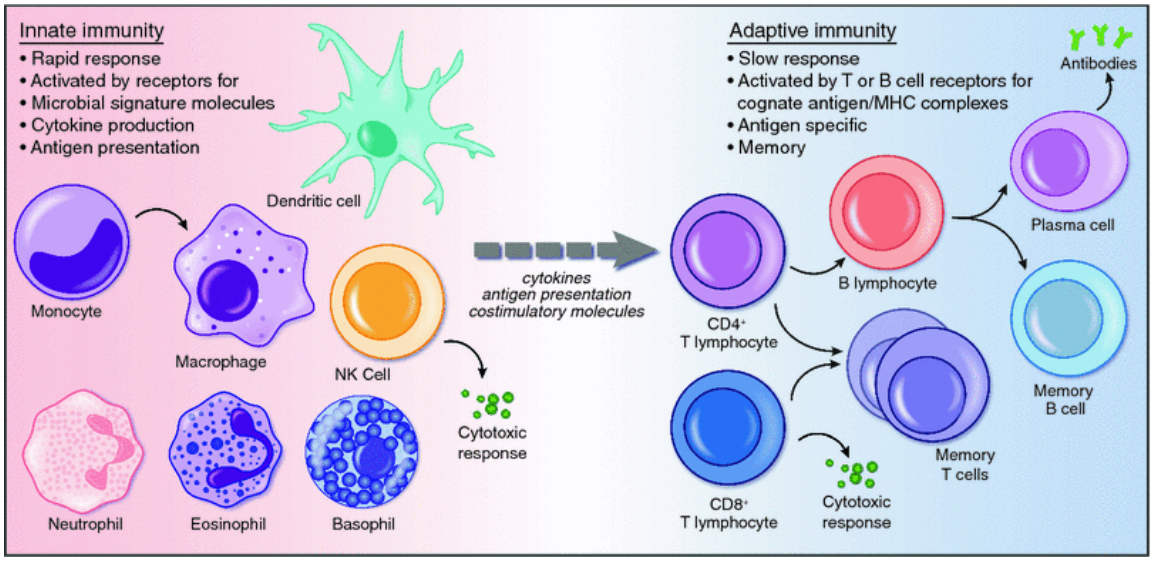

Figure 1: Overview of the innate and adaptive immunity (Beutler and Goodnow, 2011) Used with permission.

1.1.1 The Innate Immune System

The innate immune system provides a rapid, front-line response to infection. Through pattern recognition receptors (PRRs), such as toll-like receptors, the cells of the innate immune system identify common, conserved signature molecules on microorganisms called pathogen associated molecular patterns (PAMPs), as well as molecules released by endogenous cells undergoing stress or cellular injury called damaged associated molecular patterns (DAMPs)(4). Through cytokine release, activated macrophages stimulate inflammation and through chemokines initiate migration of neutrophils, monocytes and other granulocytes to sites of infection. This response occurs within minutes of infection. Natural killer (NK) cells are also activated by macrophage cytokines. The function of NK-cells is to suppress cells infected by viruses until an adaptive response is underway, and they also have a role in controlling tumour cells(1).

Dendritic cells within the tissue also bear PRRs, and when activated are the principal link between the innate and adaptive immune system. Activated dendritic cells migrate to secondary lymphoid tissue (lymph nodes, spleen and mucosal lymphoid tissue) where they present pathogen derived molecules (antigen) to naïve lymphocytes, hence their classification as antigen-presenting cells (APCs)

1.1.2 The Adaptive Immune System

While the innate system provides a fast, but unfocussed immune reaction in response to PAMPs and DAMPs, the adaptive immune system responds over days to weeks with a targeted response to specific pathogen antigens. B and T cells mature in the primary lymphoid tissue (bone marrow and thymus respectively), and then circulate in secondary lymphoid tissue as mature naïve cells. During maturation in primary lymphoid tissue, self-reactive lymphocytes are negatively selected as part of immune-regulation (5). The structure of B and T cell receptors allow them to bind a wide range of molecules and so mount an adaptive immune response to any encountered pathogen. Following activation, dendritic cells migrate to the peripheral lymphoid tissue where they encounter mature naïve lymphocytes. Dendritic cells activate T cells by 'presenting' pathogen specific antigen in association with human-leucocyte-antigen (HLA) Class 2 proteins. Activated cluster-of -differentiation(CD)8+ cytotoxic T cells enter the circulation and migrate to sites of infection where they identify infected cells by 'recognition' of specific antigen in association with MHC class 1 molecules on non-

immune cells. CD4+ T helper cells remain in the lymphoid tissue where they recognize antigen in association with MHC Class 2 on APCs and provide co-stimulatory signals that 'help' activate B cells. Activated B cells undergo a processes of affinity maturation and clonal proliferation and produce antibody (Ab) specific to the presented antigen. Ab produced by B cells protect against pathogen by three mechanisms: First, neutralization of pathogens by blocking either binding receptors or toxins; second, opsonisation which enables phagocytosis by macrophages; and finally, through activation of the proteolytic cascade of the complement system. Both antigen specific B and T cells persist as memory cells and provide long-term immunity to previously encountered pathogen. An important immune phenomenon is the secondary immunization effect, whereby on repeat stimulation memory B cells produce higher titres of higher-affinity Ab compared to primary stimulation. It is immune memory that helps prevent illness from a previously encountered micro-organism and enables immunization by vaccination.

The role of a third class of T lymphocyte, the regulatory T cell (T reg), is to control immune response. These cells are thought to play an important role in maintaining self-tolerance and prevention of autoimmunity (6).

1.2 The Role of Immunization by Vaccination in Disease Prevention

Immunization is defined as 'the process whereby a person is made immune or resistant to an infectious disease, typically by administration of a vaccine'(7). Hereafter 'vaccination' will be used to describe the administration of a vaccine with the aim of

immunization. Once administered, vaccines provoke an immune response through the mechanisms of the innate and adaptive immune as outlined in section 1.1. Vaccination plays a vital role in disease prevention at the individual, community and global level.

At the individual level, vaccination can protect against disease if infection occurs, can attenuate the severity and duration of an illness if breakthrough disease does occur, and by both mechanisms can also protect against related diseases, for example hepatocellular carcinoma associated with chronic Hepatitis B infection(8), and anogenital disease associated with human papillomavirus (HPV) infection(9).

At the community level, if a critical portion of the population is successfully immunized through vaccination, the spread of infection can be restricted through a reduction in quantity and/or duration of pathogen shedding in vaccinated individuals. This can reduce the incidence of disease and even eliminate it locally. Non-vaccinated individuals may also benefit from the reduction in transmission of pathogens, and this is known as 'herd immunity'(10).

The potential global health benefit of vaccination programmes was realised in 1980 when the World Health Organization declared the eradication of smallpox (11). Disease eradication through vaccination is not possible when pathogens are harboured in environmental or animal reservoirs.

1.3 Classification of Vaccines and Vaccine Adjuvants

Vaccines are classified by the type of antigen they contain. The main categories are summarized in *Figure 2*.

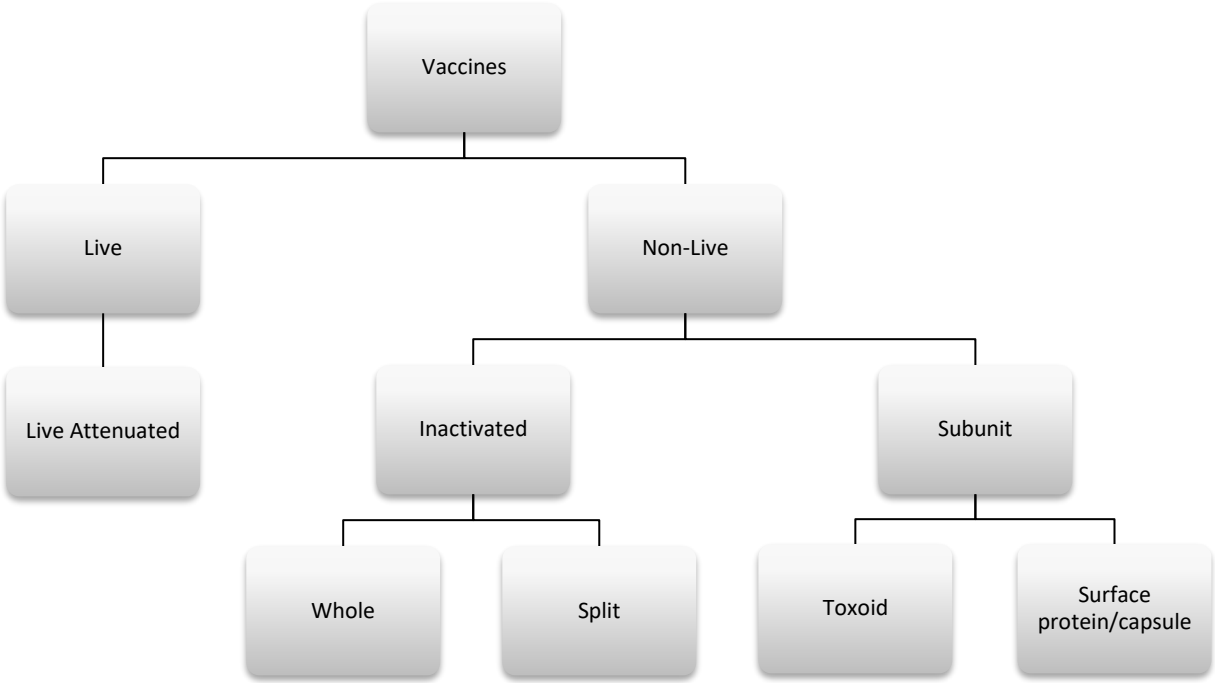

Figure 2: Classification of vaccine types

1.3.1.1 Live Attenuated Vaccines

Live vaccines are composed of attenuated or non-pathogenic strains of the microorganism. Attenuated strains may be naturally occurring, such as type 2 poliovirus (12), or strains pathogenic in animals but not humans may be isolated (for example Edward Jenner's use of cowpox to immunize against smallpox). Pathogens can

be attenuated in the laboratory by culture over multiple generations in adverse growing conditions, or through recombinant DNA technology. Due to the simpler genome, laboratory attenuation of viruses is more straightforward than bacteria. As live vaccines closely resemble a natural infection, and are able to replicate, they provoke a strong cellular and antibody response, which offers long-term protection usually with a single dose (12). Examples of live attenuated vaccines include the Measles-Mumps-Rubella (MMR) vaccines Priorix® and M-M-RVAXPRO®, and the varicella vaccine Varivax®. The disadvantages of live vaccines include the need for refrigeration, which makes the supply and storage chain more complex and expensive, and the side effect profile which includes mild forms of the disease.

1.3.1.2 Non-Live Vaccines

Non-Live vaccines are comprised of either pathogens inactivated by chemicals, heat or radiation and administered in whole or split form, or recombinant subunit vaccines. Recombinant subunits may be viral surface proteins or bacterial capsule polysaccharides, or detoxified toxins referred to as toxoids. As these killed pathogens or recombinant components are not capable of replication they are quickly eradicated by the immune system after administration, and so provoke a weaker and less durable immune response than live attenuated vaccines. However, the side effect profile is generally more favourable. Some recombinant vaccines, for examples the pneumococcal polysaccharide vaccine, provoke a weak immune response, and

therefore may be conjugated with an enhancing carrier protein. Bacterial toxoids are often used as conjugate proteins, for example the diphtheria toxoid CRM197 used in the pneumococcal conjugate vaccine Prevnar®(13).

1.3.1.3 Vaccine Adjuvants

Adjuvants are compounds added to vaccines formulations to potentiate the immune response. Although heterogenous, they can be considered in two main groups: delivery systems and immunopotentiators (12). The mechanisms of action are not fully understood, but delivery systems are thought to enhance APC uptake of antigen by provoking mild inflammation at the site of injection (14), while immunopotentiators interact directly with APCs via PRRs (12). An example of an adjuvanted vaccine is FLUAD™ , a three-component (trivalent) inactivated influenza vaccine containing MF59 which is an oil-in-water squalene, delivery system type adjuvant licensed for used in people aged over 65 (15).

1.4 Evaluating Vaccines

1.4.1 Immunogenicity, Efficacy and Effectiveness

The first two stages of vaccine evaluation are product development (basic science and pre-clinical) and safety testing (phase 1); these phases will not be discussed in detail here. The subsequent phases that evaluate a vaccine and determine how well it prevents the disease it targets, are themselves significant research challenges(16). This process of vaccine evaluation can be considered in three phases, each of which answers a different question.

1.4.1.1 Phase 2 - Vaccine Immunogenicity

Vaccine immunogenicity is a measure of a vaccines ability to provoke an immune response in an individual, and asks the question does the immune system recognize the vaccine as foreign and respond to it with a specific and durable immune response? Immunogenicity is determined by identifying a change, and the duration of this change, in a measurable component of the immune response. Vaccine immunogenicity is typically determined by a change in a specific antibody level in response to administration of a vaccine. A vaccine immunogenicity study may evaluate different dosing and scheduling strategies to determine the most immunogenic.

1.4.1.2 Phase 3- Vaccine Efficacy

This is a measure of a vaccine in 'ideal' conditions and so is usually assessed in the context of a double-blinded prospective randomized control trial. Studies of vaccine efficacy ask the question 'does the immune response provoked by the vaccine actually serve to prevent illness in a study population?'(16). Vaccine efficacy is dependent on the immune response within the study population being sufficient both qualitatively (magnitude of immune response) and also quantitively (avidity of immune response) (17). Vaccine efficacy studies are required before a new vaccine is licensed for use.

1.4.1.3 Phase 4 – Vaccine Effectiveness

Vaccine effectiveness is the most difficult aspect to evaluate. This is a measure of whether a vaccine works in the real-world setting, and asks the question, 'does the vaccine actually help to prevent infection and disease spread in the population?'(16). Vaccine effectiveness is typically assessed retrospectively in a case-control or cohort study. Vaccine effectiveness is dependent on immunogenicity and efficacy but is also affected by vaccine coverage of the population, targeting of at risk groups, timing of vaccination (particularly applicable to seasonal vaccines), logistical aspects such as vaccine storage conditions and supply chains, and other non-vaccine variables which

determine how well a vaccine performs in the real world. Vaccine effectiveness studies can only be performed after a vaccine has been licensed and administered in to target population.

1.4.1.4 Correlates of Protection

A correlate of protection (COP) is a measurable immune response that is associated with a reduction in risk from a specific illness. A COP is determined by identifying an association between an immune response to a vaccine and a clinical outcome measure. For example, the haemagglutination inhibition (HAI) assay measures Ab to the influenza virus hemagglutinin (HA) protein. An HAI Ab titre of 1:40 is considered to correlate with a 50% reduction in risk of infection from influenza virus(18). A negative (<1:10) pre-vaccination Ab titre increasing to ≥ 1:40, or a fourfold increase from pre to post vaccination titre, is considered a marker of seroconversion following vaccination or exposure to virus(19). COPs are important as they provide a benchmark against which to measure new vaccines, and a way to estimate efficacy of a vaccine without performing complex, time-consuming and expensive efficacy studies. In the case of the seasonal influenza vaccine it would be impossible to perform phase 3 efficacy studies each year, therefore immunogenicity studies are conducted in studies of relatively limited size, and the new annual vaccine must achieve specific licensing criteria, which have until recently been based on the following COPs referred to as the Committee for Medical Products for Human use (CHMP) criteria:

1. >40% patients aged 18-60 (>30% in patients aged over 60) achieving seroconversion or a significant increase in HAI titre
2. 70% patients aged 18-60 (>60% in patients aged over 60) achieving an HAI titre ≥ 1:40
3. A mean geometric mean titre increase >2.5

COPs and their limitations, and recent changes to the CHMP criteria are discussed in further detail in section 3.1.7.

1.5 The United Kingdom National Health Service Vaccination Schedule

In the United Kingdom, a schedule of vaccinations is offered routinely on the National Health Service (NHS) and administered in Primary Care. In addition to routine vaccines for the general population, additional vaccines are offered to at risk individuals. The national vaccination schedule is summarized in Table 1.

Age Group / Population Group	Vaccine
8 Weeks	5-in-1: diphtheria, tetanus, pertussis, polio, Haemophilus influenzae B (Hib) pneumococcal conjugate (PCV) rotavirus[a] meningitis B (MenB)
12 Weeks	5-in-1 (dose 2) rotavirus[a] (dose 2)
16 Weeks	5-in-1 (dose 3) PCV (dose 2) MenB (dose 2)
1 year	2-in-1: Hib (dose 3), meningitis C (MenC) (dose 1) PCV (dose 3) MenB (dose 3)
2-7	paediatric intranasal influenza vaccine (PIIV)
3 years 4 months	measles-mumps-rubella (MMR) [a] 4-in-1: diphtheria, tetanus, pertussis, polio
12-13 years (girls only)	human papillomavirus (HPV)
14 years	3-in-1: diphtheria, tetanus, polio meningitis ACWY (MenACWY)
65 Years	pneumococcal polysaccharide vaccine (PPSV)
Over 65 years	annual seasonal inactivated influenza vaccine (IIV)
70 years	herpes zoster (Shingles) [a]
Pregnant Women	inactivated influenza vaccine pertussis
Long-term health conditions	inactivated influenza vaccine
Other at-risk groups	PCV / PPSV hepatitis B (HepB) tuberculosis (TB) varicella zoster (VAR) (Chickenpox)

[a]live attenuated vaccines

Table 1: Summary of United Kingdom NHS vaccination schedule(20)

1.6 Overview of Haematopoietic Stem Cell Transplant

Haematopoietic stem cell transplant (HSCT) is a potentially curative therapy used in the treatment of a range of malignant and non-malignant conditions (21). The number of

transplants performed in the United Kingdom and Republic of Ireland is increasing year-on-year, with 4113 transplants performed in 2015(22). The basic principle of HSCT is the eradication of disease, or consolidation of previous treatment with 'conditioning' therapy, comprised of high-dose chemotherapy alone or in combination with total body irradiation(TBI), followed by reconstitution of a non-diseased haematopoietic system from HSCs. The HSC source may be autologous, harvested from the patient prior to administration of conditioning, or allogeneic from a related or volunteer unrelated donor (VUD).

1.6.1 Autologous HSCT

Autologous HSCT (autoHSCT) is a therapy used in the treatment of several haematological malignancies, autoimmune diseases and solid tumours. In the latter setting, damage to haematopoietic tissue is a side-effect of high-dose therapy, rather than the primary intent. The ablated haematopoietic system is reconstituted using the patient's previously harvested HSCs. The therapeutic effect of autoHSCT is derived solely from the conditioning chemotherapy.

1.6.2 Allogeneic HSCT

Allogeneic HSCT (alloHSCT) is a therapy used in the treatment of several haematological malignancies, bone marrow failure syndromes, haemoglobin disorders, immune deficiencies and metabolic disorders. In alloHSCT, conditioning regimens are defined as non-myeloablative (NMA), reduced intensity (RIC) or myeloablative (MAC), based on the duration and reversibility of the cytopenia induced (23) . HSCs may be harvested by apheresis from a donor's peripheral blood (PBSC) following granulocyte-colony stimulating factor (G-CSF) mobilization, directly by unstimulated bone marrow harvest (BMH), or from frozen umbilical cord blood (UCB) units.

Donor and recipient histocompatibility is determined by HLA type. HLA is encoded by the hyper-polymorphic genes of the major histocompatibility complex (MHC) on the short arm of chromosome 6. There are 2 classes of HLA and both play a role in antigen presentation and self-tolerance. HLA Class 1 is present on all nucleated cells and platelets and presents intra-cellular viral or bacterial peptide to CD8+ cytotoxic T cells (previously activated by dendritic cells) which then mount an immune response against the infected cell. HLA Class 2 is present on APCs, and presents phagocytosed extracellular peptide to CD4+ T helper cells. Crucially, the immune effector cell receptors interact with the MHC-peptide complex so either foreign MHC (on transplanted tissue) or foreign peptide provokes an immune response, hence the role of HLA in histocompatibility. Current guidelines recommend high-resolution allelic level

donor and recipient matching at 3 class 1 alleles (HLA-A, -B and –C) and 2 class 2 alleles (HLA –DRB1 and –DQB1)(24).

In the setting of malignant disease, the therapeutic effect of alloHSCT is derived from the anti-tumour effect of conditioning therapy, but also and importantly from immune control of disease by donor lymphocytes harvested and transplanted with the HSCs. Where indicated, lymphocytes may be collected and infused months to years after HSCT as a donor lymphocyte infusion (DLI). This targeting of minor histocompatibility antigen on tumour cells by the transplanted donor immune system is known as the graft-versus leukaemia (GvL) effect(25). The therapeutic effect of GvL means that alloHSCT is not dependent on myeloablation, and this understanding has promoted the routine use of RIC regimens for patients who are older or with co-morbidities, in whom MAC would carry an unacceptable risk of therapy related morbidity and mortality. However, the therapeutic benefits of GvL are limited by the linked pathological process, graft-versus host disease (GvHD) where the transplanted immune system also attacks and damages healthy recipient tissue. One of the major challenges of HSCT is the dissociation of GvL from GvHD, so recipients may benefit from the former with improved progression-free survival and reduced relapse rates, without suffering the deleterious effects of the latter.

In the setting of non-malignant disease, the donor HSCs reconstitute a healthy haematopoietic system free from immune deficiency or haemoglobinopathy or correct a metabolic disorder. In this context, there is no requirement for a GvL effect, and therefore clinicians seek to minimise GvHD by various means.

1.6.3 Complications of HSCT

While offering potential cure for high-risk diseases, HSCT carries a significant risk of morbidity and mortality from transplant related complications. The major complications of alloHSCT are organ toxicity and sinusoidal obstructive syndrome (SOS) related to conditioning chemotherapy, and infections related to impaired and dysregulated immunity. As outlined in 1.6.2 immune reactivity between the transplanted donor immune system and healthy host tissue manifests as GvHD which can take acute (aGvHD) and chronic (cGvHD) forms. Finally, disease relapse and secondary malignancies remain major challenges in HSCT.

Impaired and dysregulated immunity places HSCT recipients at increased risk from infection, and may restrict the immunogenicity of vaccines.

1.7 Immune Impairment after HSCT

HSCT recipients are significantly immunocompromised for months to years post-transplant. This is related to the underlying disease, conditioning chemotherapy and immunosuppressive therapy (IST), GvHD and its treatment, and the natural pattern of immune reconstitution following HSCT.

1.7.1 Conditioning and Immunosuppressive Therapy

HSCT recipients may have impaired immunity because of their underlying haematological disease, and this will certainly be compounded by chemotherapy, conditioning and IST administered during and after HSCT. The key components of HSCT conditioning and immunosuppressive therapy are outlined in *Figure 3*.

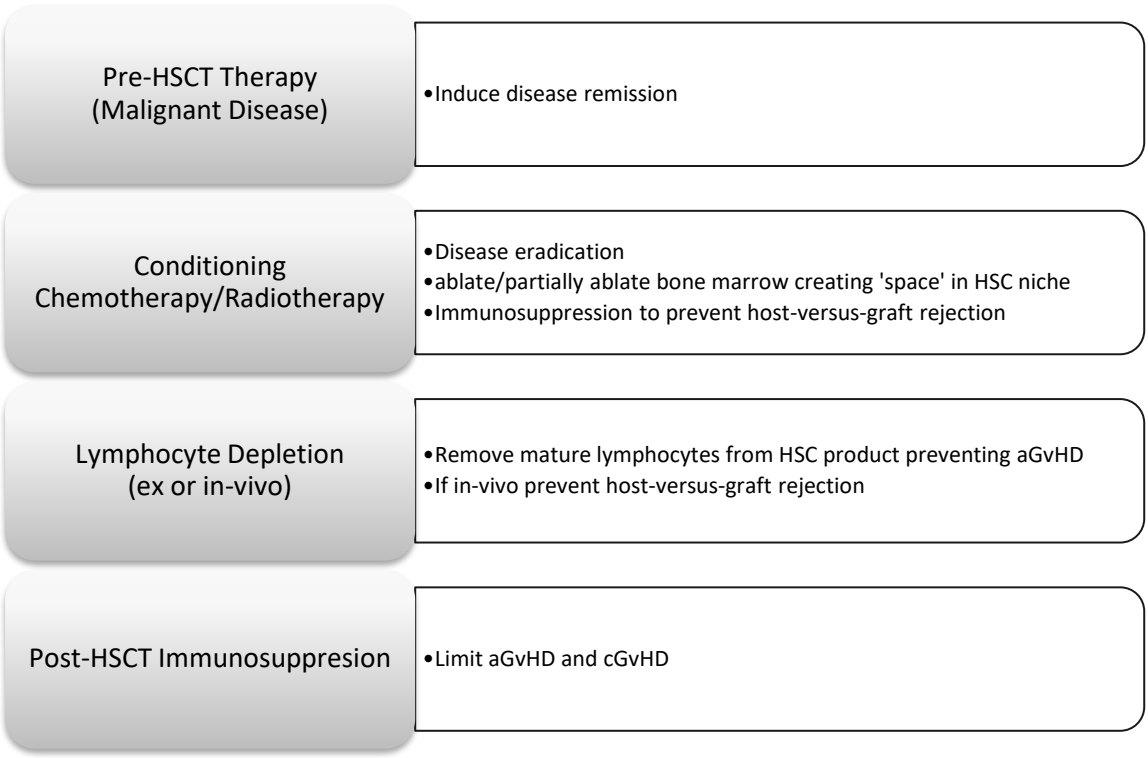

Figure 3: HSCT conditioning and immunosuppressive therapy

IST is typically weaned over the first few months after HSCT, but depending on manifestations of acute and chronic GVHD may need to be continued for considerably longer.

1.7.2 Graft Versus Host Disease

GvHD is the major complication of alloHSCT. GvHD is classified as acute or chronic. Historically, classification was based on time of onset from transplant with 100 days delineating aGvHD and cGvHD. Although nomenclature remains the same, current classification is based on clinical features. aGvHD affects skin, liver and gastrointestinal tract, while cGvHD may be an organ specific or multi-system disorder resembling autoimmune disease (26). Lymphocyte depletion of the HSC product has contributed to a reduction in rates of severe aGvHD (27). However the reported incidence of cGvHD is 30-70% and is the main contributor to non-relapse mortality amongst alloHSCT recipients(28). GvHD is thought to be initiated by injury of recipient tissue by the conditioning therapy, which activates APCs and stimulates production of pro-inflammatory cytokines. Activated recipient APCs interact with donor derived T and B cells targeting an adaptive immune response towards healthy host tissue(29). In addition to precipitating tissue inflammation and fibrosis, GvHD contributes to immune impairment and dysfunction through thymic atrophy (30,31) and functional hyposplenism (32). What is more, the mainstay of GvHD treatment is IST. Unsurprisingly the major cause of cGvHD associated non-relapse mortality is infection (33).

1.8 Immune Reconstitution

Both RIC and MAC regimens render patients aplastic, and without HSC rescue they would almost invariably succumb to infection. Immune reconstitution describes the process of a functioning, disease-free immune system maturing from transplanted HSCs and donor lymphocytes. The effector cells of the immune system reconstitute at different time-points post HSCT, and qualitative recovery may not coincide with quantitative. Full immune recovery may take years or more(34). Many factors impact on time to quantitative and qualitative immune reconstitution including initial disease, donor-recipient HLA match, recipient age, HSC source, conditioning intensity, lymphocyte depletion, GVHD, infectious complications, and use of serotherapies such as monoclonal antibodies (35). Broad patterns of immune recovery are outlined below and summarised in *Figure4*.

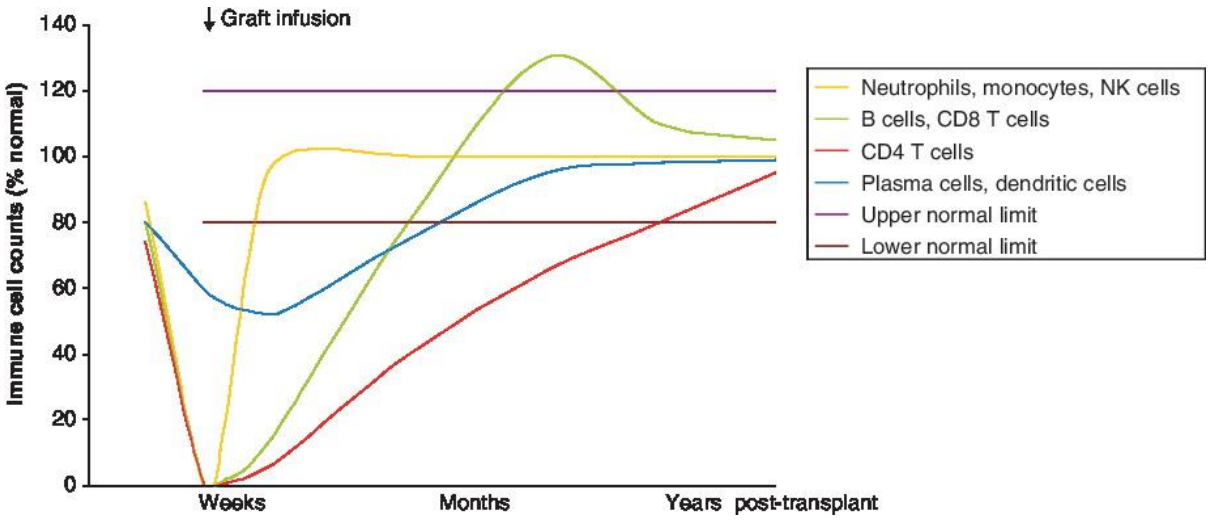

Figure4: Approximate immune effector cell reconstitution (percentage of normal counts) after MAC HSCT. (36) *Used with permission*

1.8.1 Reconstitution of Innate Immune Response

The effector cells of the innate immune response are the earliest to recover after HSCT. Granulocyte counts, particularly neutrophils recover within the first month of transplant. This is dependent on stem cell source with recovery taking approximately 14 days for PBSC, 21 days for BMH and 30 days for UCB(34). Monocytes also recovery within the early post-HSCT phase. However, functional recovery may take several months (37). Quantitative and qualitative reconstitution of NK cells occurs within 1-2 months and does not seem to be affected by HSC source, recipient age or GvHD (38). Likewise, dendritic cell recovery occurs within 3 months of HSCT although time to functional recovery is unclear (39).

1.8.2 Reconstitution of Adaptive Immune Response

B Cell counts are reported to return to normal numbers within 12 months of HSCT(40), although function as determined by Ab production may take 2 years or more. Reconstitution of B Cells is through the same adaptive process as outlined in 1.1.2. This is referred to as 'recapitulation of ontogeny'. Reconstitution of B cells is impaired by GvHD (41).

T cell recovery is HSC source dependent with recovery post UCB reported at 6-9 months(42) and post PBSC around 12 months in the absence of GvHD(43). Importantly, early CD8+ T cell recovery may occur swiftly within 3 months by thymic-independent peripheral expansion of memory lymphocytes transplanted with the HSC product or later administered as DLI (44). This is thought to contribute to a narrow T cell repertoire, and may have a role in the pathogenesis of immune phenomenon post-HSCT including cGvHD and autoimmune disease (45). The pathogenesis of autoimmune disease post HSCT is discussed in more detail in Section 5.1.2. Development of de-novo mature T cells from naïve T cells derived from donor HSCs is dependent on thymic function and may be significantly impaired in older patients who have undergone thymic involution, and in recipients with GvHD. CD4+ T helper cells may take up to 2 years to recover (46) and this delay may contribute to impairment of immune function despite qualitative recovery of other effector cell subsets.

A number of studies have investigated disease specific Ab levels following auto and alloHSCT. In the first months after HSCT there is a decline in all immunoglobulin subclasses. However, Ab levels specific to commonly encountered antigen may recover within the first year (35). In contrast, Ab levels to Antigen not encountered after HSCT, including the vaccine preventable diseases, may decline for months to years after transplant. Studies have identified a post-HSCT decline in Ab titres to measles, mumps and rubella (47–49), tetanus (50), poliovirus(51–53), and pneumococcus (54,55). This is most evident in alloHSCT recipients, but a marked and progressive decline in disease specific Ab titres is also seen in autoHSCT recipients(56–58).

1.9 Infection after HSCT

It is apparent that HSCT recipients are profoundly immunocompromised for weeks to months post HSCT and may experience both quantitative and qualitative deficiencies of the innate and adaptive immune system for months to years. Consequently, HSCT recipients are at significant risk of post-HSCT infection. In a large multi-centre, retrospective series of HLA-identical sibling AlloHSCT recipients transplanted between 1980 and 2001, infections accounted for 10% of all deaths, and 15% of non-relapse deaths. Bacteria accounted for 36% of infection related deaths, viruses 31%, fungi 28% and parasites 5%. 50% of infection related deaths occurred before 3 months, and 50% after (59). Importantly, the authors found an improvement in infection related mortality within the first 12 months of HSCT over the study period. A more recent single centre study reported a 6% 1 year cumulative incidence of death from infection again with bacterial and viral infection being the most common (37.5% and 25% respectively)(60).

Susceptibility to pathogenic organisms evolves over time under the influence of factors outlined in 1.7. Pathogenic organisms encountered in the post-transplant period are shown in *Figure 5*.

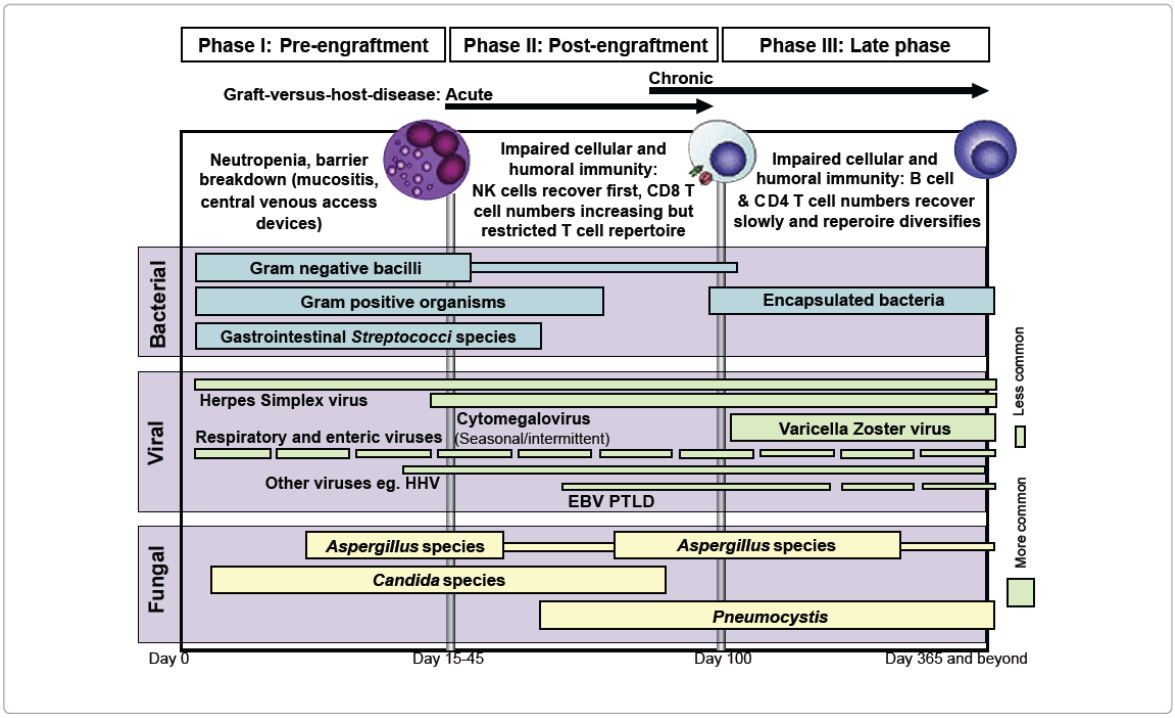

Figure 5: Phases of Infection after alloHSCT (61) *Open Access*

1.10 Vaccine Preventable Disease after HSCT

As a result of the profound immunosuppression and loss of adaptive immune memory associated with HSCT, recipients may become susceptible to diseases to which they were previously immune following past exposure or vaccination. Most vaccine preventable diseases (VPD) are rare amongst the general UK population, and given widespread vaccination HSCT recipients may derive benefit from herd immunity. For most VPDs, there are only a limited number of reports of illness post-HSCT, so the clinical significance of decreased antibody levels is not apparent from clinical data. The exceptions to this are the influenza virus and Streptococcus pneumoniae infections.

1.10.1 Influenza Virus after HSCT

Seasonal influenza accounts for 20-40% of respiratory viral infections post HSCT (62,63). As one would expect, transplantation during influenza season is associated with increased risk of infection. A 10-23% mortality rate is reported for seasonal influenza, rising to 28% in those who develop secondary pneumonia (63–66). Lower respiratory tract infection is more common in those early post-transplant and with lymphopenia (64).

The 2009 Influenza pandemic A H1N1 virus A(H1N1)pdm09 carried a higher morbidity and mortality in HSCT recipients than in the general population. Incidence of infection was reported at 4% with a higher rate among recipients of allo compared with autoHSCT (67,68). Infection carried a high reported incidence of severe complications with 21-50% progression to lower respiratory tract infection and 8-30% mechanical ventilation. Mortality was reported at 5-43%(67–73). By way of comparison, in the United Kingdom the case fatality rate in the general population was 26 per 100,000(74). Of note, these predominantly retrospective studies of HSCT populations may have missed mild cases of A(H1N1)pdm09 not presenting to HSCT centres, and so incidence may be underestimated, and complication rate overestimated. A comparative study of seasonal influenza and A(H1N1)pdm09 found a higher rate of severe complication in the latter, but a similar overall influenza associated mortality(75). The A(H1N1)pdm09 virus has continued to circulate since 2009 and up to the most recent flu season (Northern Hemisphere 2016 Season) as one of the dominant seasonal strains.

Identified risk factors for severe complications from A(H1N1)pdm09 included active GvHD and immunosuppressive treatment with steroids and mycophenolate, and lymphopenia. In the general population younger age was associated with increased morbidity and mortality with a peak mortality rate in those aged less than twenty(76) This is in contrast to HSCT recipients in whom increasing age was associated with poorer outcome. Disease was generally milder in paediatric HSCT recipients and haemato-oncology patients (77,78). It is thought that previously acquired cross-reactive antibodies to the A(H1N1) strain circulating prior to 1957 provided protection against A(H1N1)pdm09 in older members of the general population, while some immunological cross-reactivity between A(H1N1)pdm09 and the strain circulating between 1957 and 2009 provided some protection in those aged 20-60. The poorer outcome with age in HSCT recipients may of course be related to underlying disease state, but a loss of protective immune memory post-transplant may have contributed. Nosocomial infection was also found to be associated with poorer outcome(72). This may reflect underlying illness in hospitalized patients and emphasizes the need for rigorous infection control to protect the most vulnerable HSCT recipients.

1.10.2 Pneumococcal Disease after HSCT

Pneumococcal disease – illnesses cause by Streptococcus pneumoniae – is a significant cause of morbidity and mortality post HSCT. In the immunocompetent, this bacterium typically causes non-invasive diseases such as ear infections and bronchitis. However, Streptococcus pneumoniae can also cause pneumonia, bacteraemia and meningitis, and these invasive pneumococcal diseases are more common in the immunocompromised. Several large multi-centre studies have investigated invasive pneumococcal disease in HSCT recipients. Although invasive pneumococcal disease may present early post-transplant during the period of pre-engraftment neutropenia(79), the majority of cases present late with a reported median time point of 15-27 months(79–82). In recipients of both autologous and allogeneic HSCT the incidence is significantly higher than the general population; up to 965/100000 (80) in allogeneic HSCT recipients compared with a reported incidence in the general population of 11.5/100000(81). AlloHSCT and the presence of GvHD or treatment with IST have been identified as risk factors for infection, although are not consistently associated with mortality(79) which has been reported at 20-30%.

1.10.3 Other Vaccine Preventable Diseases

Other vaccine preventable diseases have not been investigated systemically post HSCT, principally owing to small case numbers. There are case reports and small case series of Haemophilus influenzae infection in HSCT recipients (83–86). Pertussis infection in HSCT recipients has also been reported (Kochethu, Clark, & Craddock, 2006; Suzuki et

al., 2003). Measles infection in the immunocompromised, including HSCT recipients, can result in severe infection and lead to disease complications such as measles interstitial pneumonia (87,88).

1.11 Prevention of Infection and Vaccination after HSCT

During and following the alloHSCT process, a range of measures are undertaken to prevent infection in recipients. Broadly speaking these can be classified as: i) measures to prevent exposure to pathogens, ii) measure to prevent occurrence of disease in event of exposure, and iii) measures to prevent disease reactivation in the case of latent infections.

Exposure prevention measures include pre-HSCT screening of recipient and donor for infection, nursing in high-efficiency particulate air (HEPA) filtered rooms, attention to hand-hygiene among both patient and healthcare staff, and a low bacterial diet. To prevent disease occurrence patients will typically be administered prophylactic antimicrobials against bacterial, fungal and viral pathogens. In addition, prophylaxis will be administered if patients are at risk from reactivation of latent infection (e.g. toxoplasmosis, viral hepatitis). Antifungals and antivirals will typically be continued until recipients are free of GvHD and no longer requiring IST, while prophylaxis against encapsulated bacteria will be continued life-long.

Given the post-HSCT loss of adaptive cellular and humoral immune memory, the decline in VPD specific Ab levels, and with evidence for increased morbidity and mortality from at least some VPDs, current recommendations are that recipients receive a schedule of vaccinations after auto and alloHSCT. Indeed, a number of immunogenicity studies have demonstrated that waning Ab titres to VPDs can be boosted by vaccination (53,89,90). However, the immune response to vaccination may be impaired after both auto and alloHSCT, and longer time from transplant to vaccination has been reported as a predictor of vaccine response(91,92). This accords with knowledge of patterns of immune reconstitution post HSCT.

There are no clinical efficacy studies of vaccines in HSCT recipients, and it is not clear whether COPs established in healthy populations are meaningful in immunocompromised subgroups, or whether suboptimal immune responses may convey some clinical benefit in high-risk populations. For VPDs that are rare in the general population, poor early immunogenicity or vaccine administration from 12 months or more after HSCT seems not to result in high infection rates based on the limited number of case reports. However, the influenza vaccine is time critical, and is available and typically administered during the influenza season from October to February. In the absence of clinical efficacy studies, the optimum seasonal influenza vaccination strategy for recipients who are within the first weeks to months after HSCT during the annual season is unknown. The evidence for influenza vaccination will be discussed in detail in Chapter 3. Invasive pneumococcal disease can present in both the early and late post HSCT period(79). However, there is evidence that the PCV is

immunogenic in recipients from 3 months post HSCT (93,94) although efficacy data is again lacking.

1.12 Vaccination Guidelines

Although evidence for vaccination post HSCT is limited, FACT-JACIE International Standards for Haematopoietic Cellular Therapy require that vaccination schedules are in place at accredited HSCT programmes (95). To define this schedule of post-HSCT vaccination, UK HSCT programmes can refer to guidelines from several major societies, along with consensus conference proceedings and recommendations from national paediatric groups (96–99). Full details of vaccination guidelines appear in appendix 1, but are summarised here. Current guidelines advise commencing post-HSCT vaccination schedules from 3-6 months post HSCT (96,98,100,101), with some paediatric experts recommending commencing at 12 months(99). Active cGvHD or concomitant IST are given universally as contraindications to live attenuated vaccines due to concerns about vaccine induced disease. However, live attenuated vaccines can be administered 2 years after HSCT providing these contraindications no longer apply. Guidelines suggest non-live vaccines should be administered regardless of concomitant GvHD, but response to vaccine may be measured (96,98). Non-live vaccines may be delayed if recipients are treated with high dose IST (100). The Current UK paediatric recommendation is that inactive vaccines are delayed if recipients have active cGvHD (99). All guidelines recommend administration of vaccines against the VPDs covered by

the routine UK NHS schedule, although where a choice of formulations are available guidelines vary or make no specific recommendation.

1.13 Current post-HSCT Vaccination Practice

A small number of studies have investigated vaccination practice after HSCT.

Ariza-Heredia et al(102) investigated vaccination rates at 6 months post HSCT in 663 patients in the United States. 38% of patients were fully vaccinated, 24% partially vaccinated and 38% had received no vaccines. At 6 month 15% had received inactivated influenza vaccine, and 40% pneumococcal conjugate vaccine. The authors conducted a physician survey and review of the case notes of unvaccinated HSCT recipients to identify possible barriers to vaccination. They suggest that physician awareness of vaccination guidelines, and awareness of contraindications to vaccination may have contributed to low vaccination rates. Furthermore, they suggest that clinical guidelines are not sufficiently encompassing to guide decision making in some of the complex clinical scenarios encountered in post HSCT care. The need for investigation of patient barriers in this specific group is acknowledged. The low rates of vaccination concord with the results of a previous study by Lerchenfeldt et al (103) who investigated vaccination rates among 137 recipient of allogeneic and autologous HSCT. 33% of patients missed at least one vaccine set, and 26% received a vaccine set later than recommended.

Audits have been performed recently at two UK transplant centres. In an audit of 30 HSCT recipients, 70% were up to date with recommended vaccine schedule at 1 year post transplant, but this fell to only 28% at 2 years (104). Uptake of seasonal influenza vaccine was 72%. Again, physician knowledge of guidelines and the perception of GVHD as a contraindication to vaccination may have contributed to low vaccination rates. In an audit of the records of 100 consecutive HSCT recipients 60% had commenced revaccination by 6 months(105). Patients unvaccinated tended to be those with a more complex clinical course, or frequent hospital admissions. Again, there was an inconsistent approach towards vaccinating HSCT recipients with GVHD.

This small number of studies shows a variable but overall low rate of revaccination and completion of schedules among this high-risk group of patients. Whether this pattern is reflected broadly across UK HSCT programmes is not known. Adoption and knowledge of guidelines, including contraindications to vaccination, a limited evidence base, and complexity of clinical scenarios were identified as possible barriers to vaccination practice.

1.14 Conclusion

This introduction describes the function of the human immune system, and how vaccination can induce long term immune memory and prevent disease at the individual, community and global level. HSCT is a profoundly immunosuppressive therapy, and immune reconstitution may take months to years. This results in a loss of

immune memory to previously encountered pathogens and vaccines, and may render HSCT recipients susceptible to VPDs, in particular to seasonal influenza virus and invasive pneumococcal disease. What is more, patterns of immune reconstitution impair response to some vaccines for at least the first year after HSCT. This is a particular issue for the seasonal flu vaccine, administration of which is time critical.

Although the evidence base for vaccination post HSCT is limited, national and international guidelines and recommendations have been published. Studies and audits suggest that implementation of vaccination schedules is poor, and this may be due to the limited evidence base, and poor physician awareness of guidelines recommendations. Although patient factors contributing to vaccine acceptance and refusal have been studied in other high-risk populations, this has not been explored in HSCT recipients. Through three related studies, the aims of this thesis are: i) To review post alloHSCT vaccination practice across UK programmes; ii) To investigate influenza vaccine immunogenicity in alloHSCT recipients in the first-year post HSCT; and iii) Explore how the health beliefs of HSCT recipients contribute to seasonal influenza vaccination intent. Expanding on the theme of impaired and dysregulated immunity, the final study in this thesis moves away from vaccination, and explores autoimmune cytopenias (AIC) as a complication of HSCT for acquired aplastic anaemia (aAA)

2 Routine Vaccination Programme (RVP) Practice after Adult and Paediatric Allogeneic Haematopoietic Stem Cell Transplant: A British Society of Blood and Marrow Transplantation Survey of UK NHS-Based Programmes.

2.1 Introduction

As outlined in Chapter 1, infection is an important cause of morbidity and mortality following HSCT (61). Impaired humoral immunity, evidenced by a decline in Ab titres to VPDs, is observed within weeks and may continue for years post-HSCT (47–55). Studies of the immunogenicity of a number of vaccines have demonstrated that waning or undetectable post-HSCT Ab titres can be boosted through vaccination(47–49,51–56,89,106,107). However, correlating immunogenicity data with clinical efficacy in a trial setting, or establishing real-world vaccine effectiveness is challenging in small patient groups, and no robust data exists for HSCT recipients. Moreover, it remains unclear whether COPs (for example a 'seroprotective' HAI titre of ≥ 40, or a pneumococcal IgG anti-capsular Ab level ≥ 0.35 ug/ml) established in the general population can be extrapolated to HSCT recipients. Although these questions about the efficacy and effectiveness of post-HSCT vaccination remain unanswered, it is considered best practice to attempt to afford recipients the same breadth of immunity to VPDs as the general population. To this end, FACT-JACIE International Standards for Haematopoietic Cellular Therapy Production Collection, Processing and Administration

state that schedules and indications should be in place for post-transplant vaccination of alloHSCT recipients(95). The current position of the Infectious Diseases Society of America (IDSA) is that regardless of donor or recipient infection or vaccination history, HSCT recipients should be viewed as 'never vaccinated' and offered a schedule of post-transplant vaccination(97).

HSCT programmes can refer to several guidelines and consensus statements at international and national level, to define this schedule of post-transplant vaccination. In 2009 the American Society for Blood and Marrow Transplantation (ASBMT) convened an international group of experts in infectious diseases, HSCT, and public health, to develop guidelines for preventing infectious complications among HSCT recipients, and this included a detailed section on post HSCT vaccination (ASBMT2009) (96). A report from the Clinical Consensus Conference on Clinical Practice in cGvHD provided updated advice in 2011 that addressed in detail vaccination of recipients suffering this post-transplant complication (CPcGVHD2011) (98). The IDSA produced 2013 guidelines (IDSA2013)(97) for vaccination of the immunocompromised host, which included a schedule for adult and paediatric HSCT recipients.

In the United Kingdom, the Department of Health (DOH) 'Green Book' details vaccination for the general population, and touches briefly on vaccination of HSCT recipients but does not provide recommendations or a schedule, instead referring the reader to publications given above for specialist information (108). The Royal College of

Paediatrics and Child Health (RCPCH2002)(109) and Children's Cancer and Leukaemia Group (CCLG2014)(99) have published recommendations addressing vaccination of paediatric HSCT recipients. Immunisation Guidelines for Ireland 2015 (IGI2015) provide recommended vaccination schedules for adult and paediatric HSCT recipients(101).

The recommendations given in these guidelines vary, and in some cases are contradictory (110). This may reflect the limitations of the current evidence base, differences in expert opinion, and different licensing of vaccines between nations, meaning some vaccines recommended by international groups may not be available and recommended at national level: for example, the full-dose diphtheria-tetanus-pertussis (DTaP) vaccine is not licensed for use in adults in the United Kingdom.

2.2 UK post-HSCT vaccination practice

UK post-HSCT vaccination practice was scoped by the British Society of Blood and Marrow Transplant (BSBMT) in 2007 prior to publication of recommendations discussed above(111). The study included auto and alloHSCT programmes and the overall response rate was 56% (n=29). All responding alloHSCT programmes (n=20) recommended post-HSCT vaccination, although there was variation in the vaccines administered (100% diphtheria-tetanus-pertussis and influenza A; 95% pneumococcal and polio vaccine; 90% Haemophilus influenzae B; and 80% meningococcal C vaccine).

The survey did not report on other aspects of post-HSCT vaccination including service organization, commencement and delay of vaccination and monitoring of response.

A recent single-centre audit identified variation in post-HSCT vaccination practice(105). Of those patients who were more than 6 months post-HSCT, 60% had commenced re-vaccination programmes. The authors reported an inconsistent approach to vaccinating HSCT recipients with GvHD or receiving immunosuppressive therapy.

2.3 Study Hypothesis and Objective

We hypothesized that variation across guidelines and the factors underlying this, contribute to variation in post-HSCT vaccination practice among the adult and paediatric alloHSCT programmes of the UK NHS.

The aim of this study conducted on behalf of the BSBMT was to bring knowledge of UK post-alloHSCT vaccination practice up-to-date and determine how current evidence, recommendations and guidelines translate into clinical care, by exploring in detail service organization, vaccine selection, commencement and delay of vaccination, and monitoring of response to vaccines.

2.4 Materials and Methods

2.4.1 Survey Development and Design

A Routine Vaccination Programme (RVP) was defined as a 'series of scheduled vaccinations administered after alloHSCT as part of standard post-transplant care'. A 25-question web-based survey was developed using SmartSurvey™. Questions were grouped into four themes: RVP service organization, RVP vaccine selection, RVP commencement and delay, and monitoring of response to vaccines. Response options were mapped to the current national and international guidelines outlined in 2.1 and where appropriate to the question format, a free-text box was also offered. The survey was developed in conjunction with an infectious disease physician, senior adult and paediatric alloHSCT physicians, and an alloHSCT nurse specialists all with an interest and expertise in vaccination. The survey was piloted with 5 HSCT specialists and optimized accordingly. The final survey can be found in appendix 2.

2.4.2 Survey Administration

An invitation to participate was e-mailed by the BSBMT to all 27 adult and 12 paediatric UK alloHSCT programme directors. Directors were invited to complete the survey or delegate to the member of staff taking primary responsibility for the vaccination programme. Programmes were not asked to submit SOPs for review. The survey was open between May and December 2015.

2.5 Results

2.5.1 Response Rate and HSCT Programme Characteristics

100% of adult, and 83% of paediatric HSCT programmes responded to the survey, with an overall response rate of 95%. The age range of patients treated by paediatric programmes was 0-20 years. The majority of surveys were completed by HSCT programme directors (54%) or consultant grade HSCT physicians (30%) with the remainder completed by HSCT nurse specialists (8%), pharmacists (5%) or non-consultant grade physicians (3%). 95% of responding programmes were JACIE accredited having completed at least 1 cycle, with 5% working towards JACIE accreditation.

2.5.2 RVP Service Organization

All responding adult and paediatric alloHSCT programmes recommend a RVP to HSCT recipients transplanted at their centre. However, only a minority of adult (8%) and paediatric (10%) programmes offer vaccination on site; the remainder refer HSCT recipients to their primary care physician for vaccine administration. Nearly two-thirds (65%) of programmes do not maintain a record of vaccine administration in patients' records. Of these programmes that do not maintain local vaccination records, 29% have audited RVP practice, compared with 54% of HSCT programmes that do. The survey did not enquire about the scope of audits undertaken.

Most adult (97%) and paediatric (80%) programmes maintain a document controlled standard operating procedure (SOP) detailing RVP schedules. Programmes were asked to indicate the main vaccination guidelines or policy document referenced in the SOP, and/or to which healthcare practitioners refer to guide RVP decisions. The majority of adult programmes (72%) draw from either international HSCT specific guidelines (e.g. ASBMT 2009, IDSA 2013) or modified versions, whereas the majority of paediatric programmes (60%) draw from national HSCT specific guidelines (e.g. RCPCH 2002, CCLG 2014). Naturally, these trends reflect the published guidelines most relevant to adult and paediatric programmes respectively.

A minority (21%) of adult programmes use locally developed HSCT specific guidelines, and 7% indicate that they use a combination of international HSCT specific, and national non-HSCT specific guidelines (e.g. DOH Green Book). In free text one programme stated that they need to use this combination as they have 'problems with getting vaccinations done if [their] recommendations are not compatible with Green Book advice'. A minority of paediatric programmes use international guidelines (10%), or locally developed guidelines (10%). 20% of paediatric programmes indicated that they use national HSCT specific guidelines combined with national non-HSCT specific guidelines.

2.5.3 Vaccines included in RVP schedules

Vaccines administered or recommended for RVP by adult and paediatric HSCT programmes are shown in Table 2.

Vaccine or formulation	Paediatric n (%)	Adult n (%)
Pneumococcal	10 (100)	27 (100)
23 valent polysaccharide	2 (20)	5 (18)
13 valent conjugate	7 (70)	21 (78)
No specific recommendation	1 (10)	1 (4)
Tetanus-diphtheria-pertussis	10 (100)	27 (100)
Full dose (DTaP)	4 (40)	17 (63)
Reduced dose (Tdap)	1 (10)	7 (26)
Full (DTaP) or reduced (Tdap) dose depending on age	3 (30)	0 (0)
No specific recommendation	2 (20)	3 (11)
Meningococcal	10 (100)	26 (96)
Men C conjugate	4 (40)	16 (62)
Men ACWY conjugate	2 (20)	6 (23)
Men B conjugate	0 (0)	0 (0)
Men C or ACWY depending on age	2 (20)	0 (0)
No specific recommendation	2 (20)	4 (15)
Haemophilus influenzae B	10 (100)	27 (100)
Inactivated poliovirus	10 (100)	26 (96)
Inactivated influenza	10 (100)	26 (96)
Measles-mumps-rubella (if measles seronegative)	10 (100)	14 (52)
Human papillomavirus – female recipients	10 (100)	4 (15)
Human papillomavirus – male recipients	4 (40)	1 (4)
Varicella (if seronegative)	2 (20)	0 (0)
Hepatitis B	2 (20)	9 (33)

Table 2: Vaccines and specific formulations (where applicable) routinely recommended post-HSCT in 10 paediatric and 27 adult alloHSCT programmes

Adult programmes appear cautious around administration of live attenuated vaccines, with only half recommending Measles-Mumps-Rubella (MMR) vaccines to measles seronegative patients. In contrast, all paediatric programmes recommend this vaccine. A minority (20%) of paediatric programmes, and no adult programmes recommend a live attenuated varicella vaccine to seronegative recipients.

Almost all adult and paediatric programmes recommend an inactivated vaccine against the VPDs covered by the UK NHS vaccination schedule (diphtheria-tetanus-pertussis, Haemophilus influenza B, pneumococcus, influenza, meningococcal and polio vaccines). The exception to this is the human papilloma virus (HPV) vaccine, which is recommended for females aged 11-26 by all paediatric programmes but only 15% of adult programmes. In the UK only high-risk individuals are immunized against hepatitis B; a minority of adult (33%) and paediatric (20%) programmes recommend this vaccine as routine.

Several formulations of the pneumococcal, diphtheria-tetanus-pertussis and meningococcal vaccines are available in the UK and are used in the NHS vaccination schedule in different age and population groups. The immunogenicity of these formulations is not equivalent, and some are poorly-immunogenic in HSCT recipients.

2.5.3.1 Pneumococcal Vaccine

Most adult (70%) and paediatric (78%) programmes recommend a vaccination schedule that includes the immunogenic pneumococcal conjugate vaccine (PCV) (93), with 18% and 20% recommending the pneumococcal polysaccharide vaccine (PPSV), which is poorly immunogenic in this patient group(112). The remaining adult and paediatric programmes do not make a specific recommendation.

2.5.3.2 Diphtheria-Tetanus-Pertussis Vaccine

A majority of adult (63%) but less than half (40%) of paediatric programmes recommend a schedule that includes the immunogenic full-dose diphtheria-tetanus-pertussis (DTaP) vaccine. The remaining adult programmes either recommend the reduced dose vaccine (dTap) or do not give a specific recommendation (11%). 30% of paediatric programmes recommend either DTaP or dTap depending on patient age, which would accord with the UK NHS vaccination schedule for the general population.

2.5.3.3 Meningococcal Vaccine

Three meningococcal vaccines are currently included in the UK NHS vaccination schedule for different age groups: a monovalent meningococcal C (MenC), a

monovalent meningococcal B (MenC), and a quadrivalent meningococcal ACWY (MenACWY) vaccine. The latter two vaccines were introduced in September 2015 during the survey period, in response to an increased number of cases of invasive meningococcal disease caused by B and W capsular groups (113,114).

Most adult (62%) and paediatric (40%) programmes recommend the MenC vaccine first line, with 8 (22%) adult and paediatric programmes recommending the MenACWY vaccine. Of these 8 programmes, 3 completed the survey prior to September 2015, and 5 after. No programmes recommend the MenB vaccine first line.

2.5.4 Commencement of RVP Vaccines

The most common time point at which adult and paediatric programmes commence RVP is 12 months post-HSCT. However, there is variation in practice with adult programmes commencing from 3 to 18 months, and paediatric programmes from 6 to 18 months. All adult and 80% of paediatric programmes commence RVP in recipients of sibling and unrelated donors at the same time point post-HSCT. 20% of paediatric programmes delay RVP in recipients of unrelated donors until 18 months post-HSCT.

Most adult programmes (74%) do not measure a marker of immune reconstitution before commencement of RVP. In contrast, 70% of paediatric programmes do assess

immune reconstitution, measuring lymphocyte subsets alone (30%) or in combination with immunoglobulin levels (40%).

2.5.5 Delay of RVP Vaccines

2.5.5.1 Chronic GvHD and Immunosuppressive Therapy

The approach to vaccination of HSCT recipients with cGvHD or on IST varies across programmes. Programmes were asked to indicate the lowest or 'threshold' cGvHD grade by NIH global severity criteria (26), and lowest or 'threshold' combination of IST, that necessitates deferral of inactivated and live attenuated vaccines. While the majority of paediatric (80%) and adult (74%) programmes defer inactivated vaccines if recipients have active cGvHD, the threshold grade prompting deferral varies (Table 3).

	Paediatric n (%)		Adult n (%)	
Timing of Commencement	Sibling or Syngeneic donor	Unrelated Donor	Sibling or Syngeneic donor	Unrelated Donor
3 months	0 (0)	0 (0)	4 (15)	4 (15)
6 months	1 (10)	1 (10)	8 (30)	8 (30)
9 months	1 (10)	1 (10)	1 (4)	1 (4)
12 months	8 (80)	6 (60)	13 (48)	13 (48)
18 months	0 (0)	2 (20)	1 (4)	1 (4)
Reasons for delaying RVP	Live attenuated vaccines	Inactivated Vaccines	Live attenuated vaccines	Inactivated Vaccines
cGvHD[b]				
Would not delay for cGvHD	0 (0)	2 (20)	1 (4)[a]	7 (26)
Mild	4 (40)	2 (20)	20 (77) [a]	8 (30)
Moderate-Severe	6 (60)	6 (60)	5 (19) [a]	12 (44)
IST [b]				
Would not delay for IST	0 (0)	0 (0)	0 (0) [a]	6 (22)
Single agent IST including corticosteroid	10 (100)	9 (90)	25 (96) [a]	15 (56)
Dual agent IST	0 (0)	1 (10)	1 (4) [a]	6 (22)
Triple agent IST	0 (0)	0 (0)	0 (0) [a]	0 (0)
Acute illness	9 (90)	8 (80)	20 (75)	22 (81)
CD4 Count < 200 cells/ul	9 (90)	9 (90)	9 (33)	4 (15)
CD20 Antibody in last 6 months	8 (80)	6 (60)	9 (33)	2 (7)
IVIg in last 6 months	4 (40)	2 (20)	2 (7)	1 (4)

Table 3: Timing of commencement and reasons for delaying routine vaccination in 10 paediatric and 27 adult UK alloHSCT programmes. [a]Data available from n = 26 programmes. [b]Lowest cGvHD grade or IST combination necessitating deferral indicated.

The remaining 20% of paediatric and 26% of adult programmes administer inactive vaccines to HSCT recipients with active cGvHD regardless of grade. All paediatric and

the majority (78%) of adult programmes defer inactivated vaccines if recipients are on IST. Again, there is no consensus on the lowest IST combination that necessitates deferral (Table 3).

2.5.5.2 Other reasons for delaying RVP

As expected, the majority of paediatric and adult programmes administer neither live attenuated (90% and 75% respectively) nor inactive (80% and 81% respectively) RVP vaccines to recipients with an acute illness. A minority of adult programmes delay RVP vaccines in recipients treated with a monoclonal CD20 antibody (7% inactivated, 33% live attenuated), or intravenous immunoglobulin (4% inactivated, 7% live attenuated) in the last 6 months. The percentage of paediatric programmes that delay RVP vaccines is greater for both recipients treated with monoclonal CD20Ab (60% inactivated, 80% live attenuated) and intravenous immunoglobulin(IVIg) (20% inactivated, 40% live attenuated).

2.5.6 Monitoring Response to RVP Vaccines

Half of paediatric programmes, and 44% of adult programmes routinely monitor serological response to vaccinations. Of the adult programmes that routinely monitor Ab response to vaccination, 100% monitor pneumococcal Ab, 58% Haemophilus

influenzae B, 50% tetanus and 17% for both meningococcal and measles Ab. Of the paediatric programmes that routinely monitor Ab levels, 100% monitor pneumococcal Ab, 100% tetanus, 60% Haemophilus influenzae B, 20% for each of mumps and rubella and diphtheria antibodies. 30% of adult programmes monitor serological response to vaccine if clinically indicated. Indications give are as follows: illness from a VPD (100%), Ongoing IST (75%), active GVHD (38%). All the 30% of paediatric programmes that monitor response if clinically indicated, give illness from VPD as the sole indication.

2.5.7 Vaccination of Family Members and Close Contacts

HSCT programmes were asked to indicate whether they recommend seasonal influenza vaccine for family members and close contacts. 15% of adult programmes did not answer this question. Of the responding adult programmes, all recommend seasonal influenza vaccination of both adult and child family members. 90% of paediatric programmes recommend seasonal influenza vaccination of family members. 20% of paediatric and 22% of adult programmes recommend child family members and close contacts are vaccinated with the live intranasal vaccine with the remainder either recommending the SIIV or giving no specific recommendation.

2.6 Discussion

With a 95% response rate, this survey provides a current and comprehensive picture of RVP practice across adult and paediatric UK NHS alloHSCT programmes. To our knowledge this is the most detailed national survey of post alloHSCT RVP practice to date, encompassing service organization, vaccine selection, commencement and delay of vaccination, and monitoring of response to vaccines.

The best-practice recommendation to offer HSCT recipients prophylactic vaccination has been adopted universally by responding adult and paediatric programmes, representing 100% and 83% of all UK programmes respectively. However, as hypothesized, we identified variation across all survey themes.

2.6.1 Service Organization

The main sources of guidance used by HCST programmes to inform local SOPs, or guide RVP decisions are broadly divided along adult and paediatric lines. In the UK, national paediatric HSCT specific guidelines were last updated by CCLG in 2014, and 60% of alloHSCT programmes identify national guidelines as their main source of information. National adult HSCT specific guidelines have not been published in the UK, and 72% of adult programmes use either international HSCT specific guidelines or adapt these for

local use. This leaves the remainder of paediatric and adult HSCT programmes using locally developed guidelines, or a combination of international and national non-HSCT specific guidelines. As programmes draw from a range of different guidelines, and in some cases, adapt guidelines locally, a degree of variation in practice is to be anticipated. This highlights the need for a harmonized guideline and/or policy that synthesizes best practice recommendations and national licensing considerations, thereby providing HSCT programmes and primary care teams who administer vaccines a single up-to-date reference source.

Almost all UK transplant programmes refer HSCT recipients to primary care for RVP with only a minority administering vaccines at the HSCT centre. In the NHS of the United Kingdom and Northern Ireland, the national vaccination schedule is delivered in primary care. It is therefore unsurprising that HSCT programmes refer patients to primary care rather than establish vaccination services locally. When several vaccine formulations are licensed in the UK, the majority of HSCT programmes make specific recommendations to primary care. However, a minority do not make specific recommendations and therefore the decision will fall to the administering primary care team. Fewer than half of adult and paediatric HSCT programmes keep a local record of vaccine administration and this may be reflected in the 80% of paediatric and 56% of adult HSCT programmes that have never undertaken an audit of RVP practice. Certainly, audit rates are lower amongst those programmes that do no keep local records of RVP versus those that do (29% v 54%). Clearly, if some HSCT programmes do not provide

detailed vaccination recommendations, or maintain local RVP records it is paramount that primary care practices have sufficient resources and familiarity with post-HSCT vaccination schedules to be able to make appropriate vaccine selections, record vaccine administration, monitor uptake and provide vaccination reminders to HSCT recipients, many of whom will be receiving vaccinations outside of the normal age ranges and time-points of the NHS national vaccination programme. Whether this is the case was beyond the scope of this survey, but warrants future investigation. These findings also suggest that many HSCT programmes may not have detailed information about vaccination uptake in their patients.

2.6.2 Vaccine Selection

2.6.2.1 Inactivated Vaccines

The inactivated vaccines recommended for administration are almost universally recommended by UK AlloHSCT programmes. However, where more than one vaccine formulation is available, selection varies across programmes.

2.6.2.1.1 Pneumococcal Vaccine

The percentage of programmes recommending a pneumococcal vaccine has increased from 95% in 2007 (115), to 100% in the current survey. Two vaccine formulations are routinely used in the UK vaccination schedule: the 23-valent pneumococcal polysaccharide vaccine (PPSV23), and the 13-valent pneumococcal conjugate vaccine (PCV13). All current HSCT vaccination guidelines recommend that adult and paediatric recipients receive a pneumococcal vaccine, although the timing of initiation varies from 3 to 15 months post HSCT. The earlier vaccination time-point is derived from studies that show the PCV is equivalently immunogenic when administered at 3 and 9 months post HSCT with complete seroprotection rates approaching 80% in a 3 dose schedule (94,116). The PPSV23 is poorly immunogenic in both 1 and 2 dose schedules (117), and when compared head-to-head with the PCV(112). Across all guidelines, the PCV is recommended for the primary vaccination course, with the PPSV23 offered as a fourth dose 12 months after commencement in recipients without cGvHD to broaden the serotype response (93). In those with cGvHD a fourth dose of PCV is recommended. In the UK routine vaccination schedule the PCV13 is administered to infants under 2 years, and the PPSV23 is administered in high risk individuals aged over 2 years, and in adults over 65. The exception to this is severely immunocompromised patients including HSCT recipients in whom the PCV13 has been recommended since the December 2013 update of the DOH Green Book. However, this indication does not appear in the British National Formulary(118). While the majority of paediatric and adult programmes recommend a vaccine schedule that includes the PCV, a fifth of paediatric and adult

programmes recommend the poorly immunogenic PPSV23 first line. A minority of programmes make no specific recommendation. This may be a particular issue for adult recipients, an age group that would normally receive the PPSV23. Whether primary care practitioners are administering the PCV13 in preference to PPSV23 is unknown. Given HSCT recipients are at high risk of morbidity and mortality from invasive pneumococcal disease, it is of concern that some patients may not be receiving the more immunogenic vaccine.

2.6.2.1.2 Meningococcal Vaccine

In the UK, the MenC-conjugate vaccine is administered to infants. In 2015, in response to a proportional increase in capsular group B and W infections(119), the MenC teenage booster given in school years 9-10 was replaced with the quadrivalent MenACWY, and a MenB vaccines for infants was introduced . Both UK formulations of the ACWY vaccine (Nimenrix®, Menveo®) are conjugate vaccines, while the MenB vaccine (Bexsero®) is a non-conjugate recombinant protein vaccine.

The optimum selection and timing of meningococcal vaccine in HSCT recipients is unclear. Three doses of the MenC conjugate vaccine resulted in seroprotective Ab titres when administered at 12 months in autologous, and 18 months in paediatric allogeneic HSCT recipients(120). A quadrivalent meningococcal polysaccharide vaccine

was found to be immunogenic from 8 months post alloHSCT in adult recipients (121). However, more recently the quadrivalent MenACWY conjugate vaccine showed limited immunogenicity when administered in a single dose at a median time point of 2.34 years post HSCT. A second dose in a subgroup of the study population resulted in higher rates of seroresponse (122). To our knowledge, immunogenicity of the MenB vaccine has not been explored in HSCT recipients.

Current guidelines including IDSA2013 and CCLG2014 recommend the MenACWY vaccine. ASBMT2009 recommends following national vaccination policy. The guidelines most recently published are the IGI2015 and MenACWY and MenB are both recommended. Across UK alloHSCT programmes practice varies with both the MenC and MenACWY recommended first line. It is likely that this variation in practice is related to the recent introduction of MenACWY and MenB in the UK. Although, this perhaps highlights the issue of responding to changes in national vaccine licensing and recommendation when working from international or locally adapted guidelines.

2.6.2.1.3 Diphtheria-Tetanus-Pertussis Vaccine

A diphtheria-tetanus-pertussis vaccine is recommended by all current HSCT vaccination guidelines, although advice around specific formulation varies. In the UK 5 vaccines containing diphtheria and tetanus toxoid are available:

-DTaP (Full dose) – Pediacel® (5 in1), Infanrix® (4 in1)

-dTaP (Reduced dose diphtheria) – Repevax® (4 in 1)

-dTap (Reduced dose diphtheria and pertussis) – Boostrix® (4 in 1)

-dT/IVP (Reduced dose diphtheria in combination with IPV) – Revaxis® (3 in 1)

In the UK primary vaccination is with 3 doses of DTaP in infancy (5 in 1) followed by a first pre-school booster of either DTaP or dTaP (4 in1). A fourth reduced dose dT (3 in 1) booster is administered in children aged 13-18.

The reduced dose dTap vaccine is poorly immunogenic in autologous HSCT recipients(123), and the full dose DTaP is recommended for primary vaccination in all HSCT recipients. DTaP is associated with an increased rate of local reaction in adult, immunocompetent individuals, but this reaction is rarely seen in the immunocompromised (96). Over a quarter of adult HSCT programmes either recommend the reduced dose vaccine, or make no specific recommendation. The majority of paediatric HSCT programmes recommend the DTaP vaccine, or vary their recommendation according to age. As an additional complication, no DTaP formulations are licensed for patients over 13 years of age in the UK, so whether

adolescent and adult HSCT recipients are receiving this formulation in primary care is unclear. Again, a limited evidence base, variation across guidelines and national licensing issues are likely to contribute to variation in practice.

2.6.2.1.4 Human Papillomavirus Vaccine

The recommendation of human papilloma virus quadrivalent (HPV4) vaccine varies by recipient age group with all paediatric but only a minority of adult programmes recommending this vaccine. Female HSCT recipients are at increased risk of genital HPV disease compared with the general population, and extensive cGvHD is a significant risk factor for disease and associated severe dysplasia (124,125). The HPV4 vaccine has been shown to significantly reduce the incidence of HPV associated anogenital diseases(9), and high grade cervical intraepithelial neoplasia(126) in healthy female populations. While there is no current evidence to recommend use of the HPV4 vaccine in HSCT recipients, a Phase 1 trial of safety and immunogenicity in female alloHSCT recipients aged 18 to 50 is ongoing (NCT01092195). Acknowledging lack of evidence, IDSA 2013 guidelines recommend considering administration of HPV4 vaccine in females and males age 11-26. IGI2013 guidelines recommend administration for females up to 45 years. In the UK NHS routine vaccine schedule the HPV4 vaccine is administered to girls aged 12 to 13 and is available on the NHS up to age of 18. Lack of evidence and restricted availability on the NHS likely contribute to very few adult alloHSCT programmes recommending this vaccine to female recipients.

2.6.2.2 Live Vaccines

The two live vaccines recommended in IDSA2013 for HSCT recipients are measles-mumps-rubella (MMR) and varicella (VAR) vaccines. These two vaccines are recommended for measles and varicella-seronegative recipients respectively, who are 24 months post-HSCT with neither GvHD nor ongoing immunosuppression and 8-11 months after last dose of IVIg. Only half of adult programmes recommend MMR and none VAR. This may be due to the relative complexity of determining the appropriate timing of vaccination(127) and concerns regarding vaccine induced disease if administered despite contraindications.

While all paediatric alloHSCT programmes recommend MMR vaccine administration, only 20% recommend VAR. The VAR vaccine is not part of the NHS routine vaccination schedule, but is offered to high-risk individuals. In contrast with IDSA2013, The CCLG2014 guidelines give VAR as contraindicated in HSCT recipients. It is likely that this contradiction between international and national guidelines contributes to the reported variation in practice across HSCT programmes.

2.6.3 Commencement and Delay of Vaccines

In broad terms, the optimum time-point for starting RVP is as soon as a vaccine will induce immunity without causing undesirable effects. This time-point is likely to vary between HSCT recipients depending on a range of pre and post-HSCT variables. Currently, no markers of immune reconstitution have been reliably associated with vaccine response. A higher lymphocyte count has been associated with achieving HAI titres≥40 in response to an adjuvanted MIIV A(H1N1)pdm09 vaccine although a cut-off value for administration was not determined(128). Similarly, adult recipients with higher CD19+ B-Cell and CD4+ T helper cell counts were more likely to respond to a SIIV (92). In contrast, CD4+ T helper cell counts of </>200 were not associated with response to PCV (94), and CD4+, CD8+ and CD19+ lymphocyte counts at 6 and 12 months post HSCT were not associated with response to DTaP in paediatric recipients(129). Given lack of evidence for using markers of immune reconstitution to guide RVP commencement, current guidelines recommend a standardized rather than tailored approach. Most adult alloHSCT programmes in the UK follow this practice, while 70% of paediatric programmes assess lymphocyte subsets alone or with Ig levels. The survey did not enquire about threshold values for commencing RVP.

Current international guidelines recommend starting RVP in adult and paediatric recipients at 3-6 months post-HSCT, commencing with the PCV13. In the UK, the RCPCH 2002 recommends commencing recipients of sibling and unrelated donors at 12 and 18

months respectively. The more recent CCLG2014 recommends most recipients should be started around 12 months, but consideration can be given to an earlier time-point on a case-by-case basis. In practice UK paediatric and adult HSCT programmes commence RVP at a range of time points from 3 to 18 months, with the majority commencing at 12 months. 20% of paediatric programmes follow RCPCH2002 guidance and commence recipients of unrelated donors at 18 months.

Guidelines universally advise against the administration of live vaccines to HSCT recipients with active cGvHD or receiving immunosuppressive therapy (IST) at any dose due to an increased risk of vaccine induced disease. It is of concern that 19% of adult and 60% of paediatric programmes indicated moderate or severe GvHD is their threshold grade for deferral of live vaccines. Similarly, a single adult programme indicated that they consider dual IST as the threshold dose for delay of live vaccines.

HSCT recipients with GvHD or on IST are at high-risk of infection, and international guidelines recommend administration of inactive vaccines in those with active or resolved cGvHD of any severity grade. CCGVHD2011 suggest that vaccination may be reasonably withheld for up to 3 months in adult recipients if they are receiving dual IST comprising >0.5mg/kg prednisolone combined with an additional immunosuppressive agent. The same guideline recommends that vaccines should not be postponed in children and adolescents with ongoing active or resolved cGvHD irrespective of immunosuppressive therapy, due to higher exposure to infectious agents in day-care

and schools, and more rapid immune reconstitution following HSCT. However, in contrast with this guidance CCLG2014 paediatric guidelines recommend that inactive vaccines should be withheld if the recipient has active cGvHD, and until they have been off IST for 6 months. While it is certainly desirable to offer patients with GvHD or on IST protection against infection, the impact of this immunosuppressive process on vaccine immunogenicity is unclear. Studies have reported impaired immunogenicity of PCV (94), SIIV(91), Hib and polio virus vaccine(130) in patients with GvHD, while others have not found this association (51,93,122,131). In practice, the majority of adult and paediatric programmes delay inactivated vaccines in HSCT recipients with cGvHD or receiving IST. This finding is in keeping with single centre studies of vaccination practice that have reported delay of vaccination in recipients with GvHD(102,104,105). While acknowledging the limited evidence base for vaccination of patients with GvHD or receiving IST, it is of concern that many UK HSCT programmes are not be offering this particularly high-risk subgroup of HSCT recipients potentially protective immunity to VPDs such as invasive pneumococcal disease and seasonal influenza.

2.6.4 Monitoring Response to Vaccination

Guidance on monitoring response to vaccines after HSCT is again varied. All immunogenicity studies in this population have investigated primary vaccination post HST. None have followed-up patients over lengthy periods or explored approaches to booster vaccinations in the setting of impaired response to the primary course. IDSA

2013 recommend monitoring of HepB Ab levels. ASBMT 2009 advises routine testing of HepB and pneumococcal Ab levels, and along with IGI2015, recommends testing every 4 to 5 years for Measles, Diphtheria, tetanus and polio Ab levels. CCGVHD2011 advises routine monitoring of Ab levels should be considered, especially in those with GvHD while RCPCH2002 explicitly counsels against routine measurement of response. It is unsurprising that reported practice across UK alloHSCT programmes varies considerably, and is perhaps guided by individual experience (e.g. of breakthrough disease) and availability of testing.

2.6.5 Vaccination of Family Members and Close Contacts

It is recommended that household members and close contacts of HSCT recipients receive the seasonal influenza vaccine annually. IDSA2013 advises that either the SIIV or live attenuated intranasal vaccine (LAIV) is administered unless the recipient is within 2 months of HSCT, or has GvHD. The evidence for this recommendation is however weak. Given the limited immunogenicity of the SIIV in HSCT recipients, particularly within the first year, this provision of herd immunity may be particularly important, and it is encouraging that most adult and paediatric programmes make this recommendation. Approximately 20% of adult and paediatric programmes recommend LAIV for child household contacts.

2.7 Conclusions

With an overall response rate of 95%, this survey provides an up-to-date, detailed and comprehensive overview of RVP practice across UK HSCT programmes. A weakness of the survey format is that it relies on self-reported practice without independent verification, and programmes were not asked to submit SOPs for review.

Reassuringly, routine post-HSCT vaccination has been adopted by all responding adult and paediatric programmes. However, as hypothesized there was variation in practice across all survey themes. This may be attributed to an evidence base insufficient to provide detailed practical guidance, conflicting recommendations between guidelines, tension between international recommendations and national vaccine licensing restrictions, and in some cases a lack of familiarity with current guidelines.

As outlined, there are various sources of post-HSCT RVP guidance. Survey findings indicate that HSCT programmes are working independently to adapt these for UK practice. While acknowledging the need for an improved evidence base for post-HSCT vaccination, a practical step would be to combine efforts and expertise through the BSBMT and the British Society for Haematology (BSH), to develop a single, national adult and paediatric guideline, synthesising current best practice recommendations and national licensing restrictions.

Responsibility for vaccine administration currently lies in Primary Care and it is unlikely that most programmes have resources to bring vaccination within the HSCT centre. Therefore, strategies are required to facilitate communication so enabling HSCT programmes to recommend immunogenic vaccine formulation at an appropriate time-point, and allow GPs to confirm vaccine administration for the purposes of audit and quality assurance. A national guideline should include a vaccination schedule and checklist that can be provided to the primary care team and can be completed and returned to HSCT programmes.

Vaccination of recipients with GvHD and IST is an area of concern, with regard to both administration of live vaccines, and delay of inactive vaccines. As an urgent interim measure, we recommend programmes review current practice alongside best practice guidance. To raise awareness of these pressing issues, a version of this survey report was disseminated through *Bone Marrow Transplantation* in January 2017(132).

3 A pilot study comparing the microneutralization assay and haemagglutination inhibition assay as measures of the immunogenicity of seasonal inactive influenza vaccine in recipients of reduced intensity conditioning allogeneic haematopoietic stem cell transplant during the first-year post-transplant

3.1 Introduction

Influenza is an enveloped, single-stranded RNA virus of the genus *Orthomyxoviridae* (133). There are 3 subtypes of influenza that cause disease in humans: influenza A, B and C. A fourth virus, influenza D, has been discovered in cattle and pigs (134). In addition to humans, Influenza A has avian, swine and equine reservoirs while Influenza B has only been identified in seal populations(135). A and B viruses both undergo gradual mutation of the viral genome resulting in subtle changes in viral protein called 'antigenic drift'. Additionally for influenza A, the large cross-species reservoirs enable rapid mutation and virus recombination called 'antigenic shift'(136). This enables the emergence of novel strains to which the human population is immunologically naïve; and so while both influenza A and B can cause moderate to severe illness and local epidemics, all influenza pandemics of the 20th century, and the most recent pandemic in 2009 have been caused by Influenza A viruses (137).

3.1.1 Influenza Virus Virion

The influenza virus virion is roughly spherical in shape. The viral envelope is a lipid bilayer derived from the host cell membrane, and contains three viral transmembrane proteins: haemagglutinin (HA), neuraminidase (NA) and matrix 2 protein (M2). These 3 proteins make up approximately 80%, 17% and 3% of surface proteins respectively (138). The influenza virion core contains eight segments of single-stranded RNA associated with nucleoprotein (NP) forming ribonucleoprotein (RNP) complexes. The RNPs are surrounded by matrix 1 (M1) protein(139).

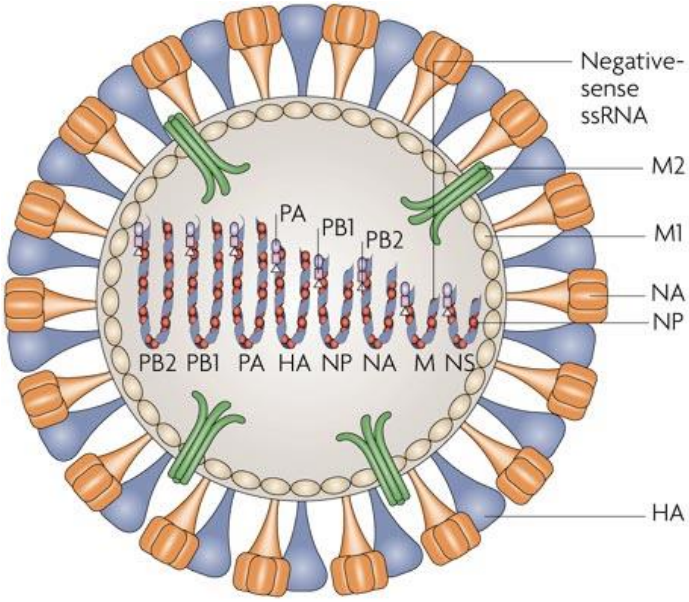

Figure 6: The structure of the influenza virus. HA = Haemagglutinin. NA = Neuraminidase. M1 = Matrix 1 Protein. M2 = Matrix 2 Protein. (140) *Used with permission.*

3.1.2 Influenza Virus Lifecycle

The first stage of the influenza virus lifecycle is virus binding and entry into the host cell. This process is initiated by the HA transmembrane protein. The HA protein is synthesised as an inactive precursor, and before fusion with the cell membrane must be cleaved by proteases of the respiratory epithelium (141). In humans, this limits site of infection to the respiratory tract. It is recognized that highly pathogenic avian influenza strains (H5 and H7) are readily cleaved by proteases in a range of tissue, and so lead to disseminated infection in the avian host (142). The HA protein, once cleaved, binds to the sialic acid component of host cell membrane glycoproteins. Sialic acid is bound to carbohydrate to form a glyocoprotein by alpha(2,6) linkage in human respiratory epithelium, and by alpha(2,3) linkage in avian intestinal mucosa (141). Human influenza viruses bind preferentially to the former, and avian viruses the latter (143) hence the dominant sites of replication and species specificity of the viruses. Swine influenza viruses bind to both forms of carbohydrate-sialic acid linkage. Cross-species virus mutation or recombination results in 'antigenic-shift' (see 3.1) and evolution of pandemic virus strains. Once binding has occurred the virion enters the host cell by endocytosis. The viral RNA (vRNA) then enters the cell nucleus where the host replication machinery is used by the virus for genome replication and transcription. The replicated vRNA and viral proteins are exported from the cell nucleus, and transported to the apical cell membrane where HA, NA and M2 are inserted prior to budding and virion release. A critical step in virion release is the removal of sialic acid from the cell membrane, which is the essential function of the NA protein (144).

3.1.3 Classification of Influenza Viruses

Influenza A subtypes are classified by HA and NA proteins. 16 HA and 9 NA subtypes can be distinguished serologically. H1N1, H2N2 and H3N2 subtypes commonly infect humans, with rare transmission of highly pathogenic avian subtypes (H5N1, H7N7 and H9N2)(139). Influenza B viruses are not classified into subtypes rather by lineage and strain.

3.1.4 Influenza Virus Infection

Influenza is a highly contagious, acute respiratory infection transmitted by droplets when infected individuals cough, sneeze or talk. The virus may also be transmitted from contaminated surfaces or hands. The World Health Organization estimates seasonal epidemics result in 3 to 5 million severe illnesses annually(145). The estimated influenza-related death rate in the United States is 9.9 per 100,000 (95% CI 7.9-11.9)(146). In the United Kingdom, Public Health England estimates the number of influenza deaths by excess all-cause deaths during the season. In 2015-2016 this was estimated at 2,291 deaths, or 0.8% deaths in excess(147).

The incubation period of influenza is 1 to 4 days. Viral shedding may begin 2 to 3 days prior to symptoms, and then persists for 6 to 7 days following symptoms onset. (148).

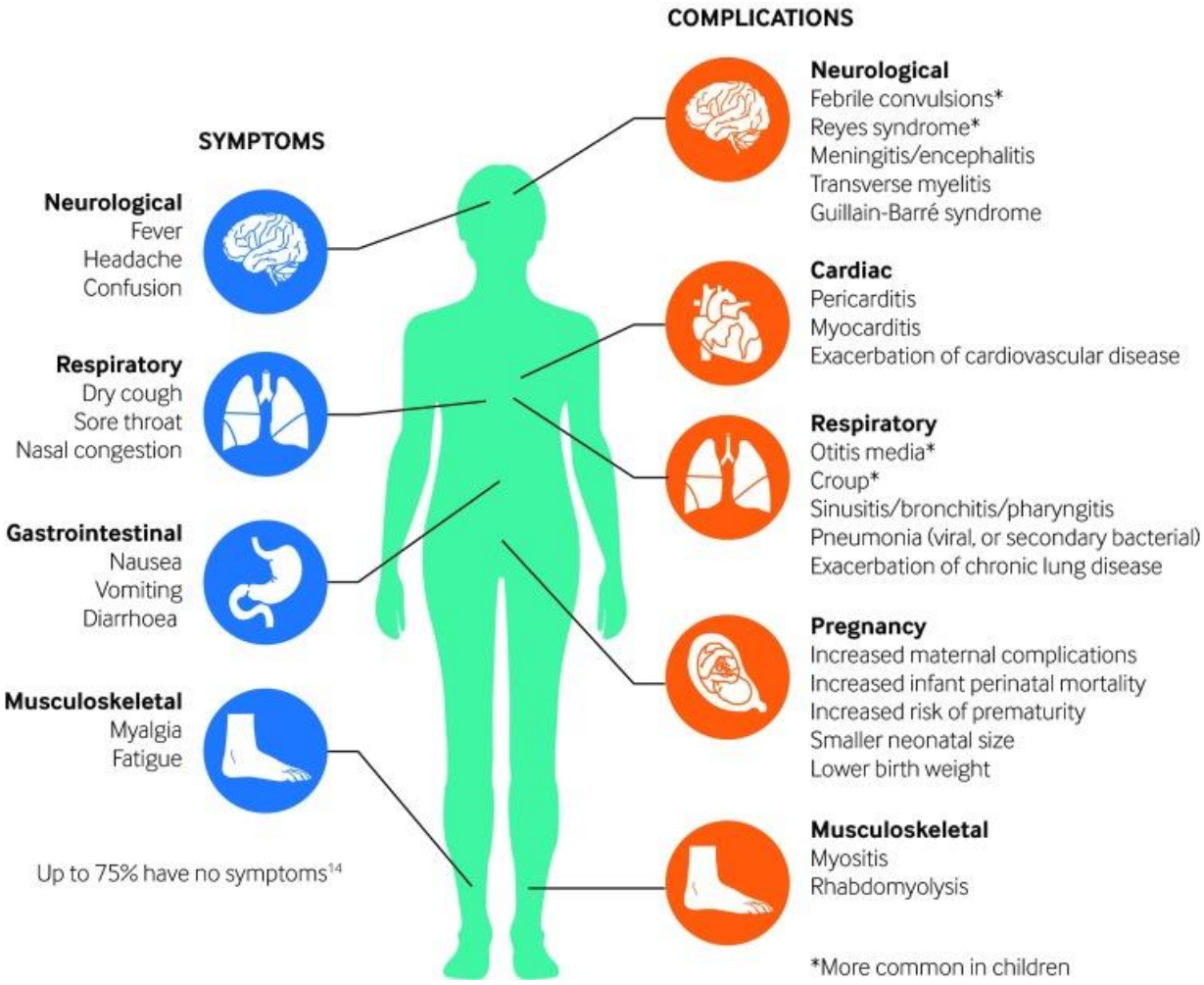

Figure 7: Symptoms and Complications of Influenza Infection. (149) *Used with Permission*

Symptoms and complications of influenza infection are shown in *Figure 7*. Those at increased risk of complications include the elderly, young children under 6 months old, pregnant women and those with chronic health conditions or the immunocompromised. Discussion of clinical management of seasonal influenza infection is beyond the scope of this introduction.

3.1.5 Global Influenza Patterns and the Seasonal Influenza Virus Vaccine

In temperate regions, the influenza virus has a seasonal pattern, with epidemics occurring during the winter months of the Northern and Southern Hemispheres. In tropical regions influenza infection appears highest during the rainy season, and some South East Asian regions having a bimodal seasonal pattern (150). Observation of global patterns from 1997 to 2005 suggests that at least for influenza A, the Tropics are a global reservoir with seasonal spread of virus away from the equator (151). A wide range of environmental and behavioral factors are thought to cause the observed seasonal pattern: Seasonal changes in humidity, levels of solar radiation and temperature may contribute to increased virus survival and efficient transmission, and may impact on host immune function; while seasonal patterns of behavior that increase contact rates enable swift virus transmission through the population (150).

Due to antigenic drift and shift the circulating subtypes and strains of influenza virus are continually evolving, and it is therefore necessary to formulate and administer vaccines annually. The WHO surveys global influenza patterns to determine dominant and emerging Influenza A subtypes and influenza B strains, and based on this makes recommendations on the composition of the seasonal influenza vaccine for the Northern and Southern hemispheres(152).

The recommended composition of the Northern Hemisphere 2015-2016 trivalent vaccine was:

- A/California/7/2009(H1N1)pdm09-like virus
- A/Switzerland/9715293/2013-like virus
- B/Phuket/3073/2013-like virus

As discussed in 1.4.1.4, the annual vaccine must meet minimum licensing criteria based on immunogenicity studies and COPs. However, during seasons where vaccine antigen is poorly matched to circulating virus strains, clinical effectiveness will be reduced despite adequate vaccine immunogenicity.

3.1.6 Human Immune Response to Influenza and Seasonal Influenza Vaccine

The human immune system mounts both innate and adaptive responses (1) to the influenza virus, and seasonal influenza vaccine. Antibodies to HA, NA, M1, M2 and NP (3.1.1) have been detected in human serum, however only HA and NA antibodies contribute to protective immunity(153). The most important target of the humoral response is the HA protein. Seasonal influenza vaccine contains HA for each virus strain. HA Ab neutralizes the influenza virus and prevents primary infection by blocking entry into the host epithelial cells(154). NA Ab blocks the sialic-acid receptor destroying function of the NA protein(155), and so prevents viral release from infected host cell, and therefore spread of infection(156). The CD8+ adaptive T Cell response is crucial for targeting infected cells and limiting disease if infection occurs.

3.1.7 Influenza Vaccine Immunogenicity and Correlates of Protection

Influenza COPs were established in the early 1970s in healthy adult populations using attenuated virus strains to induce immunity. An HAI titre of 1:18 to 1:36 was associated with 50% protection from infection. (18). Following these early studies an An HAI tire of 1:40 has been considered 'seroprotective', although for clarity the term '50% protective' may be better employed. The annual licensing of seasonal influenza vaccines has until 2016 been based on Committee for Medical Products for Human Use (CHMP) criteria, which are derived from this COP and summarized in 1.4.1.4.

Over recent years, a growing body of evidence has questioned the clinical relevance of the 1:40 seroprotective HAI titre. Firstly, a model derived from meta-analysis of 15 studies from 1945 to 1996 found a continuous relationship between HAI titre and estimated probability of protection, with the slope of the curve steepest up to a titre of 100 (approx. 0.9 probability of protection), thus questioning the validity of a single cut-off value (157). Clinical studies have reported 50% seroprotective titres ranging from 1:15 for a cell-culture based vaccine(158), up to 1:110 in a paediatric study of adjuvanated versus non-adjuvanted influenza vaccine (159). Futhermore, the HAI derived COP does not take into account mucosal IgA titres, or cellular immune response. Seroprotective titres have not been established in target groups including the elderly and immunocompromised groups including HSCT recipients and it remains unclear whether an HAI tire of 1:40 conveys the same level of protection in these groups.

The European Medicines Agency (EMA) drafted a new guideline on influenza vaccine licensing in 2014, and published these in July 2016(160). Acknowledging the 'lack of robust evidence to support immunological correlates of protection against influenza' the CHMP criteria were dropped. The new guidelines instead recommend that the immunogenicity and therefore the estimated efficacy of non-adjuvanted seasonal vaccines should be non-inferior to currently licensed vaccines. Furthermore, the EMA recommends that neutralizing antibodies are determined in all vaccine studies using viral neutralization techniques such as the viral microneutralization (VMN) assay, and recommends measurement of quantity and quality of T cell response. Pre-licensing studies of immunogenicity in immunocompromised patients is not required but the EMA suggests such immunogenicity in subpopulations should be investigated.

3.1.8 Immunogenicity, Efficacy and Effectiveness of the Seasonal Influenza Vaccine in HSCT Recipients – Literature Review

A small number of studies have investigated the immunogenicity of the seasonal trivalent inactivated influenza vaccine (TIIV) in alloHSCT recipients. In 2009 during the Influenza A pandemic, a monovalent A(H1N1)pdm09 vaccine (MIIV) was released in advance of the trivalent seasonal vaccine to expedite vaccination. This was available in adjuvanted and non-adjuvanted forms, and immunogenicity in HSCT recipients was reported. The studies of non-adjuvanted MIIV vaccines will be discussed here. All studies used HAI to determine seroresponse (for definitions of seroconversion and

seroprotection see 1.4.1.4) to vaccine. Studies are summarized in *Table 4* and outlined below

3.1.9 TIIV Immunogenicity Studies

In a study of 48 recipients of lymphocyte deplete BMH autoHSCT(n=13) and MAC alloHSCT(n=35), either 1 or 2 doses of TIIV were administered at a median time point of 14.5 months (range 2 to 82). A statistically significant association was reported between seroprotection and seroconversion rates at 4 weeks post-vaccination and time interval from HSCT to initial vaccination. TIIV was totally ineffective within the first 6 months following BMT in this group of patients receiving MAC regimens (91). A 2 dose regimen was revisited by Karras and colleagues (92) who carried out a randomized trial of a 1 versus 2 dose schedule of TIIV following. Of 65 participants, 23 received UCB HSCs, and 42 HSC from sibling or VUD (BMH or PBSC not specified). 39 were conditioned with MAC and 26 RIC and the two groups were matched for conditioning intensity, GVHD prophylaxis and stem cell source.

Reference	Vaccination Schedule and Timing of administration	Population	HSCT variables	Seroresponse Rates (SC/SP determined by HAI)	Other Findings
Engelhard et al, 1993	2 doses 28 days apart Med 14.5 (range 2-82) months	n=48 Age 21 (1-50)	n=35 alloHSCT -BMH -MAC -Lymph Dep n=13 auto HSCT -BMH	No SC before 6 months SC/SP not enhanced by second vaccine dose	Longer time from transplant associated with higher SC rates GvHD associated with lower seroconversion rates to A(H1N1) only
Pauksen et al, 2000	1 dose +GM-CSF Range 4-24 months	n=117 Age>16	n=50 alloHSCT -?HSC source -?Conditioning -7 Lymph Dep	In alloHSCT group: <12 months 9-31% SC >12 months 20-40% SC	significantly higher Influenza B seroconversion rates with GM-CSF
Gandhi et al 2001	1 dose Med 16 months (alloHSCT)	n=50 age 43.1(15-63)	n=9 alloHSCT -BMH -MAC - ?lymph Dep n=29 auto HSCT -29 PBSC, 12 BMH	Allo – 0% SP Auto BMT – 10% SP Auto PBSC – 13% SP	Equivalent response PBSC and BMH autoHSCT

Avetisyan et al 2008	5 < 6 months 9 >6 months	n=14 age 41 (21-66)	All alloHSCT -11 PBSC, 3 UCB -6 RIC, 8MAC -?lymph Dep	A(H1N1) - 29% SP A(H3N2) and Influenza B – 0% SP	B+T Cell response as early as 2 months
Karras et al, 2013	1 v 2 dose regimen Med 10 (range 2-236) months	n=65 age 40 (15-51)	All alloHSCT - 23 UCB, 42? HSC Source -26 RIC, 39 MAC -No lymph Dep	< 12 months 0-8% SC >12 months 39-64% SC At <12 months SC to any 1 strain 2-6 months = 12%SC 6-12 months = 30% SC	No benefit from 2 doses Longer time from transplant associated with higher SC rates Response rates equivalent at 2-6 and 6-12 months
Issa et al, 2011	1 dose A(H1N1)pdm09 vaccine Med 19 (range 2.5-9.4) months	n=82 age 44 (20-71)	All alloHSCT -35 Sibling, 47 VUD -3 UCB, 79 ?HSC source -38 RIC, 44MAC -No lymph Dep	SP rates: < 6months 37.5% 6-12 months 50% 12-24 months 38.7% >24 months 69%	Longer time from transplant associated with higher seroprotection rates

Table 4: Summary of studies of seasonal inactivated influenza vaccine in alloHSCT recipients. SC = Seroconversion. SP = Seroprotection

No patients received lymphocyte deplete grafts. The study found no evidence for increased serological response or T cell response in patients who received a second vaccination dose. Seroconversion was greater when vaccination was administered >12 months after HSCT versus < 12 months. At >12 months seroconversion rates to A(H1N1), A(H3N2) and Influenza B were 64%, 39% and 39% respectively. At <12 months seroconversion rates to A(H1N1), A(H3N2) and B/Victoria were 6%, 0% and 8% respectively. However, the study found that the seroconversion rate to any of the 3 vaccine strains was similar at 2-6 months and 6-12 months (12% vs. 30%, p=0.43). The authors suggest that although seroconversion rates are low at <12 months, the similar rate at 2-6 and 6-12 months suggest that vaccination may be beneficial earlier than current guidelines recommend, and that further studies are needed to confirm this finding.

In a study of the immunomodulatory effect of Granulocyte-Macrophage Colony-Stimulating-Factor (GM-CSF) on TIIV immunogenicity, 35 recipients of alloHSCT were vaccinated at <12 months, and 15 at >12months (161). In the <12-month group there was a 9 to 31% response to the vaccine subunits (A(H1N1) 31%, A(H3N2), 9%, Influenza B 20%). In the >12-month group there was a 13-40% response to the vaccine subunits (A(H1N1) 13%, A(H3N2) 40%, Influenza B 20%)

In a study of 50 patients, of whom 9 were recipients of allogeneic BMT and received MAC, a trivalent IIV was administered at a mean time point of 16 months (SD 5.8) post-

transplant. None of the recipients of allogeneic BMT demonstrated a serological response to vaccination(162).

A small study of 14 AlloHSCT recipients evaluated humoral and cellular immune response to TIIV. 6 recipients were conditioned with RIC and 8 MAC. 11 patients were transplanted with PBSC and 3 with UCB. 5 patients were vaccinated at <6 months after transplant and 9 patients >6 months. 4 patients demonstrated protective antibody titres to influenza A(H1N1) but none of the patients developed protective antibody titres to H(3N2) or influenza B. The authors do not indicate at which time point the responding group of patients were vaccinated. Evidence of early cell mediated immunity was demonstrated using pentamer staining to enumerate CD8+ cell response, and Elispot to enumerate Interleukin-3, interleukin-4 and interferon gamma producing cells. Due to small study size it was not possible to correlate cell mediated response with clinical findings to determine a protective response (163).

The non-adjuvanted 2009 MIIV vaccine was administered to 92 Allogeneic HSCT recipients at a median time point of 19 months (2.5-9.4) (164). Length of time from transplant was significantly associated with a seroprotective HI titre. There was no association between rate of seroprotective HI antibody titre and conditioning regimen, stem cell source, cGvHD or immunosuppression at time of vaccination. Rates of seroprotective titre by time from HSCT to vaccination were 37.5% at for those vaccinated at <6months, 50% at 6-12 months, 38.7% at 12-24 months and 69% at >24 months.

3.1.10 Factors Associated with Seroresponse to TIIV

3.1.10.1 Time Interval from Transplant to Vaccination

An association between vaccine response and time interval from HSCT to vaccination has been reported with seasonal TIIV (91,92), A(H1N1) and non-adjuvanted MIIV (164). However, 2 AS03-adjuvant MIIV studies showed no association with time interval from transplant to vaccination(128,165).

3.1.10.2 Conditioning Regimen, Stem Cell Source and Stem Cell Donor

A single study (166) reported a higher seroconversion rate to the H3N2 component of the Seasonal TIIV in recipients of matched sibling donor and matched unrelated donor transplants versus umbilical cord blood (24% versus 4%, p=0.04). Other studies have found no association between conditioning regimen (RIC v MAC), stem cell source (BMT, PBSC, UCBT) and donor type (sibling, unrelated donor, umbilical cord) and serological response to Seasonal TIIV, 2009 H1N1 adjuvanted or non-adjuvanted MIIV(91,161,164,165,167–169)

3.1.10.3 GvHD and IST

Engelhard and colleagues (91) found active GVHD at time of vaccination was associated with an impaired response to A(H1N1) but not the other components of the seasonal TIIV. Pauksen et al found no association between serological response to seasonal TIIV and cGvHD treatment at time of vaccination (161). Karras and colleagues (92) did not

report on GVHD directly, but found no association between steroid use at time of vaccination and serological or cellular response to TIIV.

3.1.10.4 Monoclonal Antibody Therapy

Rituximab administration within a year prior to vaccination has been associated with lower rates of seroprotection in response to 2009 H1N1 MIIV (164). Engelhard and colleagues (128) found patients treated with Rituximab within 6 months prior to vaccination did not respond to adjuvanted MIIV. This accords with previous findings from a study of non-HSCT patients with haematological malignancies(170).

3.1.10.5 Markers of Immune Reconstitution

A marker of immune reconstitution that correlates with seroconversion and seroprotection in response to influenza vaccine would allow clinicians to tailor timing of vaccination to individual patients.

Karras et al (92) found in multivariate analysis that those HSCT recipients with a higher CD19+ B Cell count had a significantly higher odds ratio of seroconverting to seasonal TIIV. In univariate analysis Mohty and colleagues (168) found haemoglobin less than 12g/L, lymphopenia less than 1g/L, IgG less than 4g/L, IgA less than 5g/L, IgM less than 0.5g/L and naïve CD4+ T cell less than 150/uL were significantly associated with a lower GMT following 2009 H1N1 AS03 adjuvanted MIIV. However, none of these associations were significant in multivariate analysis.

3.1.11 Efficacy and Effectiveness

A single study has investigated clinical efficacy of the TIIV in HSCT recipients (171). A cohort of 177 BMT recipients who presented with respiratory symptoms and had at least one nasal swab over a 1-year period were included. Vaccination records and results from nasal swabs were reviewed retrospectively. 118 patients were alloHSCT recipients. 134 patients were within 6 months of HSCT. In accordance with international recommendations only patients more than 6 months from HSCT received the TIIV. Influenza was diagnosed by direct immunofluorescence assay. Of the HSCT recipients less than 6 months from BMT 25/134 were diagnosed with seasonal influenza. Of the 43 HSCT recipients more than 6 months from HSCT 19 were vaccinated of whom 2 were diagnosed with influenza. 24 were unvaccinated and 12 were diagnosed with seasonal influenza. In the HSCT recipients eligible for vaccination (more than 6 months from transplant), TIIV administration was associated with a reduced occurrence of influenza ($p=0.015$). The authors calculated a vaccine efficacy of 80% when administered greater than 6 months from transplant. Serological response was not assessed in this study so COP cannot be determined. Unfortunately the high estimate of 80% vaccine efficacy may be inaccurate owing to retrospective assessment of vaccination status (172), no risk adjustment or matching of cases and controls (173) and the small cohort size. Furthermore, the authors have used a clinical efficacy calculation typically used in randomized-control trials rather than an effectiveness calculation used in retrospective case-control studies.

3.1.12 Side Effects

3.1.12.1 General Population

The WHO estimates local reactions to the trivalent TIIV occur in 10-64/100 doses. Estimates for severe systemic adverse events are 0.7 cases of anaphylaxis per million doses, 1-2 cases of Guillan-Barre Syndrome per million doses, and 76 cases of ocular-respiratory syndrome per million doses (174).

Placebo controlled studies have reported increased rates of local, mild, transient adverse reactions in both older (175,176) and younger, healthy working adults(177,178). Symptoms included local swelling, itching, warm feeling, pain when touched and discomfort. None of these studies reported a difference in rates of systemic symptoms (fever, myalgia, headache, fatigue) between placebo and treatment groups. A pooled analysis of 28 clinical trials of an inactivated split-virion TIIV reported mild injection site pain as the most common adverse event in children, adults and the elderly, and mild-moderate fever as the most common adverse incidence in infants (21.1%). 9 severe adverse-events across the 28 trials were possibly attributable to the vaccine and included an asthma attack, an episode of Bell's palsy, an episode of DVT, and an episode of transient meningeal syndrome. In this study, the incidence of mild-moderate fever decreased with age (4.3% in children, 1.4% in adults, 1.0% in the elderly)(179). Similarly, a pooled analysis of 76 trials of an inactivated subunit TIIV reported non-severe local reactions as the most common adverse events. The same

study reported post-licensing adverse-event rates of 14.4 events per million doses and again the most common events were local injection site reactions(180).

Current evidence is considered inadequate to accept or reject a causal link between influenza vaccination and Guillan-Barre syndrome(181). Controlled trials have not identified a link between GBS and influenza vaccination, (Govaert et al. 1993; Margolis et al. 1990; Bridges et al. 2000; Nichol et al. 1996). A retrospective Canadian population-based study between 1992-2004 identified a small but statistically signficant association between influenza vaccination and increased risk for hospitalization with GBS (relative incidence 1.45, 95%CI 1.05-1.99, p=0.02) although further analysis revealed no seasonal pattern, and identified no increase in rates of GBS after introduction of a universal influenza immunisation programme (182).

Occular Respiratory Syndrome (ORS) was reported in Canada during the 2000-2001 influenza season. The pathogenesis of ORS is unknown, but it is not considered a type 1 allergic reaction to the vaccine (Skowronski et al. 2002). The putative aetiology was high quantities of unsplit viral particles in a vaccine formulation administered that year, although cases have been reported in subsequent years and with reformulated and other manufacturers' vaccines. Symptoms are heterogenous and include ocular features of conjunctival injection and palpebral oedema, and respiratory symptoms including cough, sore throat, hoarseness, chest tightness, difficulty in breathing and swallowing. ORS is an infrequent adverse event generally considered benign with <1% cases requiring hospital admission for more than 24 hours(183) and the majority of

cases (73%) resolving fully within 48 hours without specific medical intervention (184,185).

3.1.12.2 HSCT Recipients

No published studies have identified an increased risk of TIIV side-effects in HSCT recipients relative to rates reported in the general population, and in particular TIIV administration has not been associated with an increased risk of GvHD in either paediatric or adult populations(92,162,186). The potential benefit of TIIV administration are therefore considered to outweigh the risk of side-effects in immunocompromised patient groups including HSCT recipients. (187,188).

3.1.13 Conclusions from Current Studies of TIIV in HSCT Recipients

The reported range of seroprotection rates reported across these studies to any one of the three vaccine strains in recipients of alloHSCT is 0-40%. Seroresponse rates to TIIV are lower in the first year after HSCT compared with later time points. However, in the absence of efficacy data in this population, the clinical relevance of a 1:40 HAI Ab titre is unknown. Study populations are heterogenous and data is insufficient to modify dosing schedules or timing of administration of TIIV by stem cell source, donor type, conditioning regimen or lymphocyte depletion. Similarly, the impact of GvHD and IST

on response to TIIV is unclear. A single study has evaluated cellular response to TIIV. No studies have used the VMN assay to assess vaccine response.

3.1.14 Viral Microneutralization Assay

As outlined in 3.1.9, TIIV immunogenicity studies in HSCT recipients have focused primarily on Ab levels determined by the HAI assay, and have assessed response by seroconversion and seroprotection rates. The clinical significance of the seroprotective HAI titre of 1:40 has been challenged in a growing body of evidence. The EMA now recommends that functional influenza virus neutralizing Ab is measured. The VMN assay is a highly sensitive and specific method for detecting influenza strain-specific functional antibodies that inhibit virus entry or block virus replication(189). Studies have demonstrated a correlation between HAI and VMN results, and also the higher sensitivity of VMN to detect low-titre seroconversion particularly for antibodies to influenza B(190,191) , 2009 pandemic H1N1 virus (192), and avian H5N1 virus(193). One study has demonstrated a correlation between pre-vaccination MN titres and response to vaccine by HAI. Neutralising antibody titres were determined for children who were seronegative by HAI (titre < 8) prior to receiving inactive influenza vaccine. Those who developed seroprotective HAI titres to Influenza A and B in response to IIV, had a higher geometric mean neutralizing titre pre-vaccination(194).

3.2 Gaps in Knowledge and Study Rationale

The optimum timing of seasonal IIV administration is not known. However, a consistent finding across studies is a correlation between time-point of vaccine administration post-transplant, and antibody titres. Vaccine administered at >12 months from HSCT appears most immunogenic, however recipients have been shown to respond to vaccination at 6-12 months and current guidelines are based on these findings(100). More recently a single study has shown an equivalent response among HSCT recipients vaccinated at 2-6 months and 6-12 months using HI assay(92). It is possible that despite low seroconversion and seroprotection rates, HSCT recipients derive a clinical benefit from early vaccination.

Most published studies have involved recipients of sibling or VUD HSCT treated with MAC regimens. There is limited data on response to IIV following RIC regimens, and whether IIV is beneficial in the first months following HSCT in this group of patients is unknown(195). Therefore, guidelines referenced above recommend the same vaccination schedule for all HSCT recipients regardless of conditioning regimen (96). European data from 2000 to 2011 has shown an increase in absolute numbers of HSCT recipients treated with RIC from 1436 to 5567. In the United Kingdom over 50% of HSCT recipients during this period were treated with RIC (196). It is therefore important to understand response to TIIV in this expanding group of patients.

The VMN assay offers a more sensitive technique than HAI for detecting low titre seroconversion in response to IIV particularly for Influenza A(H1N1), A(H5N1) and Influenza B. This assay has not been used to evaluate response to IIV in recipients of HSCT who are known to have low titre responses to IIV at least up until 12 months post-allograft.

The aim of this study is to compare the VMN and HAI as measures of immunogenicity of the TIIV administered in the first year after RIC alloHSCT. This study may offer further insight into TIIV immunogenicity and allow future larger studies to be appropriately powered. It may be possible to base future efficacy studies in the HSCT on the VMN assay.

3.3 Study Overview

HSCT recipients were recruited at 2 study sites during the annual influenza season from October 2015 to February 2016. Study sites were the Royal Hallamshire Hospital, part of Sheffield Teaching Hospitals NHS Foundation Trust, and the Royal Marsden Hospital NHS Foundation Trust. All participants were deemed eligible by the responsible HSCT physician to receive the seasonal TIIV as part of their standard post HSCT care. Normally patients would be referred to their General Practitioner (GP) for TIIV administration. For study purposes TIIV was administered at the HSCT centres. Sheffield Teaching Hospital recommends TIIV to HSCT recipients from 3 months post

HSCT. The Royal Marsden Hospital recommends TIIV to HSCT recipients at the beginning of the influenza season regardless of time point post HSCT. Therefore, study participants were vaccinated at a range of timepoints within the first-year post HSCT. Blood samples were collected for laboratory analysis. A 1 x 5ml blood sample was taken on day of vaccination to assess baseline Ab titre, and a second 1x5ml blood sample 28-35 days after vaccination to assess response. Blood samples were centrifuged, aliquoted and frozen locally as serum samples, and then shipped to the Public Health England (PHE) influenza virus reference department laboratory. Laboratory work was performed between February and October 2016. Pre and post vaccination samples were tested using the standard HAI assay, and the VMN assay.

The study was conducted in compliance with the study protocol, and standards of good clinical practice and regulatory requirements. All necessary local NHS research and development approvals were obtained for each centre before patient recruitment began.

See appendix 3 for patient information sheet, consent form and notice of research ethics committee approval.

3.3.1 Study Sponsorship, Insurance and Ethical Approval

The study was sponsored and insured by University College London (UCL). Ethical approval for the study was granted on 12 August 2016 by the NHS Health Research Authority (HRA) National Research Ethics Service (appendix 3)

3.3.2 Study Objectives and Endpoints

Primary Objective

To compare the rates of seroconversion to the seasonal inactivated influenza vaccine as measured by the HAI and VMN assays in recipients of RIC alloHSCT

Secondary Objectives

i) To determine whether seroconversion rates in response to IIV in recipients of RIC alloHSCT are equivalent at <6 and 6-12 months' time points post-transplant as determined by HAI and VMN assays.

ii) To determine in those patients who are seronegative by HAI assay pre-vaccination, whether a proportion have detectable antibody titres by VMN assay and whether this can predict those who will seroconvert or develop seroprotective antibody titres.

Primary Endpoint

The rates of seroconversion measured by HAI and VMN assays at 28 days after administration of IIV to the study population.

Secondary Endpoints

iii) Pre-vaccination HAI and VMN antibody titres

i) The rate of seroconversion measured by HIA and VMN

ii) The rate of seroprotective titres measured by the HAI assay

3.3.3 Study Participants

3.3.3.1 Inclusion and Exclusion Criteria

Inclusion criteria:

- Aged 16 or over
- Recipient of RIC alloHSCT for any primary haematological disease
- Between 0 and 12 months post-transplant at time of vaccination
- Deemed eligible to receive seasonal influenza vaccine by their transplant physician as part of standard clinical care at the treating HSCT centre

Exclusion Criteria:

- Any patient who has already received seasonal influenza vaccine in the period autumn-winter 2015-2016
- Any patient in whom inactivated influenza vaccine is contraindicated as per summary of product characteristics:
    - Hypersensitivity to the active substances, to any of the excipients or to any component that may be present as traces such as eggs (ovalbumin, chicken proteins), neomycin, formaldehyde and octoxinol-9

    - Any patient with febrile illness or acute infection at time of planned vaccination
- Any patient who the responsible transplant physician feels should not receive the seasonal IIV as part of routine care
- Any patient who does not wish to receive the seasonal inactive influenza vaccine as part of their routine clinical care

3.3.4 Study Flowchart

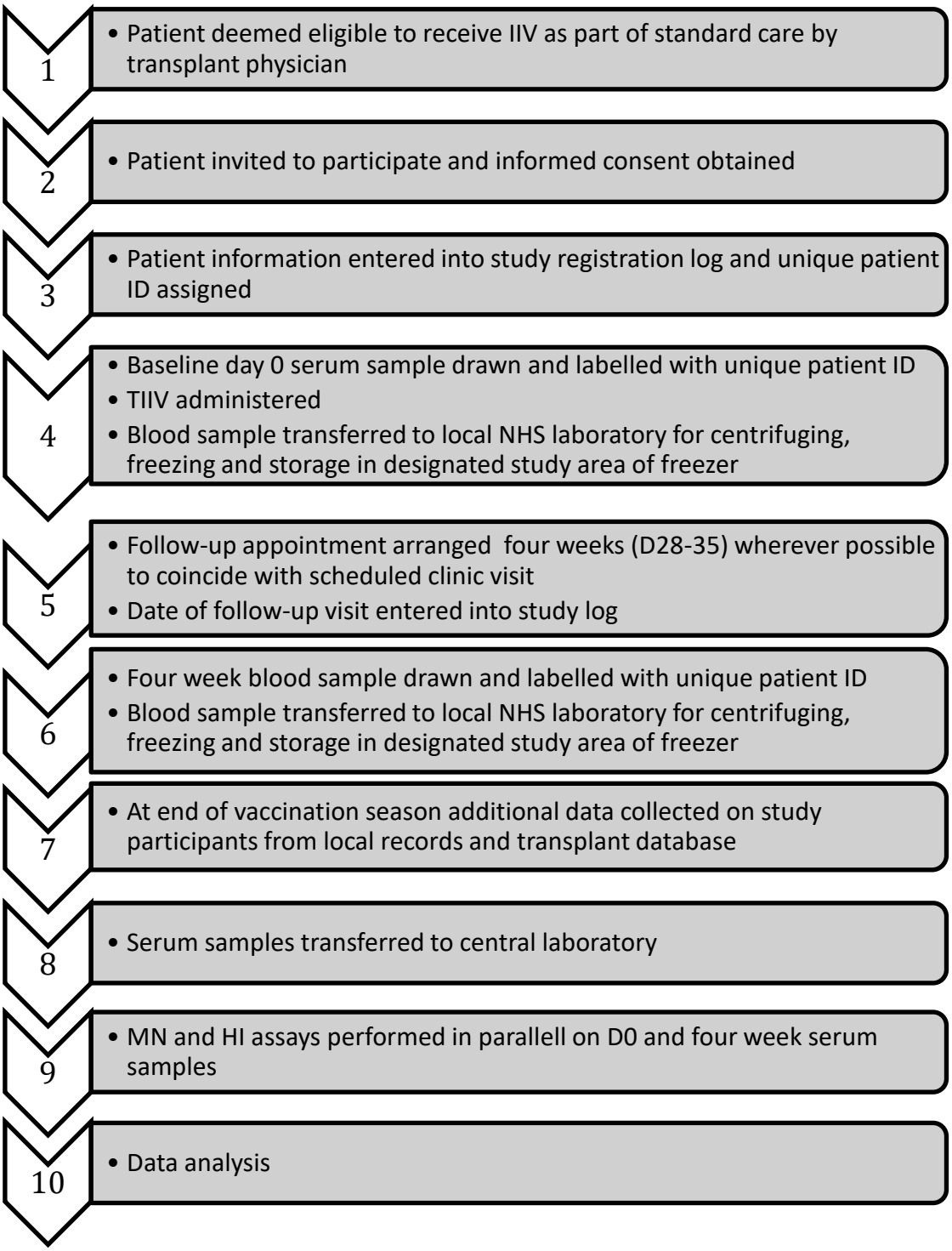

Figure 8: Study Flowchart

3.3.5 Participant Informed Consent, Registration, Confidentiality and General Ethical Considerations

All patients received Patient Information Sheets (PIS) and were offered the opportunity to ask any questions about the study. Patients were asked to give written consent and were advised that they could withdraw at any time from the study. Patients were advised that their decision to participate or to withdraw from the study would not impact on the care they received. As they were deemed eligible to receive the TIIV by their HSCT physician, they were advised that if they decided not to participate they would still be eligible to receive the TIIV at their General Practitioners. The PIS sheet and study consent form can be seen in appendix 3.

3.3.6 Vaccine Administration

A single dose of the 2015-2016 Northern hemisphere seasonal trivalent split-virion IIV (Sanofi) was administered intramuscularly to study participants by a trained member of the study team.

3.3.7 Blood Samples

Approximately 1x5ml blood samples were drawn on day of vaccination and 21-28 days later. Samples were centrifuged, aliquoted, frozen at -20oC and stored at the study site until end of study period. At the end of the study period all samples were shipped on

dry-ice to the central analysis laboratory at Public Health England. In accordance with ethics approval, and remaining serum was destroyed at the end of laboratory analysis in December 2016.

3.4 Materials and Methods

3.4.1 Solutions, cells and reagents used in Haemagglutination and Haemagglutination Inhibition Assay

Solutions/Cells/Reagents	Company	Location
Control serum (human and animal)	Public Health England	London, UK
Erythrocytes - 0.5% avian(turkey) or mammalian (guinea pig) erythrocyte suspension in PBS	Public Health England	London, UK
Influenza virus strain (Egg grown) **-A(H1N1)/Cal/7/09** **-A(H3N2)/Switz/9715293/13** **-B(Phuket)/3073/13**	Public Health England	London, UK
Phosphate buffered saline (PBS)	Gibco, ThermoFisher	Hemel Hempstead, UK
Receptor Destroying Enzyme (RDE)	Denka Seiken	Tokyo, Japan

3.4.2 Plastic material used in Haemagglutination and Haemagglutination Inhibition Assay

Plastic Material	Company	Location
50ml conical centrifuge tube	Falcon, Thermo Fisher	Hemel Hampstead, UK
1.5ml Screw cap micro tubes	Sarstedt	Numbrecht, Germany
96 well V-bottom plates	Sterilin, Thermo Fisher	Hemel Hampstead, UK
Pipettes and tips		

3.4.3 Solutions, cells and reagents used in preparation of cell monolayer, determining TCID50/ml and Viral Microneutralisation assay

Solutions/Cells/Reagents	Company	Location
Carbol Fuchsin	Sigma	Gillingham, UK
Foetal Bovine Serum (FBS)	Gibco, Thermo Fisher	Hemel Hampstead, UK
Madin-Derby canine kidney (MDCK) cell culture flask	European Collection of Authenticated Cell Cultures	London, UK
Methanol	VWR International	Leighton Buzzard, UK
Modified Eagles Medium (MEM)	Gibco, Thermo Fisher	Hemel Hampstead, UK
Phosphate buffered saline	Gibco, Thermo Fisher	Hemel Hempstead, UK
Serum free – Dulbecco's Modified Eagle's medium (SF-DMEM)	Gibco, Thermo Fisher	Hemel Hempstead, UK
TPCK-Trypsin 1mg/ml frozen aliquot	Sigma	Gillingham, UK
Trypan Blue (0.4%) solution	Gibco, Thermo Fisher	Hemel Hampstead, UK
Trypsin-EDTA (0.25%)	Gibco, Thermo Fisher	Hemel Hempstead, UK

3.4.4 Plastic Materials used in preparation of cell monolayer, determining TCID50/ml and Viral Microneutralisation assay.

Reagent / Plastic Material	Company	Location
7ml bijou container	Sigma	Gillingham, UK
50ml conical centrifuge tube	Falcon, Thermo Fisher	Hemel Hampstead, UK
1.5ml Screw cap micro tubes	Sarstedt	Numbrecht, Germany
96 well round bottom plate	Sigma	Gillingham, UK
96 well sterile culture plate	Sigma	Gillingham, UK
96 well V-bottom plate	Sterilin, ThermoFisher	Hemel Hampstead, UK
Pipettes and tips		

3.4.5 Solutions, cells and reagents used in ELISA endpoint Viral Microneutralisation assay

Solutions/Cells/Reagents	Company	Location
Phosphate Buffered Saline	Gibco, Thermo Fisher	Hemel Hempstead, UK
Modified Eagles Medium (MEM)	Gibco, Thermo Fisher	Hemel Hampstead, UK
Methanol	BDH	Poole, UK
30% H202	Sigma	Gillingham, UK
Wash Buffer PBS - Tween-20 (0.05%)	BDH	Poole, UK
Tetramethylbenzidine (TMB) liquid substrate	Europa Bioproducts Ltd	Ely, UK
0.5M Hydrogen Chloride (HCL)	BDH	Poole, UK

3.4.6 Antibodies used in ELISA endpoint Viral Microneutralisation assay

Antibody	Company	Location
Mouse anti-influenza nucleoprotein Ab (MCA400)	BioRad	California, USA
peroxidase-conjugate rabbit anti-mouse Ab (P026)	DAKO	California, USA

3.4.7 Haemagglutination Inhibition (HAI) Assay

The Haemagglutination Inhibition (HAI) assay is a method for determining the serum titre of strain specific influenza antibody targeting the HA protein. In vitro, the influenza virus HA protein binds to sialic acid receptors on avian or mammalian erythrocytes in suspension (red blood cells – RBCs) causing agglutination. Strain specific Ab binds the HA protein on the influenza virus surface, and so inhibits the virus from binding to the sialic acid receptor on RBCs. Thus the RBCs in solution are not agglutinated by virus and settle out of suspension to form a 'button' at the bottom of a V-shaped well.

Before conducting the HAI assay, the virus input concentration must be standardized by means of the haemagglutination (HA) assay.

3.4.7.1 Haemagglutination (HA) Assay

The haemagglutination (HA) assay is a method for determining the titre of influenza virus in a sample. As with the HAI assay, it is based on the in vitro property of a virus suspension to agglutinate mammalian and avian RBCs. The PHE HAI SOP requires a standardized virus input concentration of 4 HA units. 1 HA unit is defined as the minimum concentration of influenza virus in suspension required to cause complete agglutination of a 0.5% suspension of mammalian or avian RBCs.

3.4.7.2 Haemagglutination Assay Method

The HA assay was performed in accordance with Public Health England (PHE) Influenza Virus Reference Department Standard Operating Procedures (SOP). The HA assay was performed for the relevant virus strain in the morning before each HAI assay.

1. A frozen aliquot of egg grown influenza virus strain in solution was thawed at room temperature.
2. A 96 well V bottom plate in landscape orientation (Columns 1-12, Rows A-H) was prepared as follows (*Figure 9*):
    a. A 1:4 dilution of virus was prepared in column 1 rows A-C (25ul of virus and 75ul PBS)
    b. 50ul of PBS was added to columns 2-12 rows A-C.
    c. A serial, doubling dilution of virus solution was performed across the 96 well plate. Using a multi-channel pipette, 50ul of virus solution from

column 1 was transferred and mixed in column 2, then 50ul from column 2 was transferred and mixed in column 3 and continued to column 12. This gives dilutions of 1:4, 1:8, 1:16, 1:32 etc

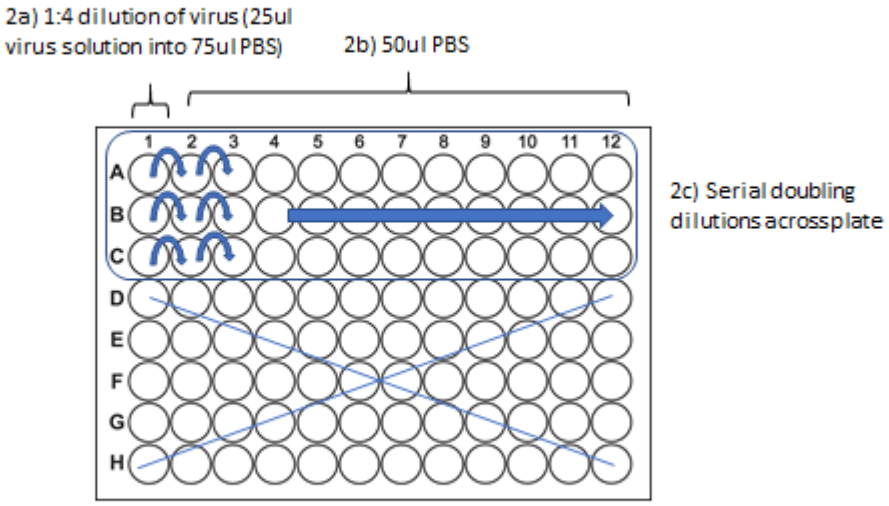

Figure 9: Preparation of 96 well plate for HA assay

3. 50ul of 0.5% RBCs were added to each well containing titrated virus solution. Based on PHE protocol, avian RBCs were used for A(H1N1)pdm09 and B(Phuket). Mammalian RBCs were used for A(H3N2) virus.
4. Plates were covered and incubated at room temperature for 30 (+/-10) minutes for avian RBCs, and 60 (+/- 10 minutes) for mammalian RBCs.
5. After incubation plates were read at an approximately 30-degree angle from vertical, against a white background.
6. A 'virus positive' agglutinated well was seen as a pink-red tinge to the solution in each well. In the absence of virus induced agglutination RBCs settled out of

suspension, formed a ‘button’ in the V bottom well, and ‘ran’ down the well when the plate was tipped at the angle above. This was read as a ‘virus negative’ well. If an RBC button did not ‘run’ this was read as a positive agglutinated well (*Figure 10*).

7. 1 HA unit of virus is defined as the highest titre at which agglutination is seen. A minimum of 2 matching rows were required. In Figure 10 the 1 A unit value is 1:512. The 1 HA unit titre was multiplied by 4 to give the 4 HA unit titre (e.g. 1:512 x 4 = 1:128).

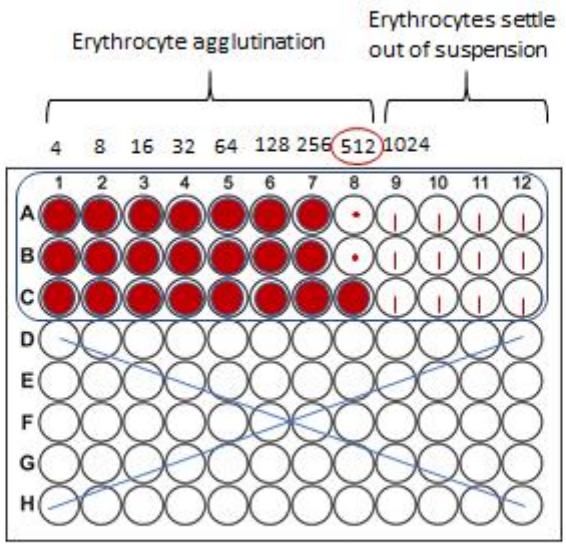

Figure 10: Reading HA assay plate

8. To achieve a more precise 4 HA unit dose, a ‘back-titration’ HA assay was performed using non-serial dilutions above and below the 4 HA unit titre. For

example, if the 4 HA unit titre were 1:128, 8 virus dilutions in PBS from 1:70 to 1:210 in 1:20 increments would be prepared in 1.5ml screw cap microtubes.

9. A 96 well V bottom plate was prepared as follows (*Figure 11*)
    a. 100ul of the lowest dilution (e.g. 1:70) duplicated in column 1 rows A and B, 100ul of the next dilution (e.g. 1:90) duplicated in column 1, rows B and C and so on to the bottom of the plate. The 5th dilution (e.g. 1:150) was duplicated in column 7 rows A and B, and again continued down the pate so that all 8 dilutions could fit on a single plate.
    b. 50ul of PBS was added to all remaining wells
    c. For each starting dilution, serial doubling dilutions were performed across 5 columns of the plate (1-6 and 7-12)
10. 50ul of appropriate RBCs were added to each well. The plate was incubated and read as described above.
11. The 4 HA unit dilution was identified as the starting dilution of the row in which agglutination was observed in but not beyond column 3 (equating to 1 HA unit). In *Figure 12*, agglutination is seen column 3 rows E and F. The 4 HA unit starting dilution in column 1 row E and F was 1:110. In this example 1:90 is too concentrated as agglutination is seen to 0.5 HA units (i.e. column 4) and 1:130 is too dilute as agglutination is only seen to 2 HA units (i.e. column 2). Duplicated dilutions needed to match to determine the 4 HA unit dilution. In this example 1:110 would be used as the 4 HA unit virus input dose in the HAI assay.

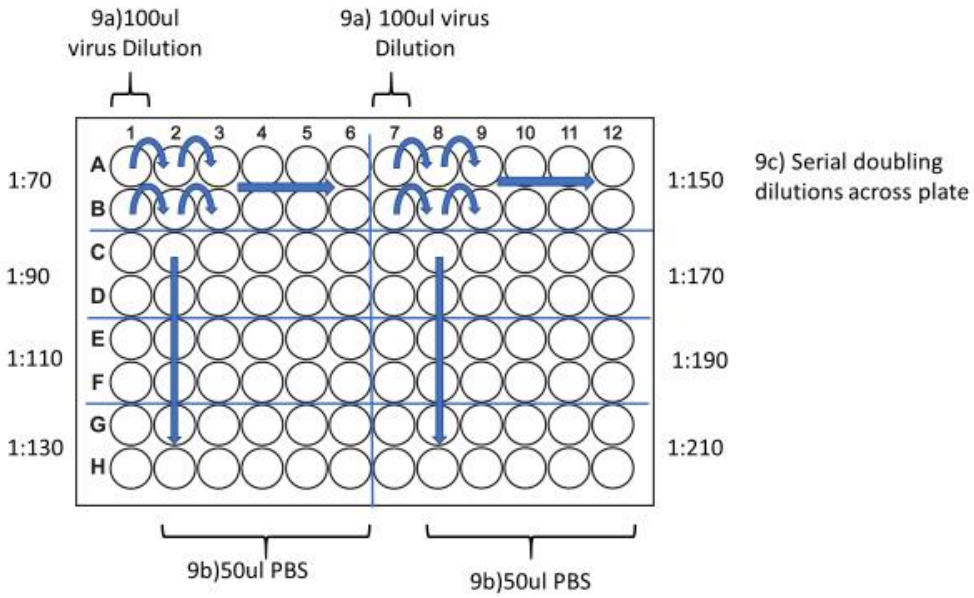

Figure 11: Preparation of 96 well plate for backtitration HA

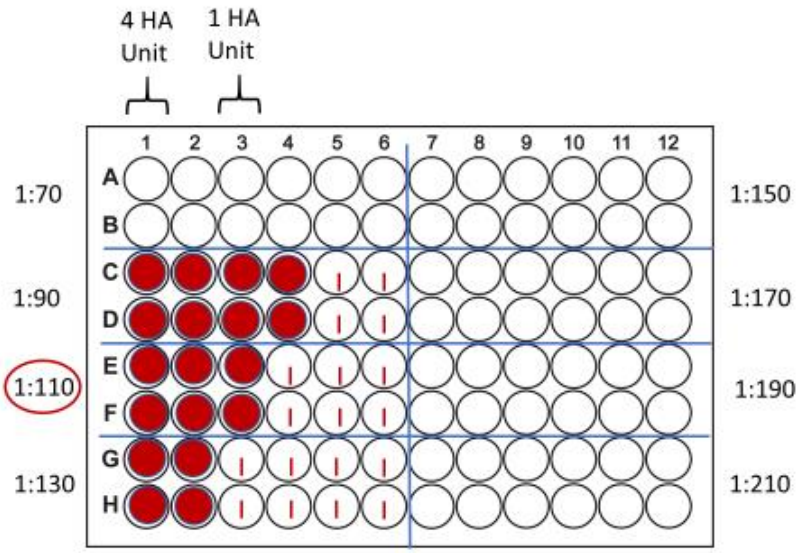

Figure 12: Reading backtitration HA assay plate

12. The volume of 4 HA unit virus solution required for the HAI was then calculated allowing 2.5ml per test plate. A solution at the concentration determined by the HA assay was prepared from the thawed virus aliquot, typically in a 50ml conical centrifuge tube (Falcon).
13. To confirm this prepared virus dilution was correct, a further back-titration was performed with starting dilution in column 1 rows A to C (i.e. in triplicate) and then diluted, incubated and read as described above. If this triplicate, back-titration of the prepared virus solution agglutinated to column 3 (equating to 1 HA unit) but not beyond, this stock was used in the HAI assay. The triplicate dilutions needed to match to confirm the 4 HA unit virus stock dilution was correct.

3.4.7.3 Haemagglutination Inhibition Assay Method

Serum Preparation

Prior to testing, patient and control sera were treated with Receptor Destroying Enzyme (RDE) to remove non-specific inhibitors.

1. RDE was dissolved in 20ml sterile saline as per manufacturer's instructions. RDE was added to aliquots of test and control sera in a 3:1 ratio (300ul RDE: 100ul sera) at room temperature.
2. RDE-Serum mixture was incubated in a water bath for 18 hours (+/- 2hours) at 37°C (+/-2°C).
3. RDE was inactivated by incubating at 56°C (+/-2°C) for 30-60 minutes.
4. The starting dilution for RDE treated sera was therefore 1:4.

Method

1. A stock of 4 HA unit virus solution was prepared as in 3.4.7.2.
2. A 96 well V-bottom plate in portrait orientation (Columns H-A, Rows 1-12) was prepared as follows (*Figure 13*):
    a. A 1:10 dilution of patient or control RDE treated sera was prepared in column H (30ul PBS and 20ul of serum) nb.starting dilution of RDE treated serum is 1:4.
    b. Patients' pre and post vaccination serum samples were paired and tested in adjacent rows. Control serum was added to row 12 of alternate plates.
    c. 25ul of PBS was added to columns G-A of rows 1-12.
    d. A serial, doubling dilution of test and control sera was performed across the 96 well plate. Using a multi-channel pipette, 25ul of serum solution from column H was transferred and mixed in column G, then 25ul from

column G was transferred and mixed in column F and continued to column A. This gives dilutions of 1:10, 1:20, 1:40, 1:80 etc.

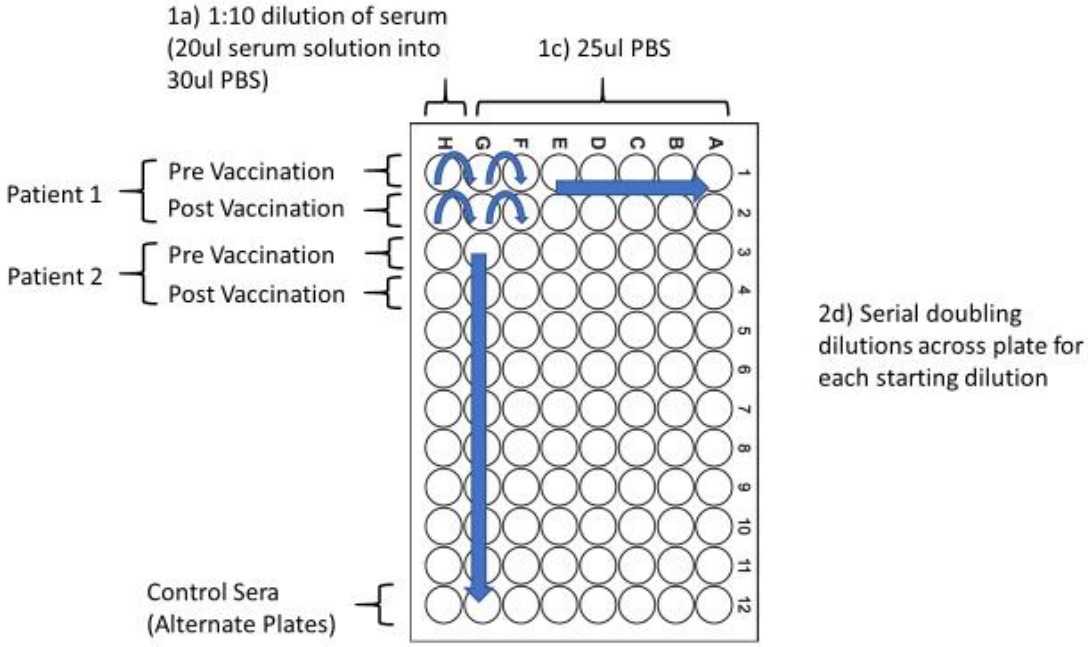

Figure 13: Preparation of 96 well plate for HAI assay

3. 25ul of prepared and confirmed 4 HA unit virus solution was added to each well.
4. Plates were covered, and virus-serum mixture was incubated for 60 (+/-10) minutes at room temperature.
5. 25ul of 0.5% RBCs was added to each well. Based on PHE protocol, avian(turkey) RBCs were used for A(H1N1) and B(Phuket). Mammalian (guinea pig) RBCs were used for A(H3N2) virus.
6. Plates were covered and incubated at room temperature for 30 (+/- 10) minutes for avian RBCs, or 60 (+/-10 minutes) for mammalian RBCs.

7. After incubation plates were read as described above.
8. For each serum or control sample, antibody titre was recorded as the reciprocal of the highest dilution at which agglutination was inhibited (see *Figure 14*)

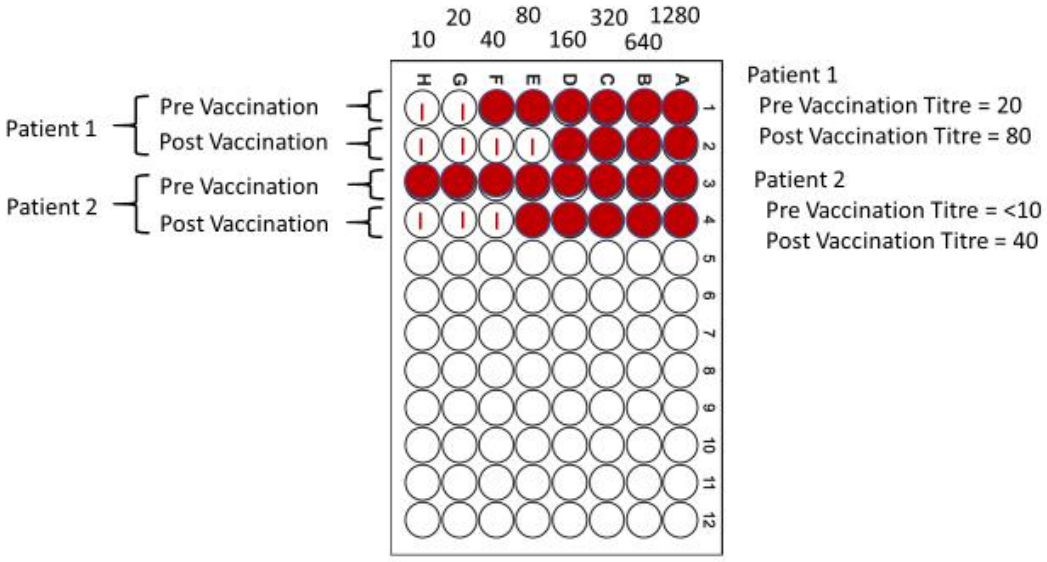

Figure 14: Reading HAI assay plate

3.4.7.4 Quality Control

Serum samples were tested in duplicate on separate plates in each assay run. 2 assay runs were performed giving 4 values for each sample. Final results were reported according to the PHE titre reporting flowchart appendix 4.

In each run the first plate comprised control samples. Control samples included as a minimum 1 positive and 1 negative human serum control, and 2 positive animal (ferret)

serum controls. Control serum was also added to alternate test plates, such that 1 plate of each duplicate pair included a control. The geometric mean titre (GMT) of each control serum used in a run had to be within two-fold of the expected value to validate the run. The expected values were taken from the PHE quality control database.

3.4.8 Virus Microneutralization (VMN) Assay

The VMN is a sensitive and specific assay for detection of strain specific neutralizing Abs that prevent virus entry into cells. There are two key stages to the assay:-

1. Virus-antibody reaction stage during which a standardized concentration of virus is mixed with serial dilutions of test serum.
2. Inoculation stage in which a cell culture monolayer is inoculated with the virus-antibody mixture. During this phase virus that has not been neutralized by Ab infects, replicates and is released from cells.

As with the HAI assay, the quantity of each virus used in the VMN assay must be standardized. The quantity of virus required to produce pathological change in 50% of cell cultures is referred to as the median tissue culture infective dose (TCID50) and is expressed as TCID50/ml. The standardized input dose for the VMN is 100xTCID50ml. The method for determining TCID50/ml and for performing the VMN assay is outlined below.

Determination of TCID50/ml was performed in accordance with the PHE influenza virus reference department SOP. The VMN assay performed was a modified version of the method previously described by the WHO(197). The first stage of both assays is preparation (seeding) of a cell monolayer in microtitre plates.

3.4.8.1 Optimisation of VMN assay

A conventional VMN assay using a cytopathic effect (CPE) endpoint takes 48-72 hours. An enzyme-linked immunosorbent assay (ELISA) gives a faster result over 24-48 hours. At the time of commencing laboratory work at the PHE influenza virus reference department, an ELISA endpoint VMN assay (E-VMN) was already established for A(H1N1). A CPE endpoint VMN assay for A(H3N2) was already established in Madin-Derby canine kidney (MDCK) - human 2,6-sialyltransferase (SIAT1) cell line. This cell line overexpresses alpha (2,6) linked sialic acids (see 3.1.2) and yields higher virus titres following inoculation (198) and is useful for assessing virus sensitivity to neuraminidase inhibitors (199). MDCK-SIAT is not routinely used for VMN assay.

Given the timeframe available for laboratory work it would not have been feasible to optimize ELISA based methods for A(H3N2) and B(Phuket) viruses. Preliminary work indicated that A(H3N2) VMN assay in MDCK-SIAT1 cell line yielded results similar to HAI assay. Therefore, VMN CPE endpoint assay was optimized for A(H1N1), A(H3N2) and B(Phuket) in MDCK cell lines. In addition, the established ELISA endpoint VMN was carried out for A(H1N1). The VMN assay method is outlined below with variables

optimized for each virus highlighted and summarized in table 5. The E-VMN method is summarized in section 3.4.8.6.

MDCK cell lines were maintained by PHE staff. The WHO recommends that cells at low-passage (<25-30) are used for VMN assay(200). Stored frozen cells were thawed at passage 30 and maintained until passage 90 in accordance with PHE policy. Due to practical limitations, it was not possible to maintain separate low-passage cell lines for the purposes of this laboratory work and therefore MDCK cells were used at the passage available.

3.4.8.2 Preparation (Seeding) of microtitre plates with Madin-Derby canine kidney (MDCK) cells

Microtitre plates were seeded with MDCK cells from cultures maintained by PHE laboratory staff.

1. The procedure outlined was performed around a Bunsen burner to create a sterile environment.
2. A tissue culture flask was observed under an inverted microscope to assess health and confluence of the MDCK cell monolayer.
3. The growth medium from the culture flask was discarded and the cell monolayer was washed twice with 20ml of PBS.
4. 3mls of Trypsin-EDTA (0.25%) was added to the flask and rocked over the monolayer for 10-20 seconds and then discarded.

5. The cell culture flask was incubated at 36°C and observed every few minutes for evidence of cell monolayer detaching.
6. When cell detachment was evident, 50ml of 10% heat inactivated fetal bovine serum (FBS) in Modified Eagles Medium (MEM) was added to the flask and pipetted vigorously to break up cell clumps. The resulting cell suspension was then transferred to a 50ml conical centrifuge tube (Falcon).
7. The cell suspension was centrifuged at 1800 revolutions per minute (RPM) at 22°C for 5 minutes with acceleration and deceleration times of 5 seconds.
8. After centrifuging, the medium was poured off and the cell pellet re-suspended in 20ml of 1% FBS-MEM.
9. In a 7ml Bijou container, 50ul of cell suspension was added to 500ul of 1% FBS-MEM and 450ul of Trypan Blue (0.4%) solution. The stained cell suspension was then introduced into a haemocytometer and viable cells were counted under an inverted microscope.
10. Total volume of cell suspension for determination of TCID50/ml or VMN was calculated, allowing 10ml suspension per 96 well culture plate. The cell suspension was then made up to the necessary volume adjusting cell concentration to $5x10^5$cells/ml.
11. 100ul of cell suspension was then transferred to each well of a 96 well culture plate.
12. Plates were incubated overnight for 18-22 hours at 37°C in 5% CO_2 and observed the following day for health and confluence.

3.4.8.3 Determination of Influenza TCID50/ml

This procedure was performed around a Bunsen burner to create a sterile environment

Preparation and titration of virus

1. A frozen aliquot of egg grown influenza virus strain was thawed at room temperature.
2. The procedure outlined was performed around a Bunsen burner to create a sterile environment.
3. A starting virus dilution (initially $10^{-4.5}$ but subsequently adjusted according to initial results) was prepared by adding 10ul of thawed virus solution to 990ul SF-DMEM in a 1.5ml microtube, and then transferring 63.2ul into 20ml of SF-DMEM in a 50ml conical centrifuge tube.
4. A 96 well round-bottom plate in landscape orientation was prepared as follows (*Figure 15*):
    a. 180ul of virus at $10^{-4.5}$ dilution was added to column 1 rows A-H.
    b. 120ul of SF-DMEM was added to all other wells
    c. A serial ½ log dilution of the virus was performed. Using a multi-channel pipette 60ul of virus solution was transferred from column 1, and mixed and ejected into well 2. Pipette tips were then discarded. With clean tips 60ul was taken from column 2 and mixed and ejected into column 3. This was repeated across the 96 well plate to column 11 replacing tips after each mixing step. N.b. virus was not transferred to column 12.

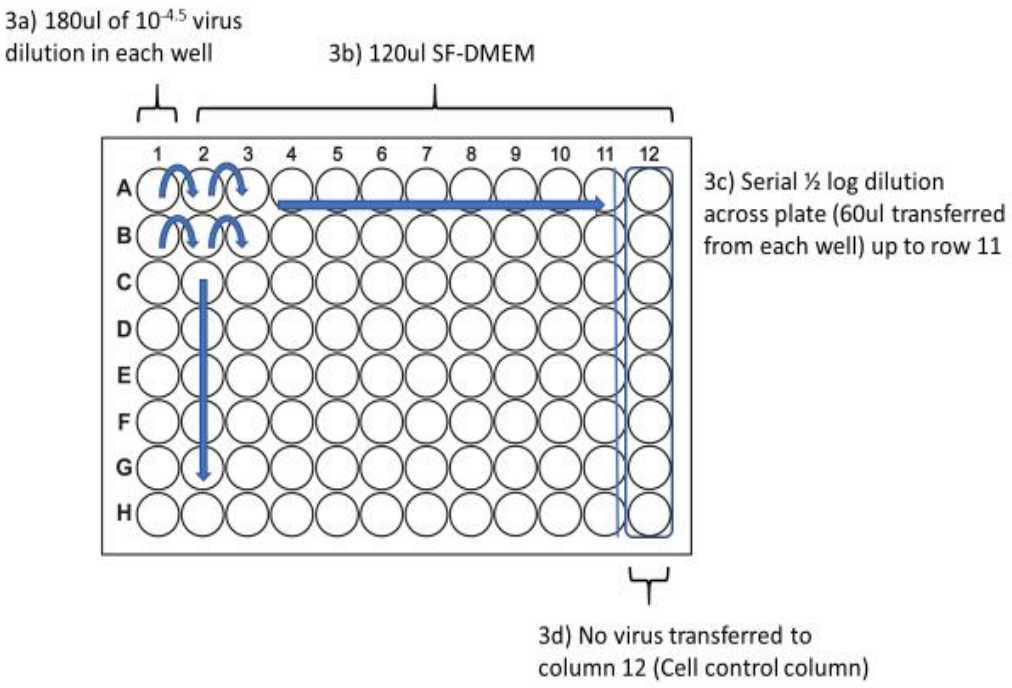

Figure 15: Preparation of 1/2 Log virus dilution in 96 well round-bottom plate

Washing and inoculation of MDCK cell monolayer

5. Using a Vacusafe™ laboratory vacuum pump, 1% FBS-MEM was aspirated from each well of the 96 well culture plates prepared in 3.4.8.2. Each well was washed and aspirated twice with 200ul/well of SF-DMEM. Care was taken not to touch the cell monolayer, and not to let the cells dry out.
6. After the final aspiration, 100ul/well of the prepared serial ½ log virus dilution was transferred column-by-column from the 96 well round bottom plate to inoculate the MDCK cell monolayer. Fresh pipette tips were used for each

transfer. This transfer included 100ul/well of SF-DMEM from column 12 (cell control column)

7. The cell culture plates with virus inoculum were incubated for 1 hour at 37C°C in 5% CO_2.

Preparation of viral growth medium (VGM) and washing of incubated cell monolayer

8. During incubation, viral growth medium (VGM) was prepared by adding 1mg/ml TPCK-trypsin to a volume of SF-DMEM to give 1ug/ml concentration. Approximately 10ml of VGM was required per plate.
9. After 1-hour incubation, the viral inoculum was removed by aspiration and washed twice with 150ul/well SF-DMEM.
10. After the final aspiration, 100ul/well of prepared VGM was added to the cell monolayer.
11. Cells were then incubated at 37C°C in 5% CO_2 for 72 hours.

Plate readout

12. After incubation, infection of cells was determined by 2 methods: -
    a. HA assay of supernatant

i. 50ul/well of supernatant was transferred column-by-column from the cell culture plate to a 96 well V-bottom plate. Fresh tips were used for transfer of each row.

ii. HA assay was performed on supernatant as described in 3.4.7.1

b. CPE stain

i. After removing 50ul for HA, the remaining supernatant was removed from each well by aspiration

ii. The cell monolayer was fixed for 30 minutes using 100ul/well ice cold methanol

iii. Methanol was shaken-off after 30 minutes and in a fume cupboard 100ul/well of 5% Carbol Fuchsin was added to each well.

iv. After 30 minutes Carbol Fuchsin was washed off with tap water and plates were left to dry.

13. HA and CPE stain endpoints were compared for each plate (*Figure 16* and *Figure 17*) to verify that CPE correlated with presence of virus in supernatant. For consistency HA was used as the assay endpoint for titre reporting. Results entered into a spreadsheet and TCID50 was calculated using the Reed-Muench formula.
14. Final TCID50/ml virus input concentration was based on minimum 3 TCID50 values obtained from separate runs each consisting of 6 plates.
15. This was repeated to establish TCID50/ml for each virus.

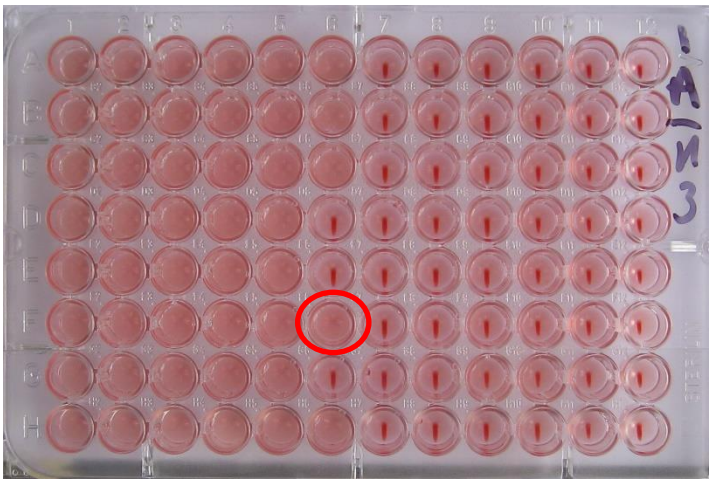

Figure 16: HA TCID/50 endpoint. Agglutination of RBCs in well demonstrates that cell monolayer was infected by influenza virus, and virus replication and release into supernatant took place over 72 hour incubation.

Figure 17: CPE Stain TCID/50 endpoint. Wells in which cells were infected and killed by virus over 72 hour incubation are not stained by Carbol Fuchsin. Living cells take up stain and appear pink. Note unstained cells correspond with agglutination on HA. Well F6 shows partial CPE and complete agglutination by HA. HA values were used for TCID50 calculation

3.4.8.4 Viral Microneutralization Assay Method

Serum Preparation

Prior to VMN testing, patient and control sera were heat-treated at 56°C(+/- 2°C) for 30 minutes to remove non-specific inhibitors.

Preparation of virus dilution

1. A frozen aliquot of egg grown influenza virus strain was thawed at room temperature.
2. The procedure outlined was performed around a Bunsen burner to create a sterile environment.
3. A virus dilution was prepared in SF-MEM according to 100xTCID50/ml dose calculated in 3.4.8.3. To confirm that this virus dilution produces pathological change in 50% of cell cultures, a backtitration is prepared alongside the VMN assay.

Preparation of virus backtitration

4. A serial ½ log dilution of the virus prepared in 2 was performed across 8 x 1.5ml screwcap microtubes (*Figure 18*).
    a. Microtubes are labelled 1-8.
    b. 730ul of virus dilution from step 2 is added to microtube 1

c. 500ul of SF-MEM is added to microtubes 2-8

d. 230ul is transferred from microtube 1 and mixed in microtube 2. The tip is then discarded. Using a fresh tip 230ul is transferred from microtube 2 to microtube 3 and this is continued to microtube 8.

e. The virus dilutions are then transferred to a 96 well round-bottom plate. Note that the backtitration dilutions could be transferred directly from the microtubes to the MDCK cell monolayer at step 11, but this is time sensitive and it proved faster to pre-prepare the dilutions on a round-bottom plate as described here. 110ul/well of virus dilution is transferred from microtube 1 to rows A-D of column 1. 110ul per well is transferred from microtube tube 2 to rows A-D of column 2 and so on up to microtube tube 8.

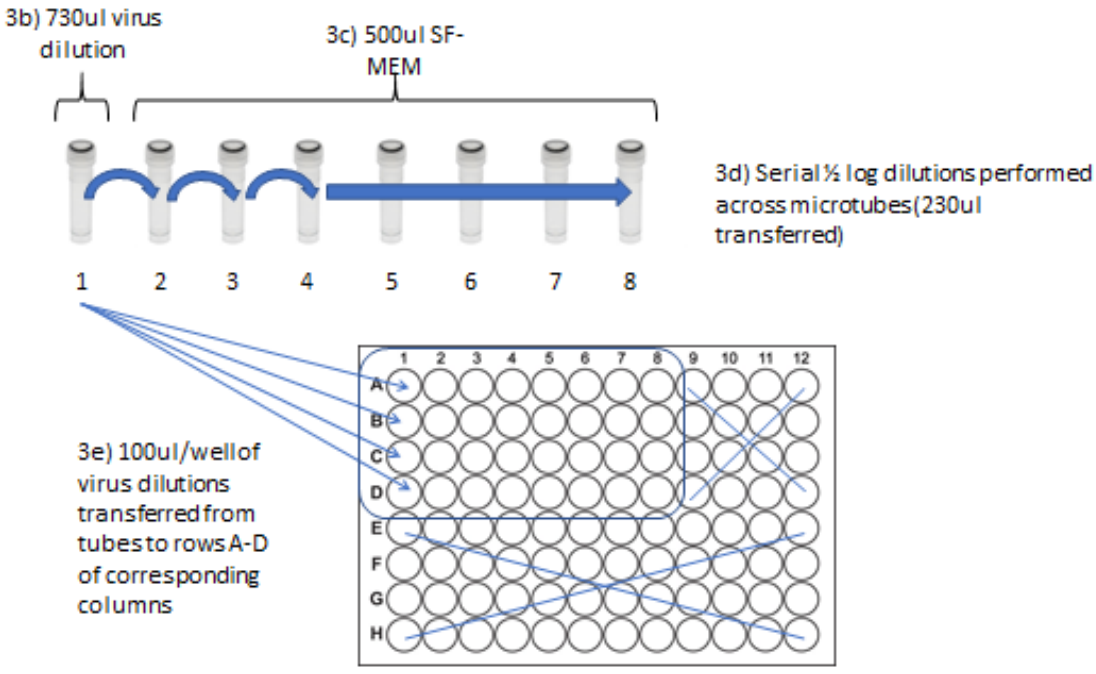

Figure 18: Preparation of virus backtitration

		Assay Stage			
		Inoculation of monolayer with virus		Incubation of infected cells	
Virus Strain	Virus dilution (100xTCID50/ml)	TPCK-Trypsin concentration (ug/ml)	Incubation (minutes +/- 15)	TPCK-Trypsin concentration (ug/ml)	Incubation (hours +/- 2)
A/California/7/09(H1N1)	1:90,000	1	120	1	70
A/Switzerland/9715293/2013 (H3N2)	1:4000	1	120	1	70
B/Phuket/3073/2013	1:140	MEM Only	180	1.5	46

Table 5: Optimised steps for A(H1N1)pdm09, A(H3N2) and B(Phuket) VMN

Titration of test and control serum

5. A 96 well round-bottom plate in landscape orientation was prepared as follows (*Figure 19*): -
    a. A 1:10 dilution of heat-treated test or control serum was prepared in column 1 by adding 11ul serum to 99ul PBS
    b. 55ul/well PBS was added to columns 2-11
    c. 110ul/well PBS was added to column 12
    d. A serial, doubling dilution of test and control sera was performed across the 96 well plate. Using a multi-channel pipette, 55ul of serum solution from column 1 was transferred and mixed in column 2, then 55ul from column 2 was transferred and mixed in column 3 and continued to column 10. This gives dilutions of 1:10, 1:20, 1:40, 1:80 etc.
    e. This is repeated for each test plate
6. 55ul/well of prepared virus dilution is added to columns 1-11 of each test plate.
7. Note columns 1-10 contain serum and virus (test wells), column 11 contains virus but no serum (virus control wells), and column 12 contains only PBS (cell control well)

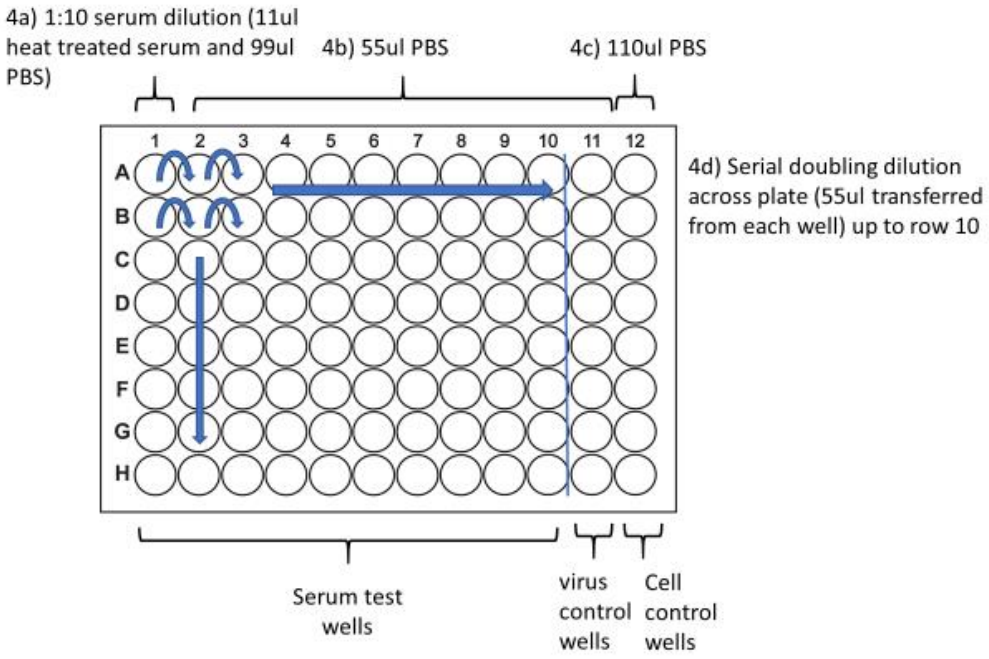

Figure 19: Preparation of serum serial doubling dilutions in 96 well round-bottom plate

a. The plates containing the virus-serum mixture are covered, gently agitated and incubated at 37C°C in 5% CO_2 for 1 hour for all viruses. The virus backtitration plate (although not containing Ab) was also incubated alongside the virus-serum mixture to ensure standardization of conditions.

8. When working with multiple plates (>10), virus was added to batches of 10 plates at 30-minute intervals. This gave adequate time for plate washing and ensured that each subsequent step was performed at the correct time point.

Washing and inoculation of MDCK cell monolayer with virus-serum mixture and virus backtitration

9. While virus-serum mixture was incubating a Vacusafe™ laboratory vacuum pump was used to aspirate 1% FBS-MEM from each well of the 96 well culture plates prepared in 3.4.8.2. Each well was washed and aspirated twice with 200ul/well of SF-DMEM. Care was taken not to touch the cell monolayer, and not to let the cells dry out
10. After the final aspiration: -
    a. For A(H1N1) and A(H3N3) 100ul/well of VGM (1ug/ml TPCK-Trypsin) was added to each well
    b. For B(Phuket) 100ul/well SF-MEM was added to each well.
11. After 1-hour incubation of the serum-virus mixture, 100ul/well was transferred column-by-column onto the corresponding wells of the cell culture plate to inoculate the MDCK monolayer. A multichannel pipette was used, and tips were changed for transfer of each row.
12. 100ul/well from the prepared virus backtitration plate was also transferred to a cell culture plate in the manner described in 11.
13. After the cell culture plate was inoculated with the virus-serum mixture, the plates were incubated as follows: -
    a. For A(H1N1) and A(H3N2) 2 hours at 37C°C in 5% CO_2
    b. For B(Phuket) 3 hours at 37C°C in 5% CO_2

Aspiration of virus inoculum and incubation of infected cells

14. After incubation, the viral inoculum was removed by aspiration and cell monolayers were washed twice with 200ul SF-DMEM as described above in 9.
15. After the final aspiration, VGM was added to the cell monolayer. The VGM concentration used was as follows: -
    a. 1ug/ml TPCK-Trypsin for A(H1N1) and A(H3N2)
    b. 1.5ug/ml TPCK-Trypsin for B(Phuket)
16. Cell culture plates were then then incubated as follows: -
    a. For A(H1N1) and A(H3N2) 70 (+/-2) hours at 37C°C in 5% CO_2
    b. For B(Phuket) 46 (+/-2) hours at 37C°C in 5% CO_2
17. After incubation infection of cells was determined by HA assay and correlated with CPE stain as described in 3.4.8.3. In all cases Ab titre was reported according to HA result.

3.4.8.5 Quality Control

Serum samples were tested in duplicate on separate plates in each assay run. 2 assay runs were performed for A(H1N1) and A(H3N2) giving 4 values for each sample. 1 assay run was performed for B(Phuket) giving 2 values for each sample. 2 titres were reported according to the PHE HAI/VMN reporting flowchart (appendix 4) and the GMT of these two values calculated to give a final titre result. All results from the single

B(Phuket) run were reportable by PHE titre reporting flowchart, and the assay was clearly less sensitive than the B(Phuket) HAI. The decision was therefore taken to use the remaining serum to perform the A(H1N1)pdm09 E-VMN assay.

In each assay run the first plate comprised control samples. Control samples included as a minimum 1 positive and 1 negative human serum control, and 1 positive animal (ferret) serum controls. Control serum was also added to alternate test plates, such that 1 plate of each duplicate pair included a control. After TCID50/ml was determined, VMN runs were performed with control samples only. GMT control values were based on a minimum of 4 titres obtained from separate runs within one-fold dilution of each other. The GMT of the sera used in a VMN test run had to be within one-fold dilution of the expected value to validate the run. Control sera used along with control values, and control results obtained for each assay along with cell passage used are shown in *Table 6*. To confirm the virus dose was correct, the backtitration was reviewed to check that 50% of infected wells showed CPE at the 1TCID50/ml dilution.

	Control Serum (dilution)	GMT Control	Run 1	Run 2
AH1N1	Cell Passage		50	86
	Human Positive 2015 (Neat)	452	640	320
	Human Negative 2015 (Neat)	40	40	40
	Human Negative 2013 (Neat)	5	5	5
	F777 (1:20)	65	113	40
AH3N2	Cell Passage		87	39
	Human Positive 2015 (Neat)	1688	1810	1810
	Human Negative 2013 (Neat)	5	5	5
	F774 (Neat)	1940	3620	1810
	F774 (1:2)	1114	1280	1810
	F763 (Neat)	970	1810	1810
B Phuket	Cell Passage		85	NA
	Human positive 2015 (Neat)	20	40	NA
	Human Negative 2013 (Neat)	5	5	NA
	F776 (1:2)	113	160	NA
	F765 (Neat)	90	56.57	NA

Table 6: Control serum GMTs, GMTs obtained from VMN runs and cell passage

3.4.8.6 ELISA endpoint Viral Microneutralisation Assay (E-VMN) assay Method

Serum Preparation

Prior to VMN testing, patient and control sera were heat-treated at 56°C(+/- 2°C) for 30 minutes to remove non-specific inhibitors.

Preparation of Virus Dilution

1. A frozen aliquot of egg grown influenza virus strain was thawed at room temperature.
2. A virus dilution of 1:2000 was prepared in a 50ml centrifuge tube in SF-MEM according to previously established 100xTCID50/ml dose.

Preparation of Serum Titration

1. A 96 well flat-bottom sterile culture plate in landscape orientation was prepared as follows (note plate orientation and control columns are as per *Figure 19* but volumes are different):-
    a. A 1:10 dilution of heat-treated test or control serum was prepared in column 1 by adding 10ul serum to 90ul PBS
    b. 50ul/well PBS was added to columns 2-11
    c. 100ul/well PBS was added to column 12
    d. A serial, doubling dilution of test and control sera was performed across the 96 well plate. Using a multi-channel pipette, 50ul of serum solution from column 1 was transferred and mixed in column 2, then 50ul from column 2 was transferred and mixed in column 3 and continued to column 10. This gives dilutions of 1:10, 1:20, 1:40, 1:80 etc.
    e. This is repeated for each test plate
2. 50ul/well of prepared virus dilution is added to columns 1-11 of each test plate.
3. Note columns 1-10 contain serum and virus (test wells), column 11 contains virus but no serum (virus control wells), and column 12 contains only PBS (cell control well)
4. The plates containing the virus-serum mixture are covered, gently agitated and incubated at 37°C (+/-2°C) in 5% CO_2 for 1 hour.

Preparation and addition of MDCK Cell Suspension

1. A $5x10^5$cells/ml MDCK cell suspension was prepared as described in 3.4.8.2
2. After 1-hour incubation of the serum-virus mixture, 100ul/well of cell suspension is added to the 96 well flat-bottom culture plates.
3. Plates were incubated for 16-18 hours at 37°C (+/-2°C) in 5% CO_2.
4. After incubation cells were checked under a microscope for even distribution and confluence.

Termination of viral replication and addition of Antibody

1. After incubation viral replication was terminated as follows: -
    a. Medium was removed from 96 well plates using a Vacusafe™ laboratory vacuum pump.
    b. 100ul/well of methanol with 0.6% hydrogen peroxide was added to the plate and left for 20minutes after which it was tipped off and plates blotted dry.
2. Plates were washed twice using an automatic plate washer and 350ul/well of Wash Buffer PBS-Tween 20 (0.05%).
3. 100ul/well of mouse monoclonal AB (mAb) specific for A(H1N1)pdm09 nucleoprotein (primary Ab) was then added to the plates.
4. Plates were then incubated for 1 hour (+/- 25 minutes) at 37°C (+/-2°C) in 5% CO_2

5. After incubation plates were washed four times using an automatic plate washer and 350ul/well of Wash Buffer PBS-Tween 20 (0.05%).
6. 100ul/well of rabbit anti-mouse IgG – horse radish peroxidase (HRP) conjugate were then added and plates were again incubated for 1 hour (+/- 25 minutes) at 37°C (+/-2°C) in 5% CO_2
7. Washing was repeated as per step 5
8. 100ul/well of Tetramethylbenzidine (TMB) was added to each well and plates were left in the dark for 18-20 minutes.
9. 100ul/well of 0.5 molar hydrogen chloride solution was added to stop the peroxidase reaction.
10. Plates were then read immediately using a flatbed scanner at a dual wavelength of 450nm and 620nm, with final optical density (OD) reading being the difference of the two values.

Quality Control

A single run was performed testing each serum sample in duplicate. The run was validated against standard PHE quality control for the E-VMN assay.

3.5 Statistical Analysis

Continuous variables are reported as median values with ranges. Categorical variables are reported as frequencies and percentages.

Immunological data is displayed in reverse cumulative frequency distributions for each vaccine component and assay. Supplementary cumulative frequency tables are displayed in appendix 5. Immunological data is summarised as pre and post-vaccination geometric mean titres (GMT), and geometric mean ratios (GMRs) of pre and post-vaccination titres. Seroconversion and low-titre seroresponse (LTS) rates are reported as frequencies and percentages. Seroconversion is defined as a change from a negative (<10) pre-vaccination titre to ≥40 post-vaccination, or a fourfold increase in baseline titre. Low-titre seroresponse is defined as a change from a negative (<10) pre-vaccination titre to a positive post-vaccination titre but not meeting criteria for seroconversion, or a less than fourfold increase in titre. For HAI, rates of titre≥40 are reported (historically considered 50% seroprotective in non-immunocompromised patients). No current or historical seroprotective titres have been defined for VMN so equivalent rates are not reported for this assay.

As Ab titre values were not normally distributed, paired patient results were compared with the Wilcoxon signed-rank test for non-parametric data. Both intra-assay GMTs (pre and post HAI, pre and post VMN) and inter-assay GMTs (Pre HAI and pre VMN, post HAI and post VMN), and inter-assay GMRs. An effect size (*r*) is presented for each result. An *r* value greater than .3 is considered a medium effect size and greater than .5 a large effect size(201). The relationship between log10 transformed HAI and VMN titres was explored using a linear regression model and equivalent titres estimated.

The relationship between categorical outcome measures (low-titre seroresponse and seroprotection rates) and categorical explanatory variables was assessed with Pearson's Chi-Square test, or Fisher's exact test when expected frequencies were less than 5. For continuous explanatory variables, binary logistic regression was used.

Correlation between non-parametric continuous outcome measures (GMT and GMR) and continuous explanatory variables were explored with Spearman's Rank Correlation. For categorical variables Mann-Whitney Test was used.

All statistical testing was 2 sided and at the 95% confidence level.

Analysis was performed with IBM SPSS version 24. Reverse cumulative frequency charts were constructed using StataCorp Stata release 14.

3.6 Results

28 adult patients in the first year after RIC alloHSCT were vaccinated as part of standard care and in accordance with local HSCT programme policies between October 2015 and February
2016. One dose of the 2015-2016 Northern hemisphere seasonal trivalent split virion IIV (Sanofi) was administered by intramuscular(IM) injection according to manufacturer's instructions by a trained healthcare practitioner. The 2015-2016 seasonal TIIV contained 15ug Haemagglutinin (HA) each of:

A/California/7/2009(H1N1)pdm09 – A(H1N1)pdm09

A/Switzerland/9715293/2013(H3N2) – A(H3N2)

B/Phuket/3073/2013 – B(Phuket)

Post-vaccination blood samples were drawn at a mean of 28.07 (21-50, SD 4.75) days from vaccination. One patient was unable to attend follow-up on the scheduled date and had a blood sample drawn on day+50 post-vaccination.

3.6.1 Patient Characteristics

Characteristics of 28 study participants are summarized in *Table 7*. All patients received HSCT as treatment for haematological malignancy. The most frequent diagnosis was AML (50%). Donor type was VUD in 71.4% of HSCTs, and sibling in 28.6%. HSC source was PBSC in 100% of cases, and all conditioning regimens included in-vivo lymphocyte depletion with alemtuzumab (89.3%) or antithymocyte globulin (ATG) (10.7%).

Characteristic (n=28)	Value
Age at HSCT, median (range)	57.8 (38.0-72.1)
Gender male, n(%)	15 (53.6)
Diagnosis, n (%)	
Acute lymphoblastic leukaemia (ALL)	3 (10.7)
Acute myeloid leukaemia (AML)	14 (50.0)
Chronic lymphocytic leukaemia (CLL)	1 (3.6)
Chronic myelomonocytic leukaemia (CMML)	1 (3.6)
Myelodysplastic syndrome (MDS)	4 (14.3)
Myelofibrosis (MF)	2(7.1)
Multiple myeloma (MM)	1 (3.6)
Non-Hodgkin Lymphoma (NHL)	2 (7.1)
Donor type, n (%)	
Sibling donor	8 (28.6)
Volunteer unrelated donor (VUD)	20 (71.4)
Stem cell source, n (%)	
Peripheral blood stem cell (PBSC)	28 (100)
Conditioning Intensity, n (%)	
Reduced intensity	28 (100)
Lymphocyte depletion, n (%)	
Alemtuzumab	25 (89.3)
Antithymocyte globulin (ATG)	3 (10.7)
Days from HSCT to vaccination, median (range)	78.5 (24-363)
Months from HSCT to vaccination, n (%)	
0-3	15(53.6)
>3-6	6 (21.4)
>6-12	7 (25)
Lymphocyte count (x10^9) at vaccination, median (range)	0.57 (0.02-2.98)
Graft versus host disease at vaccination, n(%)	8 (28.6)
Acute (stage 1, skin)	5 (17.9)
Chronic (mild, skin)	3 (10.7)
Immunosuppressive therapy (IST) at vaccination, n(%)	
Any IST	18 (64.3)
Single agent	13 (46.4)
Dual agent	4 (14.3)
Triple agent	1 (3.6)
Intravenous Immunoglobulin (IVIg) in last 12 months, n(%)	2 (7.1)
Rituximab in last 12 months, n(%)	3 (10.7)

Table 7: Characteristics of n=28 Patients

28.6% of recipients had GvHD, which was limited to stage 1 acute skin GVHD (17.9%) or mild chronic skin GVHD (10.7%) in all cases. Recipients were vaccinated at a median time-point of 78.5 (range 24-363) days. 18(64.3%) of patients were receiving IST at time of vaccination.

3.6.2 HAI and VMN Immunological Data

Pre and post vaccination Ab titres to A(H1N1)pdm09, A(H3N2) and B(Phuket) by HAI and VMN are summarised in reverse cumulative frequency charts (*Figure 20* to *Figure 23*). Log10 transformed Ab titres are plotted on the x-axis, and % of patients with > *x*-titre on the y-axis. Supplementary cumulative frequency charts are provided in appendix 5 and frequency of negative titres (<10) are summarised in *Table 8*. Reverse cumulative frequency curves display each data point and offer a visual means to compare pre and post Ab titres, and HAI against VMN assays(202). For all three vaccine components and both HAI and VMN assays the pre and post vaccination curves overlap and intersect at several points indicating minimal difference between pre and post vaccination titres. With the exception of the A(H3N2) VMN curve, the initial slope of the curves is steep reflecting low variability at the lower end of the titre range and a large proportion of patients with negative (<10) or low Ab titres both pre and post-vaccination.

Vaccine Component	Ab Titre < 10, n(%)					
	HAI		VMN		E-VMN	
	Pre	Post	Pre	Post	Pre	Post
A(H1N1)pdm09	18(64.3)	17(60.7)	15(53.6)	14(50.0)	11(39.3)	11(39.3)
A(H3N2)	15(53.6)	13(46.4)	3(10.7)	3(10.7)		
B(Phuket)	14(50.0)	19(67.9)	23(82.1)	24(85.6)		

Table 8: Frequency of negative (<10) Ab titres for A(H1N1)pdm09, H(H3N2) and B(Phuket)

While for A(H1N1)pdm09 and A(H3N2) the proportion of patients with greater than any given titre is higher for VMN than HAI and the distribution of positive titres is broadened, the reverse is true for B(Phuket). These broad patterns observed in the reverse cumulative frequency curves are explored in more detail.

3.6.3 Geometric mean titres (GMT) and geometric mean ratios (GMR)

Pre and post vaccination GMTs for A(H1N1)pdm09, A(H3N2) and B(Phuket) by HAI and VMN assays, along with GMRs are summarized in *Table 9* and *Table 10*.

Inter and intra-assay GMTs were compared with Wilcoxon signed rank test and are presented with an effect size (*r*). There was no statistically significant difference in GMTs ($p>0.05$) from pre to post-vaccination for either A(H1N1)pdm09 or A(H3N2), and GMRs were close to 1 for both viruses by both assays. However, post-vaccination GMTs for B(Phuket) were lower than pre-vaccination by both HAI (15.17 v 11.89, r=-0.32,

p=0.017) and VMN (6.98 v 6.25, r=-0.32, p=0.018). This is reflected in an HAI GMR of 0.78 and VMN GMR of 0.89.

GMTs by VMN were higher than by HAI for A(H1N1) both pre-vaccination (16.82 v 12.56, r=0.30, p=0.024) and post vaccination (16.41 v 11.45, r=0.34, p=0.011). Similarly, for A(H3N2) GMTs both pre (129.64 v 11.46, r=0.58, p=<0.001) and post vaccination (118.88 v 11.60, r=0.59, p=<0.001) were higher. However, for Influenza B GMTs were lower by VMN than HAI both pre (6.98 v 15.17, r=-0.44, p=0.001) and post vaccination (6.25 v 11.89, r=-0.37, p=0.005).

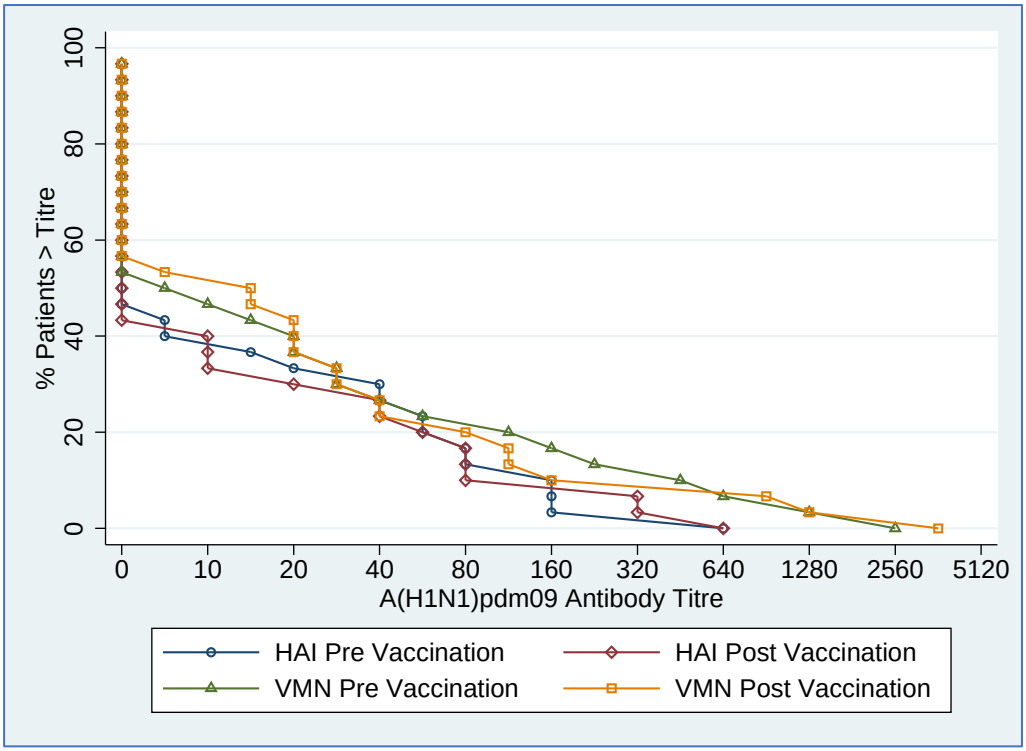

Figure 20: Reverse cumulative frequency distribution of A(H1N1)pdm09 Ab titres (Log10 transformed)

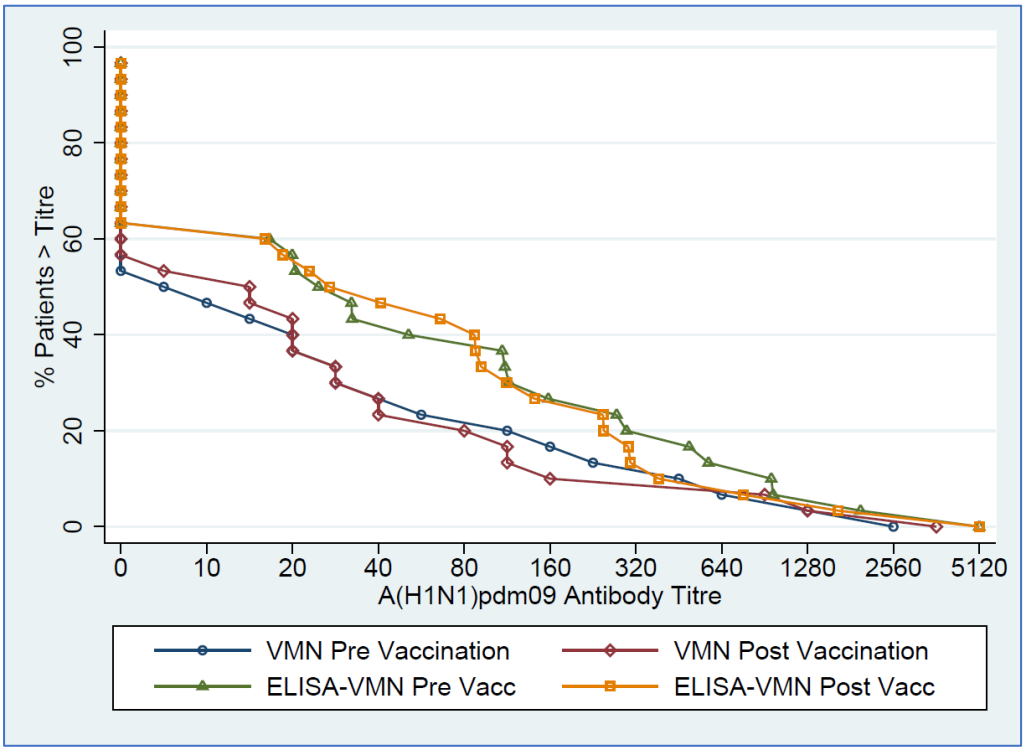

Figure 21: Reverse cumulative frequency distribution of B(Phuket) Ab titres (Log10 transformed)

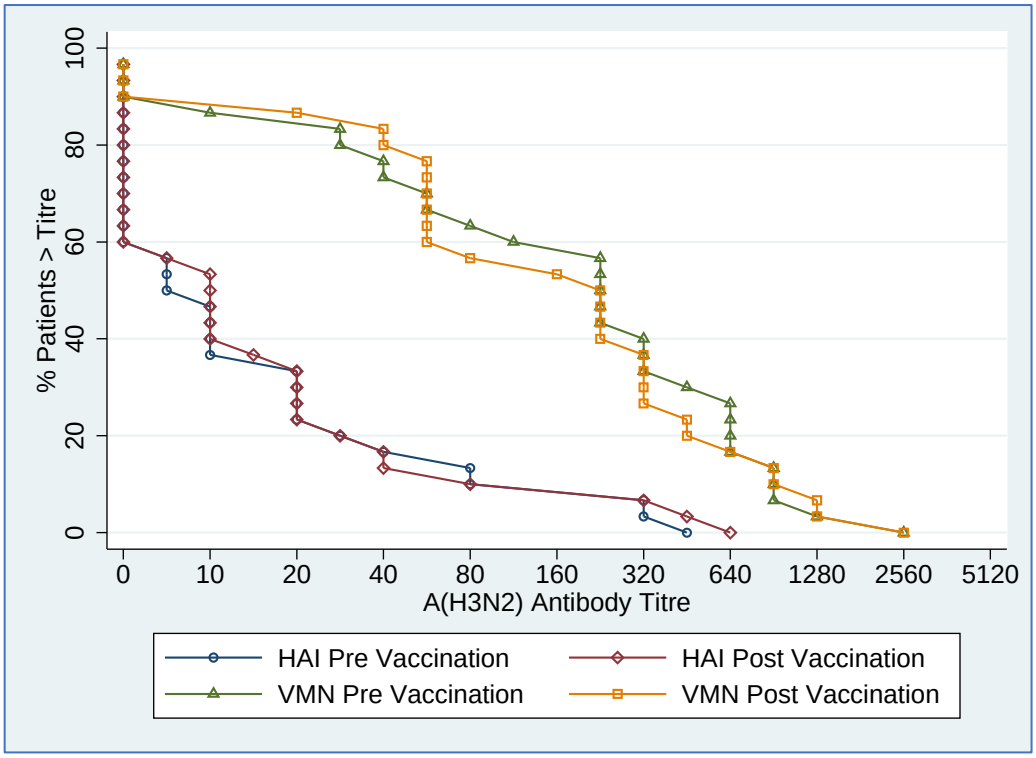

Figure 22: Reverse cumulative frequency distribution of A(H3N2) Ab titres (Log10 transformed)

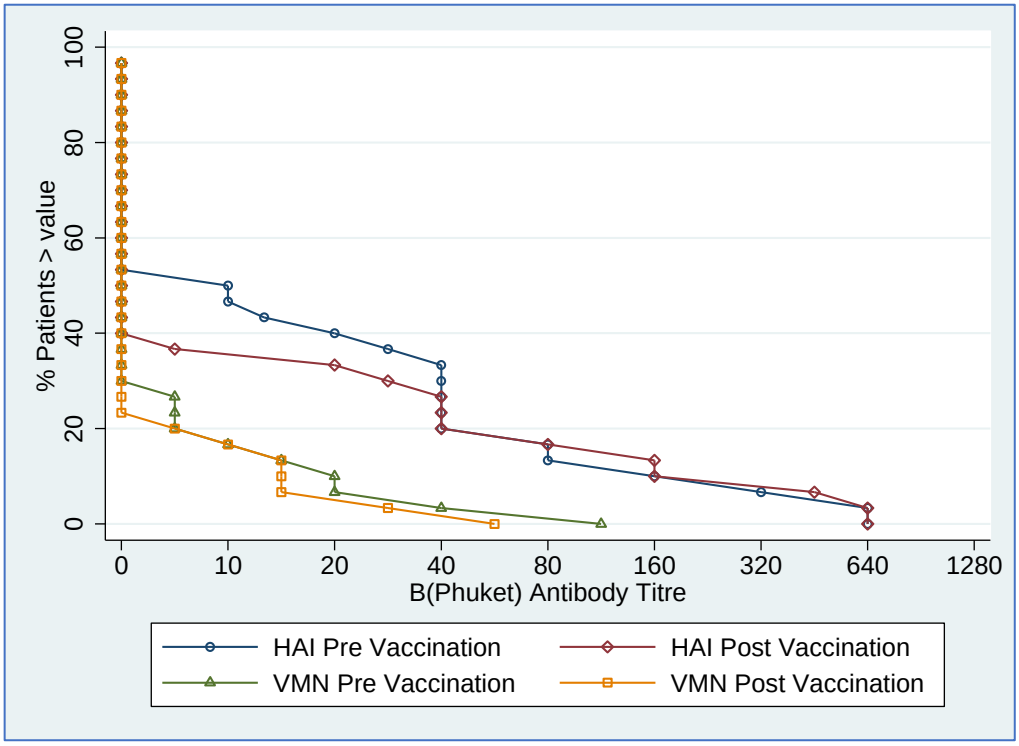

Figure 23: Reverse cumulative frequency distribution of B(Phuket) Ab titres (Log10 transformed)

Influenza strain	HAI GMT			VMN GMT			GMR		
	Pre-Vaccination	Post-Vaccination	*r* (p value)	Pre-Vaccination	Post-Vaccination	*r* (p value)	HAI	VMN	r (p value)
A(H1N1)pdm09	12.65	11.45	*-0.22* (0.093)	16.82	16.41	-0.09 (0.484)	0.91	0.98	*0.21* (0.270)
A(H3N2)	11.46	11.60	*0.07* (0.610)	129.64	118.88	-0.14 (0.277)	1.01	0.92	*-0.08* (0.968)
B/Phuket	15.17	11.89	*-0.32* (0.017)	6.98	6.25	-0.32 (0.018)	0.78	0.89	*0.16* (0.384)

Table 9: GMTs and GMRs for A(H1N1)pdm09, A(H3N2) and B(Phuket) by HAI and VMN assays. P values are derived from Wilcoxon Signed Rank test comparing: HAI pre and post vaccination GMTs; VMN pre and post vaccination GMTs; and HAI and VMN GMRs.

A(H1N1)pdm09	GMT		GMR (95% CI)
	Pre-Vaccination	Post-Vaccination	
ELISA-VMN	34.43	32.87	0.95
VMN	16.82	16.41	0.98
R (p value)	0.48 (<0.001)	0.48 (<0.001)	0.06 (0.647)

Table 10:GMT and GMR for A(H1N1)pdm09 by VMN and ELISA-VMN. P values are derived from Wilcoxon signed-rank test comparing VMN and ELISA-VMN pre- and post-GMTs and GMR

3.6.4 Rates of seroconversion (SC) and low-titre seroresponse (LTS)

Rates of seroconversion (SC) and low-titre seroresponse (LTS) are summarised in *Table 11*. A single seroconversion was detected by E-VMN in a patient vaccinated at 0-3 months post HSCT. This same patient had a low-titre seroresponse by VMN. More low-titre seroresponses were detected by VMN than HAI for A(H1N1)pdm09 (14.3 versus 7.1%) and A(H3N2) (28.6 versus 14.3%).

Vaccine component	Assay	Low- titre seroresponse*, n (%)	Seroconversion**, n(%)
A(H1N1)pdm09	HAI	2 (7.1)	0
	VMN	4 (14.3)	0
	E-VMN	2 (7.1)	1 (3.6)
A(H3N2)	HAI	4 (14.3)	0
	VMN	8 (28.6)	0
B(Phuket)	HAI	1 (3.6)	0
	VMN	0	0

*Ab titre <10 pre-vaccination to positive post-vaccination but not meeting criteria for seroconversion OR <fourfold increase in titre
** Ab <10 pre-vaccination to ≥40 post vaccination OR ≥ fourfold increase in titre from pre to post vaccination

Table 11: Low titres seroresponse and seroconversion rates in n=28 participants

Low titres seroresponses by HAI and VMN are displayed by vaccination timepoint in *Table 12*. Of note, only 1 LTS was congruent across HAI and both VMN assays in a patient vaccinated at 6-12 months. For A(H3N2) all low-titre seroresponses were incongruent across the two assays.

Vaccine component	Vaccination Timepoint	HAI low-titre seroresponse, n (%)	VMN low-titre seroresponse, n(%)	
			VMN	E-VMN
A(H1N1)pdm09	<3 (n=15)	0	3 (20)	1 (6.7)
	3-6 (n=6)	1 (16.7)	0	0
	6-12 (n=7)	1 (14.3)	1 (14.3)	1 (14.3)
	Total	2 (7.1)	4 (14.3)	2 (7.1)
A(H3N2)	<3 (n=15)	1 (6.7)	5 (33.3)	
	3-6 (n=6)	3 (50.0)	0	
	6-12 (n=7)	0	3 (42.9)	
	Total	4 (14.3)	8 (28.6)	
B(Phuket)	<3 (n=15)	1 (6.7)	0	
	3-6 (n=6)	0	0	
	6-12 (n=7)	0	0	
	Total	1	0	
≥ 1 Strain	<3 (n=15)	2 (13.3)	7 (46.7)	
	3-6 (n=6)	3 (50)	0	
	6-12 (n=7)	1 (14.3)	4 (57.1)	
	Total	6 (21.4)	11 (39.3)	

Table 12: Low titre seroresponse by months from HSCT. A(H1N1)pdm09 LTS congruent across all 3 assays highlighted

There was no statistically significant association between vaccination timepoint and LTS rate for HAI (χ^2=3.705, p=0.196) or VMN (χ^2 =5.161, p=0.76).

3.6.5 Rates of HAI titre ≥ 40

Rates of pre and post-vaccination titres ≥ 40 by HAI assay are shown in *Table 13*. Frequency of titres ≥ 40 to individual vaccine components are low, ranging from 14.3% for A(H3N2) to 32.1% for B(Phuket). For both A(H1N1)pdm09 and A(H3N2), the frequency of titres ≥ 40 fell from pre to post vaccination, and in all cases, this occurred in patients vaccinated at <3 months post HSCT. Frequency of titre ≥ 40 to any 1 or more vaccine component were 50% both pre and post-vaccination.

Vaccine Component	Vaccination timepoint	Pre-Vaccination, n (%)	Post-Vaccination, n (%)	Difference Post-Pre, n(%)
A(H1N1)pdm09	<3 (n=15)	5 (33.3)	4 (26.7)	-1 (-6.7%)
	3-6 (n=6)	2 (33.3)	2 (33.3)	0
	6-12 (n=7)	1 (14.3)	1 (14.3)	0
	Total	8 (28.6)	7 (25.0)	-1 (-3.6%)
A(H3N2)	<3 (n=15)	2 (13.3)	2 (13.3)	0
	3-6 (n=6)	1 (16.7)	1 (16.7)	0
	6-12 (n=7)	1 (14.3)	1 (14.3)	0
	Total	4 (14.3)	4 (14.3)	0
B(Phuket)	<3 (n=15)	6 (40.0)	4 (26.7)	-2 (-13.3%)
	3-6 (n=6)	1 (16.7)	1 (16.7)	0
	6-12 (n=7)	2 (28.6)	2 (28.6)	0
	Total	9 (32.1)	7 (25.0)	-2 (-7.14%)
≥1 Strain	<3 (n=15)	9 (60.0)	9 (60.0)	0
	3-6 (n=6)	3 (50.0)	3 (50.0)	0
	6-12 (n=7)	2 (28.6)	2 (28.6)	0
	Total	14 (50)	14 (50)	0

Table 13: Rates of pre and post vaccination titres ≥40 by vaccination time point

The rate of Ab titre≥40 to any 1 or more virus strain appears to decline with longer time from transplant (60% at <3 months, 50% at 3-6 months, and 28.6% at 6-12) months however this was not statistically significant (χ^2=1.886, p=0.390).

3.6.6 Negative HAI / Positive VMN status as a predictor of HAI titre ≥ 40

As rates of HAI titre ≥ 40 did not increase from pre to post-vaccination, and there were no serconversions, it was not possible to evaluate whether negative HAI/Positive VMN status at baseline predicted these two outcomes. For A(H1N1)pdm09, 3(10.7%) patients had negative (<10) pre-vaccination titres by HAI, but were positive by VMN. 7 (25%) patients had negative pre-vaccination titres by HAI but were positive by E-VMN. For A(H3N2) there were 12(42.9%) HAI negative/VMN positive patients at baseline, and for

B(Phuket) 0. HAI negative/VMN positive status did not predict LTS by HAI for either A(H1N1)pdm09 (VMN: χ^2=3.476, Fisher's Exact p=0.206), (E-VMN: χ^2=6.462, Fisher's Exact p=0.056) or A(H3N2) (χ^2=0.266, Fisher's Exact p=0.625).

3.6.7 Factors influencing immunogenicity

3.6.7.1 Seroprotection, seroconversion and minimal seroresponse

None of the categorical variables diagnosis, donor type (VUD v Sibling donor), lymphocyte depleting agent (Alemtuzumab v ATG), months from HSCT to vaccination (either categorised as <3, 3-6, 6-12 or dichotomised as <3 v >3 month, and <6 v >6 months), GvHD at vaccination, Immunosuppressive therapy at vaccination, Intravenous Immunoglobulin within last 12 months, or rituximab within last 12 months were associated with pre or post vaccination HAI titre ≥ 40, or low-titre to seroresponse rates by HAI or VMN (P>0.05 in all cases by Pearson's Chi-Square or Fisher's Exact test). Neither was there an association between these outcome variables and the continuous explanatory variables age at transplant, lymphocyte count at vaccination, and days from HSCT (P>0.05 in all cases by binary logistic regression). As only 1 seroconversion was detected by E-VMN further statistical analysis was not possible for this dependent variable.

3.6.7.2 Post vaccination Antibody titres

Post-vaccination A(H1N1)pdmo09, A(H3N2, and B(Phuket) Ab titres were not correlated with any of the categorical (P>00.5 in all cases by Mann-Whitney U Test) or continuous variables (P>0.05 in all cases by Spearman's rank correlation) given above.

3.6.8 Relationship between HAI and VMN titres

The relationship between log10 transformed HAI and VMN titres was explored using a linear regression model. Statistically significant correlation was observed between HAI and VMN for A(H1N1)pdm09 (β=1.139, 95%CI 1.021-1.255, p<0.001), A(H3N2) (β=0.871, 95%CI 0.533-1.209, P<0.001) and B(Phuket) (β=0.396, 95%CI 0.322-0.47, P<0.001). There was also statistically significant correlation between A(H1N1)pdm09 HAI and E-VMN (β=1.319, 95%CI 1.145-1.493, p<0.001). Using the linear regression equation and 95%CI for β, VMN titres equivalent to an HAI 40 with 95%CI ranges were calculated. Equations and equivalent titres are shown in *Figure 24* to *Figure 27*.

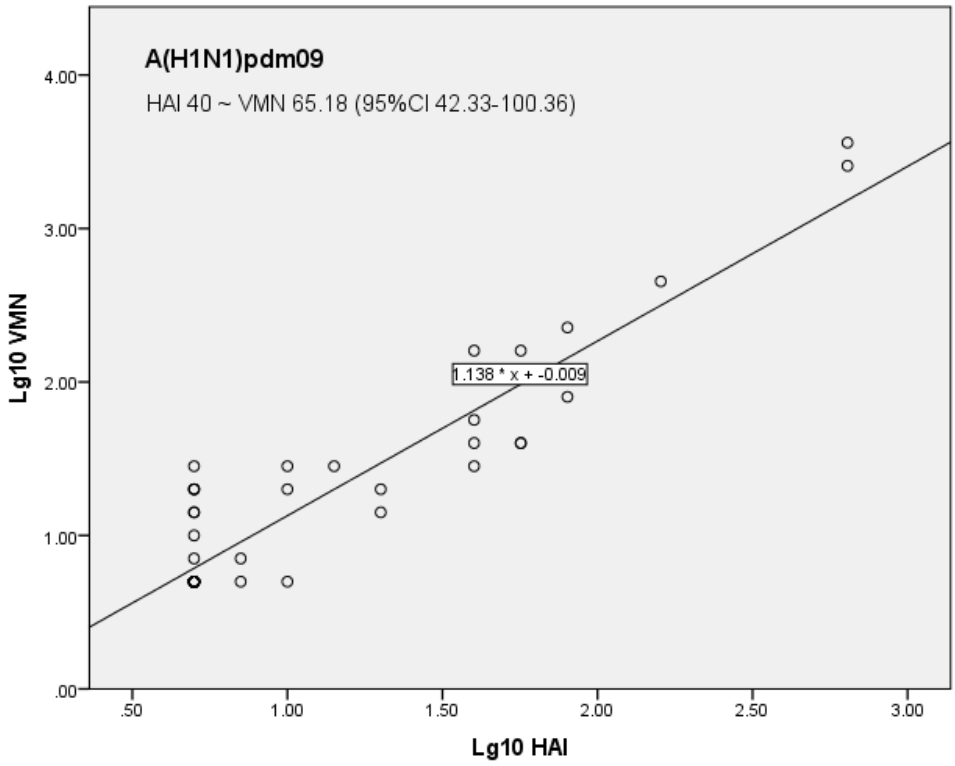

Figure 24: A(H1N1)pdm09 Lg10 transformed HAI and VMN. HAI 40 ~ VMN 65.18

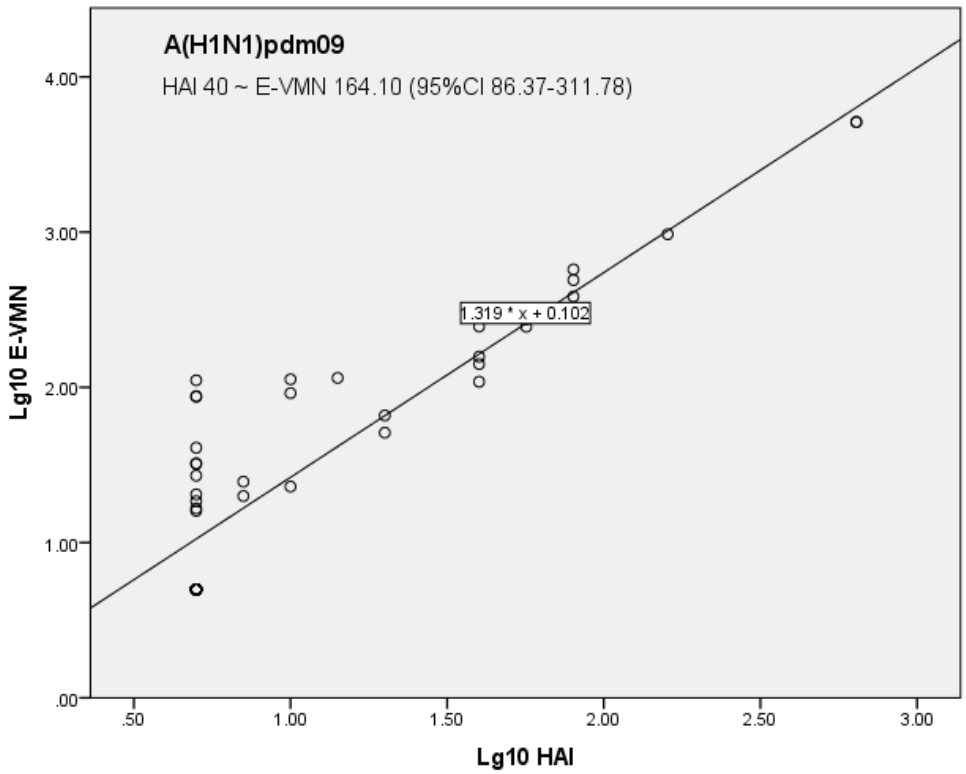

Figure 25: A(H1N1)pdm09 Lg10 transformed HAI and VMN. HAI 40 ~ E-VMN 164

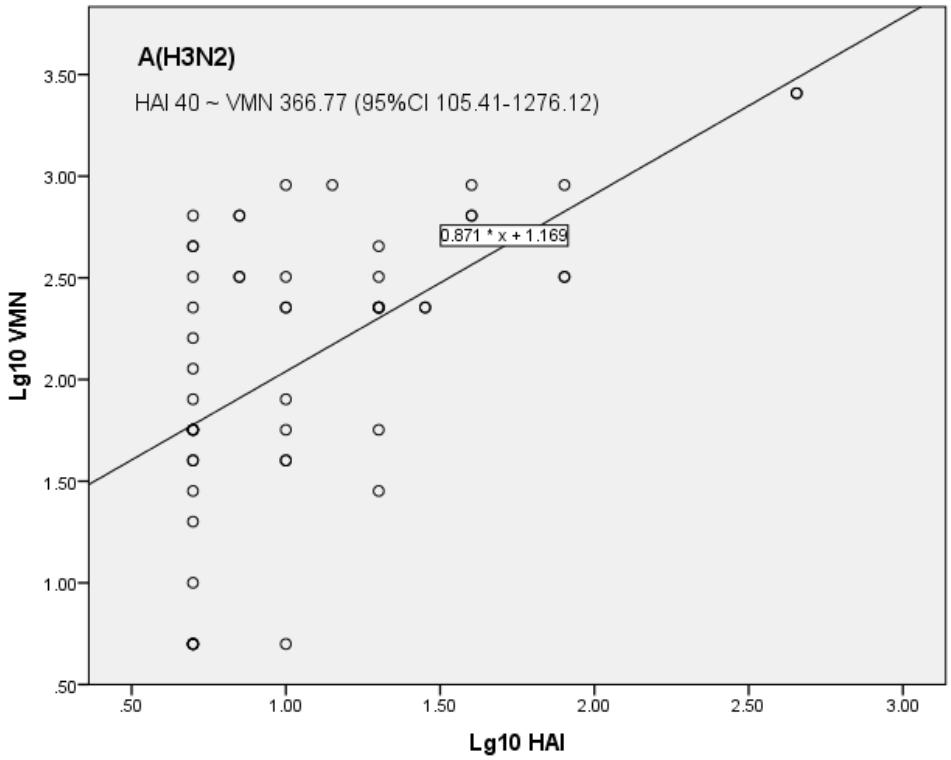

Figure 26 : A(H3N2) Log10 transformed HAI and VMN titres. HAI 40 ~ VMN 366.77

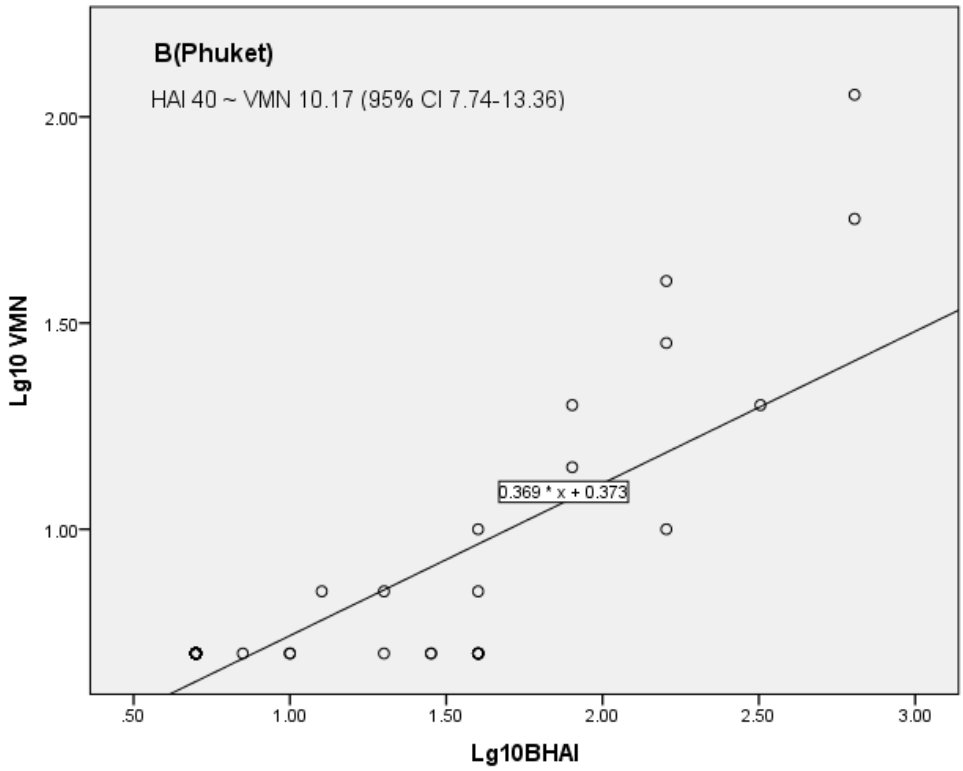

Figure 27: B(Phuket) Log10 transformed HAI and VMN titres. HAI 40 ~ VMN 10.17

3.7 Discussion

In this pilot study, the immunogenicity of the 2015-2016 seasonal IIV was evaluated in HSCT recipients using the HAI and VMN assay. This is the first study to report VMN data in this patient group. The study population was homogenous with regard to HSCT procedure: All patients received RIC regimens and PBSCs with in-vivo lymphocyte depletion. Furthermore, all patients were within the first 12 months of HSCT at time of vaccination.

GMTs for A(H1N1)pdm09 and A(H3N2) determined by VMN were statistically significantly higher than by HAI. For A(H1N1)pdm09 two VMN assays were compared; a 72-hour assay with a CPE endpoint, and a 24 hour assay with ELISA endpoint. In this study, the estimated VMN equivalent of an HAI titre of 40 was 65.18 (95% CI 42.33-100.36) for the CPE endpoint, and 164 (95% CI 86.37-311.78) for the ELISA endpoint. Previous studies in a paediatric and a healthy adult population estimated that ELISA VMN titres of 200 and 211 respectively, were equivalent to an HAI titre of 40 (77,203). The paediatric study evaluated Ab to the H1N1(A/Brisbane/59/2007) strain, and the adult study the A(H1N1)pdm09 strain. The same paediatric study estimated that a VMN titre of 140 was equivalent to an HAI titre of 40 for H3N2(A/Brisbane/10/2007). In a small study of patients infected (rather than vaccinated) by H3N2(A/SouthAfrica/114/95/7) GMTs by HAI and ELISA VMN were 29.19 and 362.98 respectively. Based on reported results, the estimated equivalent of an HAI titre of 40 was a VMN titre of 480(193). The comparative HAI and VMN titres in this present study

are consistent with previous research findings. A(H3N2) viruses have been dominant circulating strains and a component of the seasonal IIV since at least 1998 (204), while the A(H1N1)pdm09 virus is by definition antigenically dissimilar to H1N1 strains predating 2009. The presence of cross-reacting neutralizing Ab to A(H3N2) may explain why the titres by VMN were markedly higher than for the more recent A(H1N1)pdm09 virus.

Unexpectedly, for B(Phuket) the VMN assay GMT was statistically significantly lower than the HAI titre. The estimated equivalent VMN titre of HAI 40 was 10.17 (95% CI 7.74-13.36). A previous study comparing HAI and VMN reported increased rates of seroconversion by VMN compared with HAI although GMTs were not reported for direct comparison(191). The HAI assay has previously displayed poor sensitivity to some influenza strains (205). PHE Respiratory Virus Reference Department experience with 2015-2016 B(Phuket) virus indicated very poor HAI sensitivity with unexpected negative results in routine testing of samples (personal communication, Dr Katja Hoschler). Therefore egg-grown B(Phuket) virus used in reference laboratory testing was ether treated to improve HAI sensitivity. Ether treatment cleaves the virion and increases Ab binding sites(156,205) however the virion is rendered unable to replicate and therefore unsuitable for use in VMN assay. In this study, ether treated B(Phuket) was used for the HAI assay, and non-ether treated for the VMN. One may therefore hypothesise that the VMN would have compared more favourable against HAI using non-ether treated virus, however this was not assessed as part of the study.

In this study population, seroconversion by HAI was completely absent for all 3 vaccine components. A single seroconversion to A(H1N1) was detected by E-VMN in patient who was administered IIV at 0 to 3 months. This patient also had the highest detected low-titre seroresponse by VMN (<10 to 20) but falling below seroconversion criteria. Absence of seroconversion by HAI was an unexpected finding, as previous studies have reported modest rates. Pauksen and colleagues observed seroconversion rates by HAI to IIV administered in the first 12 months post HSCT of 31% for A(H1N1, 9% for A(H3N2), and 20% for influenza B. Conditioning intensity, HSC source and use of lymphocyte depletion were not reported (161). Karras and colleagues found seroconversion rates of 0% to A(H3N2), 6% for A(H1N1) and 8% for Influenza B(92). In this study 44.6% of patients received RIC and the remainder MAC and this was not identified as factors predictive of seroconversion. None of the participants in this latter study received lymphocyte deplete grafts. In a study of BMH alloHSCT patients who universally received lymphocyte deplete grafts, there were no seroconversions within the first 6 month post HSCT(91). Although the homogenous population does not allow for analysis, it may be the case that universal in-vivo lymphocyte depletion in this population impacted on immunogenicity. As all were recipients of RIC, the median age in our study was 57.8, compared with 40.8(92) and 21(91) in the studies above. Older age is associated with poorer influenza vaccine immunogenicity in the general population and this may have been a contributing factor to poor response in this study population(206).

Despite vaccination, rates of titre ≥ 40 by HAI were stable from pre to post vaccination for A(H3N2) and fell for A(H1N1) and B(Phuket). Baseline seroprotection rates were 28.6% for A(H1N1)pdm09, 14.3% for A(H3N2), 32.1% for B(Phuket) and 50% to any 1 or more strain. In an immunogenicity study of the monovalent A(H1N1)pdm09 vaccine, Issa and colleagues reported seroresponse rates to the study vaccine, but also HAI titres ≥ 40 to the seasonal influenza strains(164). These ranged from 20.7% for Influenza B, and 57.4% for A(H3N2). However, these patients were evaluated at 2.5 to 92.7 months post HSCT, and some had received the seasonal IIV in previous post-HSCT influenza seasons. In contrast, patients in this current study were all seasonal IIV naïve following HSCT. Other studies have reported baseline seroprotection rates to Influenza A and B of 12-16% (161) and 0-29% (163). Thus. baseline rates in this study population are comparable with previously published data. Pre-vaccination rates of HAI titre ≥ 40 fell with time from HSCT (60% at 0-3 months, 50% at 3-6 months, 28.6% at 6-12 months) and this is consistent with previous studies that have reported a waning of disease specific Abs within the first-year post HSCT. Our findings, however, were not statistically significant.

Low titre seroresponse (LTS) has not been correlated with clinical outcome and has not previously been reported. This reflects the minimum response detectable by serological techniques (i.e. 1-fold change in titre) and it must be acknowledged that this falls within the margin of error of such assays (a 1-fold difference in control values being acceptable) and the poor correlation between assays would attest to this. In this study rates of LTS were higher by VMN than HAI but low overall (7.1-14.3% for HAI; 14.3

to 28.6% for VMN). There was no correlation with time from transplant possibly suggesting that even this minimum detectable response is impaired throughout the first 12 months.

None of the evaluated patient characteristics correlated with seroresponse measures or with GMT or GMRs. As a pilot study the sample size was small, and this was compounded by absent and low measures of seroresponse. Neither active GvHD nor concomitant IST correlated with post vaccination HAI titre ≥ 40. The association between IST, GvHD and response to influenza vaccination has not been reported consistently. Our findings are consistent with previous studies reporting low response by HAI in the first 12 months. While Karras and colleagues suggest that equivalent seroconversion rates to 1 or more strains at 2-6 and 6-12 months (12 % v 30% p=0.43) may justify earlier vaccination, our findings of almost entirely absent humoral response throughout the first year would argue against this strategy in RIC PBSC lymphocyte deplete alloHSCT recipients. On the other hand, our study of course did not evaluate whether GMTs, GMRs and rates of HAI titre >40 would have dropped significantly without vaccination, which may have played a role in maintenance of response.

3.7.1 Study Strengths and Weaknesses

3.7.1.1 Study Population

The study was conducted in a homogenous population with regard to HSCT procedure. Inclusion criteria were broad with regard to GvHD, IST and previous serotherapy, and

participants were vaccinated as part of standard care at their respective HSCT programmes. While it is not possible to extrapolate our findings to other HSCT sub-populations (e.g. autologous, BMH or UCB alloHSCT, non-lymphocyte deplete), it does point towards poor seroresponse to IIV in this specific population. Although as the sample size for this pilot study is small, results should be interpreted cautiously, and we would not recommend change in practice based on current findings. Vaccination timepoint was not evenly distributed over the first year, with 50% of recipients vaccinated within the first 3 months. Based on immune reconstitution patterns low responses at this time point are to be expected and this may have contributed to the lower rates of seroresponse than previously reported. Although, there was no difference between 0-3, 3-6 and 6-12 months, and the only seroconversion detected at any timepoint was in the 0-3-month group. Study participants were all seasonal IIV naïve from point of HSCT, however pre-HSCT vaccination status was not reported, and we did not have access to pre-HSCT serum samples to determine change in Ab status from pre to post HSCT. Due to sample size, it would not have been meaningful to correlate clinical outcome data with immunological data, and therefore this was not undertaken.

3.7.1.2 Laboratory Procedures

Correlations between HAI and VMN assays estimated in this study are consistent with previous studies in other population groups. For VMN, it was not possible to select low passage MDCK cells as recommended by WHO, however all runs passed Public Health

England quality control criteria, with acceptable control values and back titrations. Neither the HAI nor the VMN have been standardized internationally, and inter-laboratory variability is a recognized issue. Due to timing and funding constraints it was not possible to establish an ELISA endpoint for A(H3N2) and B(Phuket). The A(H1N1)pdm09 E-VMN appears more sensitive the CPE endpoint VMN, and this assay detected the only seroconversion. It may be that E-VMN would have yielded additional seroresponse data for A(H3N2) and B(Phuket).

3.8 Conclusion

In conclusion, this study has shown almost complete absence of seroresponse to seasonal trivalent IIV administered in the first-year post HSCT in a small cohort of RIC PBSC alloHSCT recipients who underwent in-vivo lymphocyte depletion. This is the first study to use the VMN assay to assess immunogenicity of seasonal IIV in HSCT recipients. In other study populations, this assay has proven more sensitive with higher GMTs than by HAI, and also the detection of higher rates of seroconversion. The correlations between HAI and VMN titres estimated in this study are similar to those reported previously in immunocompetent adult and paediatric cohorts, and GMTs were statistically significantly higher than HAI by VMN and E-VMN for A(H1N1) and VMN for A(H3N2). Despite this, the E-VMN detected only a single seroconversion to A(H1N1) below the threshold of detection by HAI. It seems that a single dose of seasonal TIIV is minimally immunogenic in this study population. There is a clear need for novel

vaccination schedules and vaccine formulations in this patient group, particularly in the first-year post HSCT, and it may be that the VMN provides useful immunological data in this context.

4 The impact of seasonal influenza infection and vaccination health beliefs on vaccination intent amongst adult recipients of autologous and allogeneic haematopoietic stem cell transplant

4.1 Introduction

As reviewed in Chapter 1, all components of the innate and adaptive immune system are impaired for months to a year or more post HSCT. As part of this broad immunosuppression, antibody titres to VPDs decline from weeks to months post HSCT, and this may continue for years without revaccination. In particular, HSCT recipients are at risk from invasive pneumococcal disease(207), and have a high morbidity and mortality associated with the influenza virus(208). Current international guidelines recommend that after autologous and allogeneic HSCT, recipients should be regarded as 'never vaccinated' and receive a full schedule of revaccination (100,209).

4.2 Post HSCT Vaccination in Context

Recipients of HSCT may experience numerous post-transplant complications. Significant complications common to auto and alloHSCT include toxicity from

conditioning chemotherapy, marked immunosuppression, recurrence of primary disease, secondary malignancy and psychosocial consequences of such intensive treatment. This latter group of complications includes fatigue, anxiety and depressive symptoms, and post-traumatic stress disorder(210). Other long-term complications contributing to morbidity and mortality are related to impaired immunity and dysregulated immune reconstitution including infection and autoimmune complications. Unique to alloHSCT is the alloreactivity between donor immune system and host tissue that is defined clinically as acute and chronic GvHD. GvHD can cause multisystem dysfunction and is an immunosuppressive process. As a result of such post-transplant complications, recovery is rarely straightforward and may be marked by periods of acute and chronic ill-health requiring specialist treatments and hospital admission(211).

This complex recovery pathway, from both the clinician and patient perspective, and the recommendation of a full re-vaccination schedule, may contribute to unique healthcare beliefs and barriers to vaccination. Few studies have sought specifically to describe barriers to vaccination among this group, and none have investigated how health beliefs influence HSCT recipients' vaccination intent (See 1.13). Understanding barriers to vaccination and the factors that influence a patient's vaccination intent and uptake, is considered fundamental to developing a successful vaccination program(212).

It is recognized that vaccination intent is vaccine and context specific(213) so it is rational for a health belief study to focus on a single vaccine rather than vaccines and vaccination in general. As referenced above, HSCT recipients are at particular risk from seasonal influenza infection, and the influenza vaccine is unique in that it is administered annually. Therefore, this study will focus on health beliefs surrounding the seasonal influenza virus, and the seasonal inactivated influenza vaccine (SIIV).

4.3 Seasonal Influenza Vaccination rates post-HSCT

Based on a small number of single centre studies the post-HSCT influenza vaccination rate in HSCT recipients is around 60-70% in the first 2 years(104,105). In the 2015-2016 influenza season, UK influenza vaccination rates were reported at 71% in adults over 65 and 55.4% in immunosuppressed adults under 65(214). The NHS England target for the 2016-2017 influenza season is 75% for adults aged over 65, and at least 55% in all under 65 high risk groups(215). So, based on limited data, reported vaccination rates in HSCT recipients are in keeping with the influenza vaccination rate of patients aged over 65 in the UK, and exceeds the rates amongst immunocompromised adults. However, HSCT recipients are vulnerable to seasonal influenza and its complications, and it is desirable to continue to improve and maximize vaccination rates.

4.4 Factors Influencing Vaccine Uptake in other Patient Groups

A number of published studies have investigated influenza vaccine uptake amongst specific patient groups. These groups include older adults(216–219), high-risk patients (220,221) including oncology patients(222) and those with secondary immunodeficiency(223), Healthy adults in the workplace(224–228), Healthcare workers(229–234), and pregnant women(235–240). Following the 2009 H1N1 pandemic a number of studies investigated uptake of the H1N1 vaccine(241–247).

Studies from the USA and UK investigating the groups above have identified common themes in patient social and psychological factors that influence IIV intent and uptake. Key themes that emerge are as follows:

1. Social Influence
    a) Recommendation from a healthcare professional (248–265)
2. Disease-Related Factors
    a) Perceived susceptibility to influenza (250,252,254,256,259,260,264,266–270)
    b) Perceived severity of influenza (250,253,256,258,259,264)
    c) Anticipated regret associated with contracting influenza if not vaccinated(255,267,271)(250,251,258,259,264)

3. Vaccine Related Factors
    a) Perceived vaccine effectiveness(251,252,255,256,258–261,263,264,266,268,269,271,272)
    b) Perceived or previously experienced side effects(250,253,256,258,259,262,268,269,272–277)
    c) Perception that vaccine causes influenza or may worsen pre-existing condition(249–254,258–260,262,270,272,273)
4. Past behaviour - previous vaccination against influenza(251,252,255,258,259,262,264,268,271)
5. Knowledge - awareness of recommendation/need to receive influenza vaccine(250,260,270,275,276,278)
6. Altruism - protecting others(258,267)

Many of these factors influencing vaccination intent and uptake will be relevant to HSCT recipients to a greater or lesser extent. However, post HSCT care is a unique and complex clinical situation and it is possible that particular influences will dominate, and unique influences may exist. A better understanding of the patient beliefs that influence vaccination intent post HSCT may allow the development of targeted strategies and interventions that address concerns specific to this group, which may in turn contribute to improved vaccine uptake.

4.5 Theoretical Framework for Investigating Vaccination Intent

Theories of health behaviour are largely predicated on the principle that the antecedent of a health behaviour is an individual's intent towards that behaviour. Additional variables will clearly impact on whether the behaviour is ultimately carried out, but intent is considered a key predictor of health behaviour(279,280). The Health Belief Model (HBM)has been used in various healthcare contexts to investigate why people engage in behaviours to prevent, screen for or diagnose disease(281). The HBM is founded on the theory that three key factors determine intent and behaviour: 1) An individual is motivated to undertake a behaviour, for example due to a health concern, 2) an individual perceives themselves to be at risk from a particular condition or outcome, and 3) a given behaviour will reduce risk, and balances favourably in the individual's cost-benefit analysis(282). These broad determinants are further dissected to give 5 measurable constructs: 1) perceived risk of illness, 2) perceived severity of illness, 3) perceived effectiveness or benefits of intervention, 4) perceived barriers to intervention, and 5) and cues to action. These constructs are considered among the most important predictors of influenza vaccination, and in particular perceived risk from and severity of disease are reliably associated with vaccination uptake(283). Since the conception of the HBM in 1950's, additional modifying constructs have been proposed. Self-efficacy, or an individual's perceived ability to successfully bring about an outcome is considered an important modifier of the HBM(284). In addition to the cognitive constructs of the HBM, emotional constructs are considered important in predicting behaviour. In particular, worry about disease has been identified as a factor that modifies the impact of perceived risk on vaccination behaviour: a patient may perceive themselves to be at risk, but unless this is something that worries them they

may not engage in a preventative behaviour(271). Furthermore, anticipated regret of illness if a health behaviour is not performed is also considered a predictor of intent(285).

4.6 Framework for Investigating Vaccination Intent in HSCT Recipients

A framework for investigating vaccination intent amongst HSCT recipients was adapted from the themes outlined in 4.4, and the HBM and modifying constructs outlined in 4.5 *(Figure 28*).

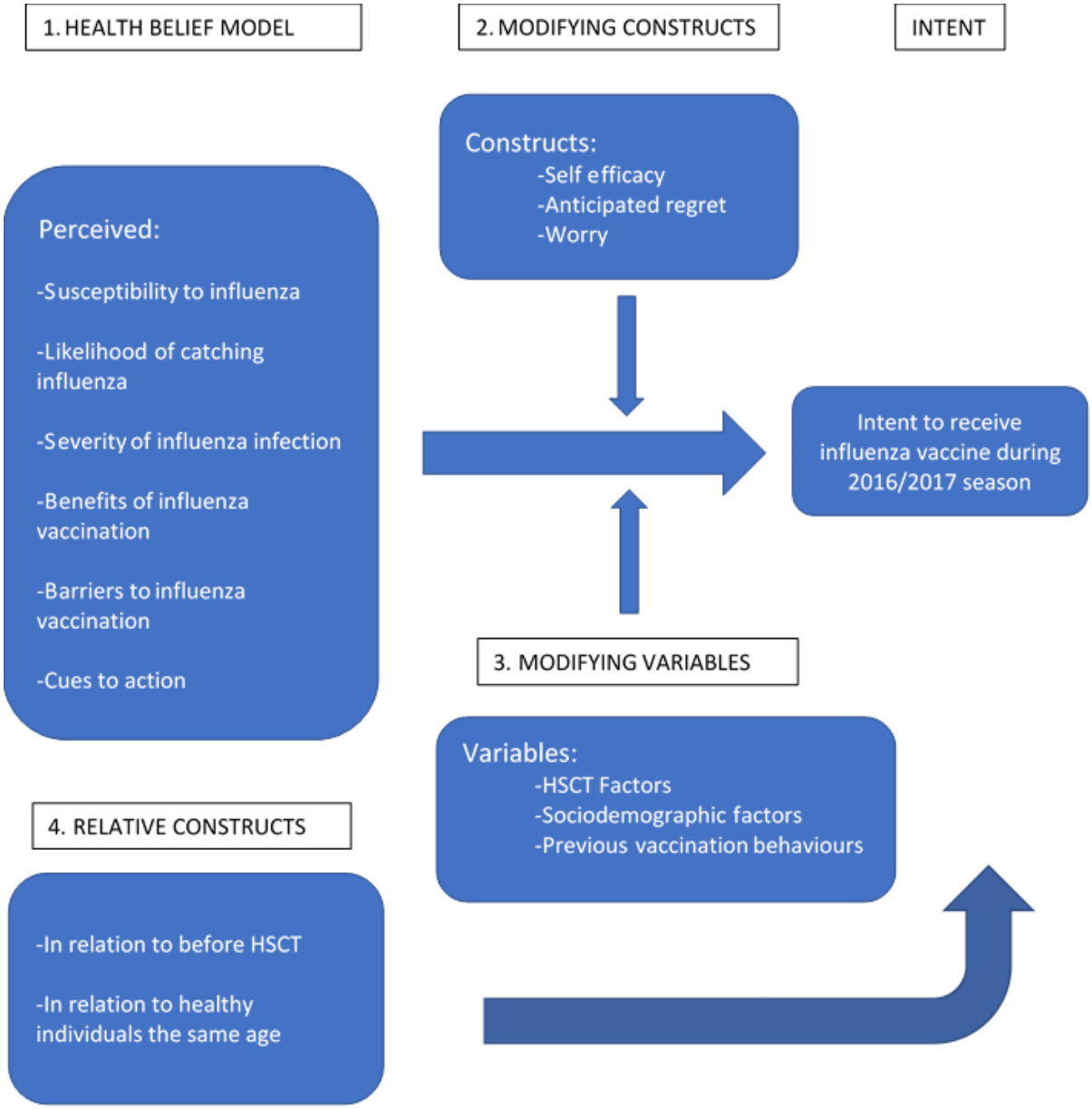

Figure 28: Framework for investigating vaccine intent in HSCT recipients. The constructs of the HBM (1) were investigated as predictors of vaccination intent among HSCT recipients. Modifying constructs (2) were investigated as independent predictors of intent and then significant constructs were incorporated into the HBM to form a modified HBM (M-HBM). The impact of HSCT factors (e.g., HSCT type, time from HSCT, disease indication), sociodemographic factors and previous vaccination behaviour were also considered as variables modifying intent (3). Finally, a subset of the risk, severity and barrier constructs of the HBM were framed as constructs relative (4) to 'before HSCT' and to 'other healthy individuals of the same age', and investigated as independent predictors of intent.

4.7 Study Overview

Auto and alloHSCT recipients were recruited at 3 study sites between the 2015-2016, and 2016-2017 influenza seasons. Study sites were the Royal Marsden NHS Foundation Trust, St George's Hospital, St George's NHS Foundation Trust, and the Freeman Hospital, Newcastle Hospitals NHS Foundation Trust. Eligible patients were asked to complete a study specific health belief questionnaire.

The study was conducted in compliance with the study protocol, and standards of good clinical practice and regulatory requirements. All necessary local NHS research and development approvals were obtained for each centre before patient recruitment began.

See Appendix 6 for patient information sheet, consent form and notice of research ethics committee approval.

4.7.1 Study Sponsorship, Insurance and Ethical Approval

The study was sponsored and insured by University College London (UCL). Ethical approval for the study was granted on 15 March 2016 by the NHS Health Research Authority (HRA) National Research Ethics Service (Appendix 6)

4.7.2 Study Aims, Hypothesis and Objectives

4.7.2.1 Study Aim

The aim of this study was to investigate the potentially unique factors that may influence SIIV vaccination intent in adult recipients of auto and alloHSCT using a questionnaire based on the health belief framework outlined in 4.6.

4.7.2.2 Study Hypothesis

There is an association between seasonal influenza and SIIV healthcare beliefs and vaccination intent in adult recipients of autologous and allogeneic haematopoietic stem cell transplant.

Individuals who perceive that their transplant has placed them at higher risk from seasonal influenza virus will be more likely to intend to receive the vaccine compared to those who do not perceive themselves to be at higher risk following their transplant.

Individuals who perceive themselves to be at higher risk from seasonal influenza virus relative to healthy individuals of the same age will be more likely to intend to receive

the vaccine, compared to those who do not perceive themselves to be at higher risk relative to healthy individuals of the same age.

4.7.2.3 Study Objectives

Primary Objective:

To describe, using a theoretical health belief framework, the barriers to vaccination perceived by recipients of allogeneic and autologous haematopoietic stem cell transplant and correlate this with vaccination intent.

Secondary Objectives:

To determine whether perceived higher seasonal influenza infection risk post-transplant predicts vaccination intent among adult recipients of autologous and allogeneic haematopoietic stem cell transplant

To determine whether perceived higher seasonal influenza infection risk relative to healthy individuals predicts vaccination intent among adult recipients of autologous and allogeneic haematopoietic stem cell transplant

To determine potential barriers to vaccination amenable to targeted intervention

4.7.3 Study Participants

Inclusion Criteria:

- Aged 16 or over
- Recipient of autologous or allogeneic HSCT using any conditioning regimen or intensity for any primary haematological disease
- No absolute contraindications to receiving the SIIV during the next flu season as per summary of product characteristics.
- Not received seasonal influenza vaccine between date of transplant and date of completion of study questionnaire
- Able to read English

Exclusion Criteria:

- Unable to read English
- Received seasonal influenza vaccine between date of transplant and date of completion of study questionnaire

4.7.4 Participant Informed Consent, Registration, Confidentiality and General Ethical Considerations

The setting for the study was the haematology outpatient department at the NHS Trusts given in section 4.7. All patients received Participant Information Sheets (PIS) and were offered the opportunity to ask any questions about the study. Patients were asked to give written consent and were advised that they could withdraw at any time from the study. Participants were advised that their decision to participate or to withdraw from the study would not impact on the care they received. Patients consenting to the study were asked to complete an anonymous health beliefs questionnaire either in the outpatient waiting area or in their own time at home, and return the questionnaire in a sealed envelope so it could not be read by either the healthcare team or site study team. All required information was recorded on the questionnaire by the participant, so further access to their healthcare records was not necessary. The investigators at the study site were responsible for maintaining patient confidentiality throughout. Study IDs were recorded on the local site log alongside local hospital number only, to ensure that participants were not approached twice. It was not necessary to inform GPs of patients' participation.

The study was conducted outside of the influenza season so as not to have any immediate impact on vaccination decisions. Participants were offered the opportunity to raise any questions or concerns raised by the questionnaire with an HSCT nurse specialist at each site.

4.7.5 Data Handling

The study specific paper-based questionnaire was completed anonymously and contained no personal details through which a specific individual could be identified. Demographic data was collected on the questionnaire but in a format that could not be used to identify a specific individual (i.e. age brackets, ethnicity). The healthcare team at no point had access to individuals' questionnaires.

The anonymous paper questionnaires were stored securely in the research office at the NHS sites until the end of the recruitment period. At the end of the recruitment period the paper questionnaires were physically transferred by courier to the Anthony Nolan Research Institute. The paper questionnaires were securely stored in the Anthony Nolan Research Institute office. Anonymous data from the paper questionnaire was transferred to a study specific password protected electronic spreadsheet.

4.8 Materials and Methods

4.8.1 Study Power Calculations

Based on a medium effect size ($f^2 = 0.15$), an α error probability of 0.05, a power (1-β error probability) of 0.8 and with 9 predictors in multivariable analysis, a sample size of n=114 was calculated.

4.8.2 Study Questionnaire

A 42-item study-specific questionnaire was developed with invaluable advice and input from Dr Alice Forster, Senior Research Fellow, Faculty of Population Health Sciences, UCL.

The questionnaire was based on the framework summarised in 4.6. Health belief statements were devised and clustered to address each construct. The statements clustered by health construct are given in Table 14. Participants were asked to indicate their agreement with each health belief statement on a 5-point Likert scale ranging from 'strongly disagree' to 'strongly agree'. The dependent variable in the questionnaire was vaccination intent and this was assessed by 2 statements. The statements were specific with regard to behaviour (receiving the seasonal influenza

vaccine) and time frame (during the next winter season), and agreement was assessed on a forward and reverse scale.

A subset of statements from the risk, severity and barrier clusters were phrased in relation to before HSCT (Table 14, Statement 4,7 and 15), and in relation to other healthy people of the same age (Table 14, statement 3,6,10 and 13). These were sub-grouped into 'health relative' and 'HSCT relative' constructs.

Statements pertaining to GP and HSCT team cues to vaccination were phrased to explore perception of HSCT team and GP knowledge about seasonal influenza vaccine in the context of HSCT, and potential impact of advice from both sources (Statements 20-23, Table 14).

Questions about preferred vaccination location and ease of access to GP services were also included (Statements 16 and 27).

Background questions enquired about type of HSCT (auto or alloHSCT), disease indication for HSCT, time from HSCT, past vaccination receipt and information received about influenza vaccine since HSCT. Basic socio-demographic questions established age, gender, ethnic background, educational background, relationship status and

residential circumstances. For all potentially sensitive questions 'Prefer not to answer' options were given.

4.8.2.1 Questionnaire Development and Piloting

After preparing the initial questionnaire draft, detailed think-aloud sessions were conducted in October and November 2015 with 3 members of the Anthony Nolan patient panel. Participants were asked to read each question aloud, outline their interpretation of the question and then indicate their response and the reason for this. The purpose of this was to ensure that questions were clear, easy to understand, that interpretation of each question was as intended, and that answers were consistent with the question asked. Following these sessions modifications were made to question content, order and phrasing.

In December 2015, the questionnaire was piloted with 10 patients at the Royal Marsden Hospital NHS Foundation trust. Participants were asked to complete the questionnaire, keeping note of the time taken, and to highlight any questions that they had difficulty answering or otherwise found problematic. The questionnaires were all completed within 10 minutes and no participants reported difficulty or concerns about the questions.

The final version of the study questionnaire can be found in appendix 7.

Construct (Cronbach's α value)
HEALTH BELIEF MODEL
Intention (α = 0.87)
1. I intend to receive the seasonal flu vaccine next winter 2. I will choose not to receive the seasonal flu vaccine next winter
Susceptibility to seasonal influenza (α = 0.83)
3. Now I have had a stem cell transplant I can catch the seasonal flu more easily than other people my age 4. Now I have had a stem cell transplant I can catch the seasonal flu more easily than before my transplant
Likelihood of contracting seasonal influenza (α = 0.91)
5. My chances of catching seasonal flu next winter will be high if I do not receive the seasonal flu vaccine 6. I am more likely than other people my age to catch seasonal flu next winter if I do not receive the seasonal flu vaccine 7. Now I have had a stem cell transplant it is more likely that I will catch seasonal flu next winter if I do not receive the seasonal flu vaccine
Severity of seasonal influenza infection (α = 0.91)
8. If I do not receive the seasonal flu vaccine and caught the seasonal flu next winter this would be a serious illness for me 9. If I do not receive the seasonal flu vaccine and caught the seasonal flu next winter this would have a negative impact on my recovery from my stem cell transplant 10. If I do not receive the seasonal flu vaccine and caught the seasonal flu next winter I would become more unwell than other people my age
Barriers to vaccination (α = 0.84)
11. I am worried about side effects of the seasonal flu vaccine 12. If I receive the seasonal flu vaccine next winter it may make me feel unwell with the flu or a flu-like illness 13. If I receive the seasonal flu vaccine next winter I am more likely to experience side effects than other people my age 14. If I receive the seasonal flu vaccine next winter it may have a negative impact on my recovery from my stem cell transplant 15. Now I have had a stem cell transplant the seasonal flu vaccine may not work as well for me as it does for other people my age 16. I would prefer to have the seasonal influenza vaccine next winter at my transplant centre instead of my GP surgery
Benefits of vaccination (α = 0.66)
17. If I receive the seasonal flu vaccine next winter it may help to prevent me from catching the seasonal flu 18. If I receive the seasonal flu vaccine next winter it may help to prevent me from passing the seasonal flu to other people around me 19. If I receive the seasonal flu vaccine next winter, but still catch the flu, it may help to prevent me from becoming seriously unwell

Cues to vaccination (HSCT Team and GP) (α = 0.76)
20. If my transplant team advised me to receive the seasonal flu vaccine next winter I would definitely have it
21. If my GP advised me to receive the seasonal flu vaccine next winter I would definitely have it
22. My GP understands my condition enough to know if the seasonal flu vaccine is right for me
23. My transplant team understand my condition enough to know if the seasonal flu vaccine is right for me
EXTENDED HEALTH BELIEF MODEL
Worry (α = 0.47)
24. If I receive the seasonal flu vaccine next winter I will worry less about catching the seasonal flu
25. The thought of catching seasonal flu next winter worries me
Self-efficacy with regard to vaccination (α = 0.29)
26. I have enough information and am able to decide whether the seasonal flu vaccine is right for me
27. I would find it easy to attend my GP surgery next winter to receive the seasonal flu vaccine
Anticipated regret if not vaccinated (α = 0.15)
28. I would regret it if I decided not to receive the seasonal flu vaccine next winter and became unwell with seasonal flu
29. I would regret it if I decided to receive the seasonal flu vaccine next winter and became unwell with side effects
AGE AND HSCT RELATIVE CONSTRUCTS
Constructs in relation to others of the same age (α = 0.64)
Statements 3, 6, 10, 13 (reverse scale)
Constructs in relation to before HSCT (α = 0.55)
Statements 4, 7, 15

Table 14: Study questions grouped by health belief construct. Value for Cronbach's α test of scale reliability (see 4.10) is given in parentheses for each construct

4.8.3 Study Recruitment

The study was open at 3 study sites from June to September 2016. 93 participants were recruited in total by HSCT Nurse Specialists.

4.9 Measure of Vaccination Intent

Vaccination intent was measured by 2 statements on a five-point Likert scale. The positive intent statement was scored from 0-4 (strongly disagree to strongly agree) and the scale was reversed for the negative intent statement. Scores were summed, and the neutral score value (neither agree nor disagree to both statements) was 4. Participants were dichotomized to 'low intent' (intent score of ≤4) and 'high intent' (intent score ≥ 5). This cut-off was chosen as it was considered the most relevant to the study question, and provided groups of sufficient size to carry out analysis.

4.10 Statistical Analysis

Categorical patient characteristics and socio-demographic factors are reported as frequencies and percentages. Associations between these variables and vaccination intent was examined with Pearson's chi-squared test, and Fisher's exact test when expected values were less than 5.

Health belief statements were grouped into health belief constructs (Table 14). Agreement on 5-point Likert scales were scored from 0 to 4 (4 to 0 for reverse scale questions). Internal scale reliability for each cluster of statements was assessed using Cronbach's α. Although there is no absolute cut-off values(286), >0.6 was considered indicative of acceptable internal scale reliability and statement scores were summed to give a total agreement score. Values <6 were considered unacceptable and construct clusters were reviewed and modified. Scale reliability was acceptable for all constructs of the HBM. Scale reliability was unacceptable for the modifying clusters relating to self-efficacy, worry, anticipated regret and the HSCT relative construct. These constructs were therefore adjusted for analysis. For the self-efficacy construct, statements 26 (*I have enough information and am able to decide whether the seasonal flu vaccine is right for me*) and 27 *(I would find it easy to attend my GP surgery next winter to receive the seasonal flu vaccine*) were evaluated separately. For anticipated regret constructs, statements 28 (*I would regret it if I decided not to receive the seasonal flu vaccine next winter and became unwell with seasonal flu*) and 29 (*I would regret it if I decided to receive the seasonal flu vaccine next winter and became unwell with side effects*) were analysed separately. For the worry construct statements 24 (*If I receive the seasonal flu vaccine next winter I will worry less about catching the seasonal flu)* and 25 (*The thought of catching seasonal flu next winter worries me) were also analysed separately.* For the HSCT relative constructs removal of statement 15 (*Now I have had a stem cell transplant the seasonal flu vaccine may not work as well for me as it does for other people my age)*

yielded a Cronbach's α value of 0.83 and therefore this statement was dropped from the construct.

Summed agreement scores for each construct were analysed as continuous scales. For vaccination intent, and health belief constructs, mean agreement score values, standard deviations and 95% confidence intervals are presented. Histograms, and mean and median values were reviewed to confirm normality of distribution for each scale. Analysis of Variance (ANOVA) was used to compare mean agreement scores between low and high vaccination intent groups for each construct. Mean agreement scores between age groups, and between participants grouped by time from HSCT were also compared with ANOVA. Homogeneity of variances was confirmed with Levene's statistic.

Binary logistic regression was used to examine the effects of health belief constructs on seasonal influenza vaccination intent. Three models were examined: 1) the HBM, 2) the M-HBM, 3) age and HSCT relative model. For each model, a hierarchical regression method was used. The assumption of a linear relationship between each independent variable and log of the outcome variable was tested and confirmed using the Box-Tidwell procedure(287). Multicollinearity across all constructs was assessed. No variance inflation factor was greater than 10, and the mean of values was acceptable at 1.92(201).

Questions 22-25 (HSCT and GP cues), and 16 (Preferred vaccination location) were not included in the binary logistic regression model, as statements were phrased such that they could not be used as predictors of intent. For these variables, mean values for the low and high intent groups were compared with ANOVA. HSCT and GP cue scores *within* low and high intent groups were compared with a paired sample T-Test. The number of participants favouring vaccination at their GP practice or HSCT programme were compared between the high and low intent groups with Pearson's Chi-square test.

Missing data was minimal. 1 participant left 1 response blank, 1 participant left 3 responses blank, and a further 1 participant left 6 responses blank totalling 10 missing data points for 3 participants across the whole study. All missing data points were from the high-intent group, and were within the likelihood, severity, barrier and GP cues to vaccination constructs. Summed agreement scores were not calculated in these cases and participants were excluded from analysis for the affected construct only.

Power calculations were performed with G*Power version 3.1. Statistical analysis was performed with IBM SPSS version 24.

4.11 Results

4.11.1 Participant Characteristics

Characteristics of 93 study participants are given in *Table 15*. 78.5% were recipients of alloHSCT and the most frequent disease indication was AML (28.0%). The majority (68.6%) were within the first 6 months post HSCT. 40.9% of participants had received the IIV before HSCT, and only 4.3% had received a non-influenza vaccine since HSCT. 52.7% of participants were male, and most (84.9%) were of a white ethnic group.

Characteristic, n=93	**n(%)**	**High Vaccination intent (%)**	**P Value**
Gender			
Male	49 (52.7)	81.6	
Female	44 (47.3)	70.5	0.23
Age group			
16-34	22 (23.7)	68.2	
35-54	36 (38.7)	91.7	
55-64	20 (21.5)	75.0	
65+	15 (16.1)	53.5	0.02*
HSCT Type			
Allogeneic	73 (78.5)	80.0	
Autologous	20 (21.5)	75.3	0.78
Disease Indication			
Acute lymphoblastic leukaemia (ALL)	11 (11.8)	72.7	
Acute myeloid leukaemia (AML)	26 (28.0)	76.9	
Aplastic Anaemia (AA)	5 (5.4)	60.0	
Chronic myeloid leukaemia (CML)	5 (5.4)	100	
Hodgkin Lymphoma	9 (9.7)	88.9	
Myelodysplastic syndrome (MDS)	5 (5.4)	60.0	
Myelofibrosis (MF)	2 (2.2)	50.0	
Multiple myeloma (MM)	22 (23.7)	77.3	
Non-Hodgkin Lymphoma (NHL)	8 (8.6)	75.0	0.79

months from HSCT	64 (68.8)	81.3	
0-6	20 (21.5)	70.0	
7-12	9 (9.7)	55.6	0.20
> 12			
Seasonal flu vaccine before HSCT			
Yes	38 (40.9)	89.5	
No	55 (59.1)	67.3	0.01*
Any vaccine since HSCT			
Yes	4 (4.3)	100	
No	89 (95.7)	75.3	0.26
Information about flu vaccine from HSCT Team			
Yes	25 (26.9)	80.0	
No	68 (73.1)	75.0	0.62
Ethnicity			
White	79 (84.9)	77.2	
Mixed	2 (2.2)	50.0	
Asian	8 (8.6)	87.5	
Black	3 (3.2)	66.7	
Other	1 (1.1)	0.0	0.32
Educational Background			
Secondary Education	49 (52.7)	81.6	
Higher Education	30 (32.3)	80.0	
Other	3 (3.2)	66.7	
Prefer not to answer	11 (11.8)	45.5	0.07
Living Circumstances			
Renting	25 (26.9)	76.0	
Home Owner	54 (58.1)	79.6	
Other	10 (10.8)	70.0	
Prefer not to answer	4 (4.7)	50.0	0.56
Relationship Status			
Single	23 (24.7)	78.3	
Married / Cohabiting	56(60.2)	80.4	
Divorced / Separated	10 (10.8)	50.0	
Prefer not to answer	4 (4.4)	75.0	0.22

*Table 15: Characteristics of n=93 study participants. *Statistically Significant*

4.11.2 Vaccination Intent

The mean value was 5.92 (SD 2.0, 95% CI 5.5-6.36. 71 (76.3%) participants had positive vaccine intent, and 22 (23.7%) neutral or negative vaccine intent.

4.11.3 Socio-demographic variables and vaccination intent

There was a statistically significant difference in vaccination intent between age groups (χ^2 9.91, P=0.02). High intent rate was greatest in the 35-54 age group at 91.7%, and lowest at 53.3% in the 65+ age group (*Figure 29*). There was no statistically significant association between gender(χ^2 1.60, P=0.23), ethnicity (χ^2 4.74, P=0.32), educational background (χ^2 6.95, P=0.07), living circumstance (χ^2 2.908, P=0.56), or relationship status (χ^2 4.39, P=0.22) and intent (*Table 15*).

To determine whether there was a difference in health beliefs between age groups, mean agreement scores for all constructs were compared. There was no difference between mean agreement scores for the 16-34, 35-54 and 55-65 age groups for any construct (p >0.05 in all cases). The >65 age group had lower mean agreement scores for susceptibility statements compared with 16-34 age group (4.40, 95%CI 3.50-5.22 v 6.14, 95% CI 5.30-6.90, p=0.008) and 35-54 age group (5.86, 95%CI 5.44-6.31 p=0.02),

and also for severity statements compared with the 16-34 (6.57, 95%CI 5.45-7.67 v 8.82, 95%CI 7.71-9.89, p=0.008) and 35-54 (8.77, 95%CI 7.97-9.58, p=0.005) age groups.

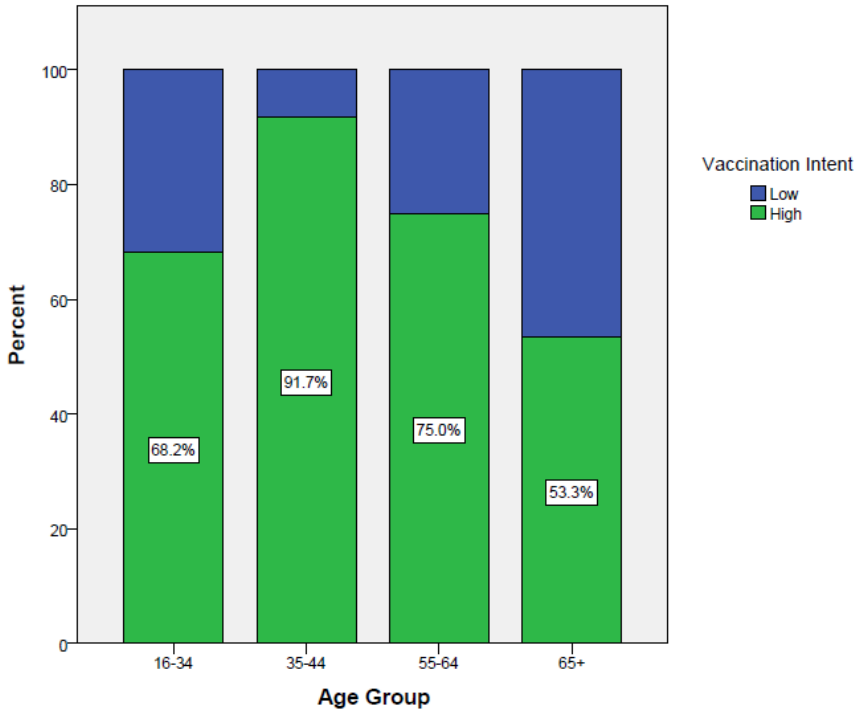

Figure 29: Seasonal influenza vaccination intent by age group

4.11.4 Transplant variables and vaccination intent

There was no association between type of HSCT (χ^2 0.19, p=0.78), or disease indication (χ^2 4.68, p=0.79) and vaccination intent (*Table 15*). Vaccination intent was 80% in the first 0-6 months post HSCT, 70% at 6-12 months, and lowest at 55.6% at >12 months from HSCT although this finding was not statistically significant (χ^2 3.45, p=0.18).

To determine whether there was a difference in health beliefs between participants at different time points post HSCT, mean agreement scores for all constructs were compared. There was no difference in mean agreement scores between participants at 0-6 and 6-12 and >12 months post HSCT ($p>0.05$ in all cases).

4.11.5 Past vaccination behaviour, vaccine information and vaccination intent

There was no association between vaccine intent and previous influenza vaccination information from the HSCT team (χ^2 0.25, p=0.62) or receipt of any non-influenza vaccine since HSCT (χ^2 1.30, p=0.26).

The lowest frequency of influenza vaccination prior to HSCT was 22.7% in the 16-34 age group and highest at 53.3% in the over 65 age group (*Figure 30*). Influenza vaccination prior to HSCT was associated with intent in the over 65 age group. 87.5% of those who had received the vaccine before HSCT expressed high intent compared with only 14.3% of those who had not (Fisher's exact test, p=0.01). This was not seen in any of the other age categories. Across the combined 16-64 age group vaccination intent was 90% amongst those who had received the vaccine prior to HSCT, and 75% in those who had not (χ^2 2.67, p=0.14).

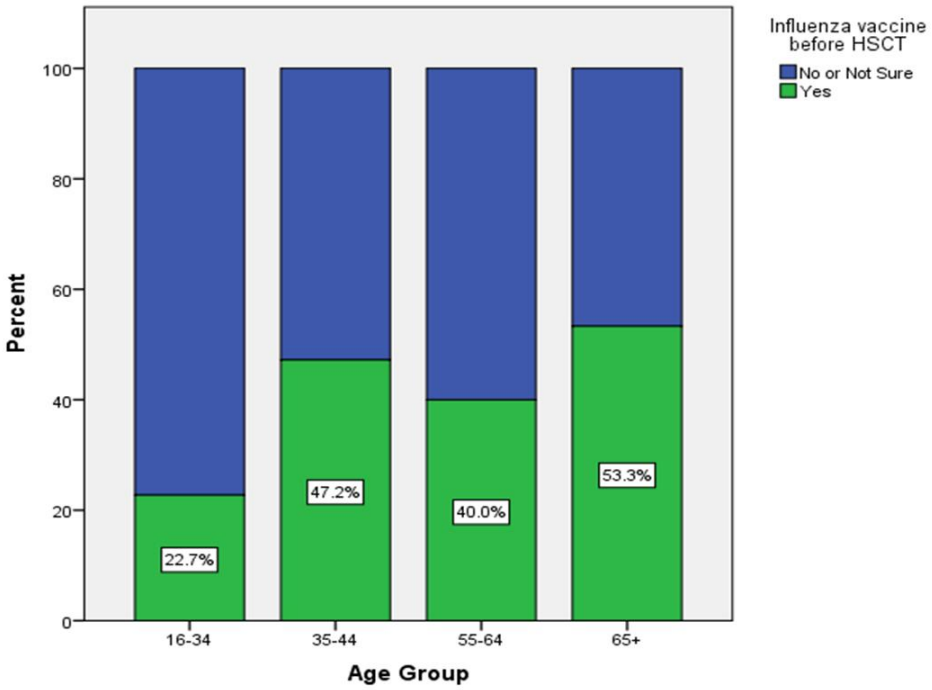

Figure 30: Influenza vaccine receipt prior to HSCT by age group

4.11.6 Health belief constructs and vaccination intent.

Table 16 summarises the mean agreement scores for each health belief constructs (after modification as described in 4.10) separated by low and high intent groups. ANOVA was used to compare mean agreement scores between groups and p values are presented. For all health belief constructs, with the exception of anticipated regret of side effects if vaccinated, there was a statistically significant difference between mean agreement scores for the low and high vaccination intent groups. In all cases the difference between agreement scores was in the expected direction; the high intent

group had statistically significantly higher mean scores for perceived susceptibility to influenza (6.09 v 4.05, P<0.001), likelihood of contracting influenza (8.58 v 5.55, P<0.001), severity of influenza infection (8.65 v 6.77, P=0.002), benefits of vaccination (8.56 v 5.85, P<0.001), worry statement 24 (2.80 v 1.77, P<0.001), self-efficacy statement 26 (2.81 v 2.41, P=0.007), self-efficacy statement 27 (3.10 v 2.32, P<0.001), anticipated regret statement 28 (3.35 v 2.27, P<0.001) and. The low intent group had a higher mean agreement scores for perceived barriers to vaccination (11.27 v 8.45, p=0.001). The high intent group also had higher mean agreement scores than the low intent group for both age (11.09 v 7.91, P <0.001) and HSCT relative (7.74 v 5.82, P<0.001) constructs. There was no difference between high and low intent groups for worry statement 25 (2.39 v 2.14, p=0.34), and self-efficacy statement 29 2(2.13 v 1.19, p=0.40)

The high intent group had statistically significantly higher mean agreement scores than the low intent group for HSCT team cues (7.23 v 5.68, p <0.001) and GP cues (5.89 v 3.55, P<0.001) to vaccination, indicating that knowledge and perceived impact of recommendation was not equal between the two groups. This is explored further in 4.11.8.

Health Belief Constructs	Number of Questions (scale range)	Mean Agreement Score (SD, 95% CI)		
		Low Vaccination intent (n=22)	High Vaccination Intent (n=71)	P value
Health Belief Model Constructs				
Susceptibility to Influenza	2 (0-8)	4.05 (1.59, 3.30-4.70)	6.09 (1.38, 5.75-6.39)	<0.001
Likelihood of contracting influenza	3 (0-12)	5.55 (2.06 4.61-6.40)	8.58 (2.48, 8.00-9.18) [b]	<0.001
Severity of influenza infection	3 (0-12)	6.77 (2.24, 5.83-7.72)	8.65 (2.37, 8.09-9.23) [b]	0.002
Barriers to vaccination	5 (0-20)	11.27 (2.96, 10.11-12.44)	8.45 (3.27, 7.66-9.20) [a]	0.001
Benefits of vaccination	3 (0-12)	5.95 (2.10, 5.00-6.78)	8.56 (1.80, 8.13-9.00)	<0.001
HSCT team cues to vaccination	2 (0-8)	5.68 (1.17, 5.16-6.15)	7.23 (0.97, 7.00-7.45)	<0.001
GP cues to vaccination	2 (0-8)	3.55 (1.95, 2.73-4.33)	5.89 (1.47, 5.54-6.26)[c]	<0.001
Modifying Constructs				
24 - Worry less about catching flu if vaccinated	1 (0-4)	1.77 (0.81, 1.40-2.07)	2.80 (0.77, 2.61-3.00)	<0.001
25 - Worry about catching the flu	1 (0-4)	2.14 (1.35, 1.57-2.71)	2.39 (1.01, 2.17-2.63)	0.34
26 - Self-efficacy: Enough information to decide about vaccination	1 (0-4)	2.14 (1.08, 1.68-2.58)	2.81 (0.98, 2.61-3.00)	0.007
27 - Self efficacy: Easy to attend GP	1 (0-4)	2.32 (1.04, 1.88 -3.72)	3.10 (0.88, 3.89-4.00)	<0.001
28 - Anticipated regret of catching flu	1 (0-4)	2.27 (1.08, 1.79-2.74)	3.35 (0.69, 3.18-3.52)	<0.001
29 - Anticipated regret of side effects	1 (0-4)	1.91 (1.15, 1.42-2.37)	2.13 (1.06, 1.88-2.39)	0.40
Relative Constructs				
Constructs relative to healthy individuals the same age	4 (0-16)	7.91 (2.04, 6.95-8.75)	11.09 (2.06, 10.61-11.64 [b]	<0.001
Constructs relative to before HSCT	2 (0-8)	5.82 (1.53, 5.17-6.42)	7.74 (1.47, 7.40-8.09)[c]	<0.001

Table 16: Mean agreement score values for each health belief construct separated by high and low vaccination intent groups. Results for each construct are presented as mean score value with standard deviations (SD) and 95% confidence intervals. [a]n=68, [b]n=69,[c]n=70

4.11.7 Prediction of Vaccination Intent

The impact of participants' health beliefs on vaccination intent was examined with hierarchical binary logistic regression. All constructs statistically significant in univariate analysis were included in the regression models (*Table 16*). As the modifying variables age and vaccination prior to HSCT were statistically significantly associated with intent, these were adjusted for by inclusion in the first hierarchical regression blocks. As age was an ordinal categorical variable, this variable was dichotomized to < and >65 years of age.

4.11.7.1 Health Belief Model Constructs

The impact of the HBM constructs on vaccination intent were first examined. The addition of the HBM constructs to age>65 and no pre-HSCT influenza vaccination statistically significantly improved the predictive value of the model (χ^2 42.91, p<0.001). The logistic regression model was statistically significant (χ^2 57.24, p<0.001) and explained 70.8% (Nagelkerke R^2) of the variance in intent to receive influenza vaccine next season. Sensitivity was 93.9% and specificity 77.3%. Age>65 (OR 0.05, 95% CI 0.04-0.65, p=0.02), no influenza vaccination prior to HSCT (OR 0.06, 95%CI 0.01-0.62, p=0.02) and higher perception of barriers to vaccination (OR 0.68, 95%CI 0.48-0.97, p=0.03) were statistically significantly associated with low intent to receive influenza vaccine, while higher perceived benefits of vaccination were associated with high intent

(OR 3.37, 95%CI 1.47-7.70, p=0.04). Studentized residuals were reviewed. 2 (2.15%) cases lay outside values of +/-2.58(201). Data entry was checked, and responses reviewed. Although responses for these participants were not consistent across the questionnaire, and both participants had selected multiple neutral responses, there was no clear rationale for excluding these cases and therefore they were left in the model. The logistic HBM constructs regression model is summarised in *Table 17*

HBM Construct	SE	Wald	P	Odds Ratio	95% CI
Age >65	1.31	5.22	0.02	0.05	0.04-0.65
No pre HSCT influenza vaccination	1.20	5.56	0.02	0.06	0.01-0.62
Susceptibility to Influenza	0.45	0.24	0.62	1.24	0.52-2.99
Likelihood of contracting influenza	0.28	0.96	0.33	1.31	0.76-2.26
Severity of influenza infection	0.23	0.42	0.51	0.86	0.55-1.35
Barriers to vaccination	0.18	4.48	0.03	0.68	0.48-0.97
Benefits of vaccination	0.42	8.63	0.04	3.37	1.47-7.70

Table 17: Logistic regression predicting intent to receive influenza vaccine during next season across all age groups based on Health Belief Model constructs and adjusting for age and influenza vaccination prior to HSCT.

In univariable analysis, the impact of pre-HSCT vaccination was seen only in the over 65 age group. Therefore, the logistic regression was repeated for the subgroup of patients aged under 65 excluding age as a variable, but including no pre-HSCT influenza vaccination. Both benefits of vaccination (OR 5.19, 95% CI 1.72-15.69, p=0.04) and barriers to vaccination (OR 0.57, 95% CI 0.34-0.94, p=0.027) remained statistically significantly associated with vaccine intent, but pre-HSCT influenza vaccination was not significant in this subgroup.

4.11.7.2 Modifying Constructs

The impact of the modifying constructs on vaccination intent was examined with binary logistic regression. First, they were examined as a discrete group of constructs. Significant variables were then added to the HBM in 4.11.7.1 to determine whether the predictive value of the HBM could be improved.

The addition of the E-HBM constructs to age>65 and No pre-HSCT influenza vaccination statistically significantly improved the predictive value of the model (χ^2 57.68, p<0.001). The final logistic regression model was statistically significant (χ^2 44.06, p<0.001) and explained 56.7% (Nagelkerke R^2) of the variance in intent to receive influenza vaccine next season. Sensitivity was 93.0% and specificity 72.7%. Age>65 (OR 0.19, 95%CI 0.16-0.80, p=0.03) was statistically significantly associated with low intent to receive influenza vaccine, while the worry construct was associated with high intent (OR 4.85, 95%CI 1.76-13.36, p=0.02). In this model, influenza vaccination prior to HSCT was not statistically significantly associated with intent(OR 0.19, 95%CI 0.03-1.06, p=0.06). Studentized residuals were reviewed. 3 (3.2%) cases lay outside values of +/-2.58(201). Again, there was no clear rationale for excluding these cases and therefore they were left in the model. The M-HBM logistic regression is summarised in *Table 18*.

HBM Construct	SE	Wald	P	Odds Ratio	95% CI
Age >65	1.07	4.72	0.03	0.19	0.16-0.80
No pre HSCT influenza vaccination	0.89	3.59	0.06	0.19	0.03-1.06
Self-efficacy	0.35	2.77	0.10	1.80	0.90-3.60
Worry less about catching flu	0.51	9.32	0.02	4.85	1.76-13.36
Anticipated regret of catching flu	0.57	2.01	0.16	1.76	0.81-3.84

Table 18: Logistic regression predicting intent to receive influenza vaccine during next season across all age groups based on Health Belief modifying constructs and adjusting for age and influenza vaccination prior to HSCT.

As in 4.11.7.1, the logistic regression was repeated for the subgroup of patients aged under 65 excluding age as a variable, but including no pre-HSCT influenza vaccination. Emotional response remained statistically significant (OR 4.78, 95% CI 1.63-13.99, p=0.04) and pre-HSCT influenza vaccination was not significant in this subgroup.

To determine whether the worry construct would improve the HBM in *Table 17*, this construct was introduced as an additional block in the hierarchical regression. The addition of the modifying worry constructs to the HBM significantly improved the predictive value of the model (χ^2 4.52, $p<0.03$) and explained 74.7% (Nagelkerke R^2) of intent to receive influenza vaccine next season. The overall model was statistically significant (χ^2 61.76, $p<0.001$).

All variables in the original model remained statistically significant. The final M-HBM is summarised in *Table 19*.

HBM Construct	SE	Wald	P	Odds Ratio	95% CI
Age >65	1.65	5.28	0.02	0.02	0.01-0.57
No pre HSCT influenza vaccination	1.39	5.28	0.02	0.04	0.02-0.56
Susceptibility to Influenza	0.54	0.01	0.64	0.96	0.33-2.78
Likelihood of contracting influenza	0.51	2.32	0.13	1.68	0.86-3.26
Severity of influenza infection	0.29	1.65	0.20	0.69	0.39-1.21
Barriers to vaccination	0.19	3.87	0.05	0.69	0.57-0.99
Benefits of vaccination	0.43	6.50	0.01	2.96	1.29-6.81
Worry less about catching flu	0.82	3.86	0.05	4.99	1.01-24.77

Table 19 Logistic regression predicting intent to receive influenza vaccine during next season across all age groups based on Modified Health Belief Model, and adjusting for age and influenza vaccination prior to HSCT.

Studentized residuals were reviewed. 3 (3.2%) cases lay outside values of +/-2.58(201) and after review, there was no clear rationale for excluding these cases and therefore they were left in the model.

4.11.7.3 Relative Constructs

This subgroup of HBM constructs explored HSCT recipients' health beliefs in relation to before their HSCT, and in relation to healthy adults of the same age. The HSCT relative constructs explored susceptibility to and likelihood of catching influenza, while the age relative constructs explored susceptibility to, and likelihood of catching influenza along with benefits of and barriers to vaccination. A logistic regression model was used to explore whether these constructs predicted vaccination intent independently of the other M-HBM constructs.

The addition of the age and HSCT relative constructs block to age>65 and No pre-HSCT influenza vaccination statistically significantly improved the predictive value of the

model (χ^2 24.86, p<0.001). The logistic regression model with odds ratios and 95% confidence intervals is summarised in *Table 20*. The final logistic regression model was statistically significant (χ^2 40.10, p<0.001) and explained 53.6% (Nagelkerke R^2) of the variance in intent to receive influenza vaccine next season. Sensitivity was 91.2% and specificity 63.6%. Only the age relative constructs were statistically significant (p=0.01), and in this model age >65 was not statistically significant. Studentized residuals were reviewed. 3 (3.2 %) cases lay outside values of +/-2.58(201). There was no clear rationale for excluding these cases and therefore they were left in the model.

HBM Construct	SE	Wald	P	Odds Ratio	95% CI
Age >65	0.86	2.98	0.08	0.23	0.04-1.22
No pre HSCT influenza vaccination	0.79	2.45	0.12	0.29	0.61-1.37
Age relative construct	0.29	6.35	0.01	2.09	1.18-3.71
HSCT relative construct	0.36	0.10	0.74	1.12	0.56-2.26

Table 20: Logistic regression predicting intent to receive influenza vaccine during next season across all age groups based age and HSCT relative constructs, and adjusting for age and influenza vaccination prior to HSCT.

4.11.8 GP and HSCT Team Cues to Vaccination

As discussed in 4.11.6 the mean agreement values for GP cues and HSCT cues were higher in the high intent than the low intent group. The statements presented were '*My transplant team understands my condition enough to know if the seasonal flu vaccine is right for me'* and '*If my transplant team advised me to receive the seasonal flu vaccine next winter I would definitely have it*'. The same questions were asked

substituting transplant team for GP. Therefore, this difference in mean agreement scores *between* the low and high intent group may indicate that the latter may have a relatively more favourable perception of both GP and HSCT team knowledge, and may be more inclined to follow advice regarding vaccination.

To explore GP and HSCT cues *within* high and low intent groups, mean agreement scores for GP and HSCT statements were compared with a paired sample T-Test. Within both low and high intent groups the mean agreement score for HSCT team cues were higher than for GP cues. In the low vaccine intent group, the mean agreement score for HSCT cue was 5.68 (SD 1.17, 95% CI 5.16 to 6.15) and for GP cue was 3.55 (SD 1.95, 95%CI 2.73-4.43) with a difference between the means of 2.13 (1.33 to 2.90, $p<0.001$). In the high vaccine intent group, the mean agreement score for HSCT cues was 7.23 (0.98, 6.98-7.45) and GP was 5.89 (1.43, 5.54-6.23) with a mean difference of 1.34 (1.54, 0.96-1.70; $p<0.001$). So, within both low and high intent groups participants' agreement scores for HSCT cue statements was significantly higher than for GP cue statements.

To further assess this difference, GP and HSCT agreement scores were dichotomized at the neutral value (4), to those with low/neutral agreement, or high agreement (Figure 31). Of those 71 patients with high vaccine intent, 100% had high agreement with HSCT statements, and 84.3% high agreement with GP cue statements. Of those patients with

low vaccine intent, 81.8% had high agreement with HSCT statements, while only 31.8% had high agreement with GP cue statements.

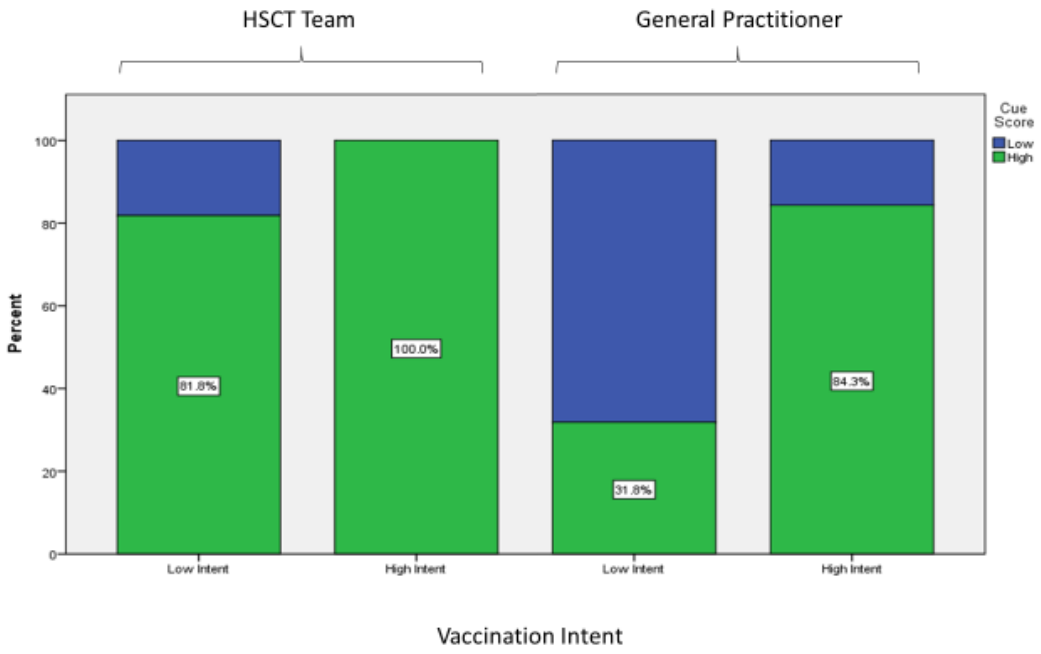

Figure 31: Comparison of low and high cue scores for HSCT Team and General Practitioner by vaccine intent

4.11.9 Preferred vaccination location

Participant responses to statement 16 (I would prefer to have the seasonal influenza vaccine next winter at my transplant centre instead of my GP surgery) were categorized into favours HSCT centre, favours general practitioner and neutral. Of those with low intent over half (54.5%) favoured vaccination at their HSCT programme, with only a minority (4.5%) favouring vaccination at their GP practice. Of those with high intent

43.7% favoured vaccination at their HSCT programme, compared with 29.6% at their GP practice (*Figure 32*). This finding was approaching statistical significance (χ^2 (2)=5.99, p=0.05).

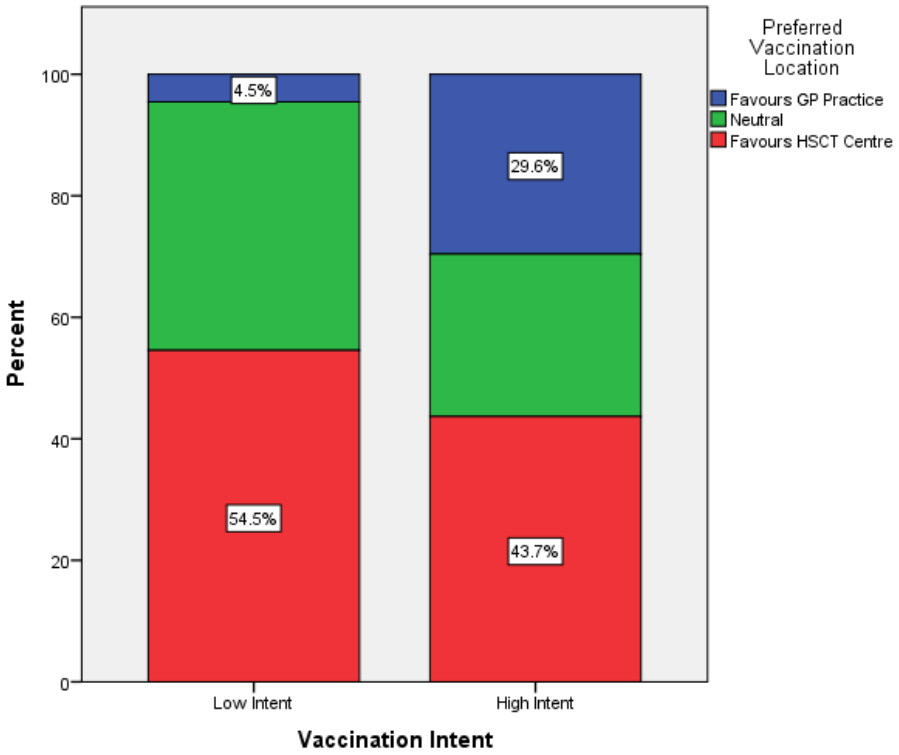

Figure 32: Comparison of preferred vaccination location by vaccine intent group

4.12 Discussion

This study is the first to explore the impact of sociodemographic, behavioural and health belief factors on SIIV intent in HSCT recipients. Overall vaccine intent was 76.3%, which is similar to the vaccine uptake rate of 71% in people aged over 65, and in excess

of the 45.1% rate in under 65 at risk groups in the UK population during the UK 2015-2016 influenza season. The reported SIIV uptake rate amongst HSCT recipients in 2 single centre studies is 60-70%(104,105). SIIV uptake during the 2016-2017 UK influenza season was not evaluated, and it must be acknowledged that uptake rates may not be equivalent to intent rates reported here.

4.12.1 Transplant Factors

None of the transplant factors assessed were associated with vaccination intent. Current influenza vaccination guidelines are standardized for all HSCT recipients as evidence is insufficient to recommend modification according to donor type, stem cell source or conditioning(96,100). Influenza infections however do appear to occur with greater frequency in allo than autoHSCT recipients (65,288) and may have higher associated morbidity and mortality(289) although this latter finding has not been consistently reported(64). There was no difference in vaccination intent between auto and alloHSCT recipients, and this perhaps reflects an awareness among both groups that vaccination is recommended. It certainly suggests that the unique complications of alloHSCT, principally GvHD, do not contribute to increased influenza vaccination intent. The study did not enquire about concomitant medications, including IST, or active GvHD and it remains possible that these factors do impact on vaccine intent within the alloHSCT group.

Rates of vaccination intent were greatest in those recipients within the first 0-6 months' post-HSCT (81.3%) and lowest at more than 12 months (55.6%) although this finding was not statistically significant. Longer time from HSCT may be associated with a reduction in perceived risk of infection, or increased concern about vaccine side effects. However, there was no statistically significant difference between mean agreement scores with health belief construct statements at 0-6, 6-12 and > 12 months from HSCT.

4.12.2 Socio-demographic factors

None of gender, ethnicity, educational background, living circumstances or relationship status were associated with vaccine intent in this study. In the United States lower rates of seasonal IIV vaccination intent have been reported amongst Black African Americans aged over 65(290), and a UK study of intent to receive A(H1N1)pdm09 vaccine reported lower rates amongst Black ethnic groups compared with people of Asian and White ethnicities, although the number of participants reporting ethnicity in this latter study was low. A recent meta-analysis of international studies found inconsistent association between ethnicity and influenza vaccine uptake in population groups including healthcare workers, pregnant women, the elderly and those with chronic health conditions(291). In our current study the ethnic distribution across participants is similar to that among UK alloHSCT recipients registered in 2014 with 89% White, 3% Black, 7% Asian, and 1% other. Reported UK Figures for autoHSCT recipients are 91% white, 3% Black, 5% Asian and 1% other (21).

Living alone and being unmarried have been associated with low vaccine uptake (291) and it has been suggested that this is related to reduced social cues to vaccination from a partner, and in those over 65 related to restricted access to healthcare services(219). In our study population, the majority of patients were within 12 months of HSCT and so interacting regularly with healthcare professionals. Agreement with self-efficacy scores pertaining to ease of access to GP vaccination services was the same across age categories.

In this study population, vaccination intent varied by age. It was reassuring to find very high vaccination intent at 91.7% in the 35-53 age bracket, but concerning that in those over 65, intent at 53.5% fell below the UK uptake rate in this age-group. Intent rate was also lower in the 16-34 age group at 68.2%, but in line with UK uptake rates. Older age has previously been reported as a barrier to vaccination in a cohort of oncology patients, including some with haematological malignancy (292). However, a study of patients with secondary immunodeficiency, including haematological disorders, reported higher vaccination rates in those aged over 65 compared with younger patients(223). Likewise, in a UK study, age was found to be a predictor of A(H1N1)dpm09 vaccine uptake amongst high-risk adults(241). A recent meta-analysis of international studies found inconsistent association between age and vaccination intent and uptake in the general public, older patients, and those with chronic disease (291). It is not apparent from these studies why age impacts on intent, and there are likely to be a range of social, psychological, financial and healthcare access issues

specific to each study population. In this current study, the over 65 age group was the smallest at 16.1% of the study population. However, this age group had higher mean agreement scores for susceptibility to and severity of influenza statements compared with 16-54-year olds. Furthermore, the impact of previous vaccination behaviour was most pronounced in this age group.

4.12.3 Previous vaccination behaviours

A strong association between past vaccination behaviours and future vaccination intent has been reported(291). Previous influenza vaccination has been associated with high intent or uptake in all at risk groups (219,293) and cancer patients(292). Our findings accord with this, although notably the association was only evident in the over 65 age group. The highest rate of influenza vaccination prior to HSCT was in those over 65 (53.3%) followed by the 35-54 age group (47.2%). Lowest rates of previous vaccination were amongst the 16-34 age group (22.7%). Given the over 65 age group is considered an at-risk group regardless of comorbidity, and is the target of UK NHS public health vaccination campaigns, this is perhaps not surprising. In the 16-54 age groups intent was high regardless of previous vaccination status. However, in the over 65 age group, there was a strong positive but also negative association with high intent amongst 87.5% of those previously vaccinated, but only 14.3% amongst those not vaccinated. Although the reason for this finding is not apparent, it may be that those in the over 65 age group (some of who would have been eligible to receive the vaccine in the previous

flu season on the routine NHS schedule) had actively declined the vaccine in the past, whereas those under 65 who had not received the vaccine may simply not have been eligible or may not have been offered it. Therefore, in the older age group low intent amongst those not previously vaccinated may be the continuation of pre HSCT vaccine refusal. However, as the questionnaire enquired about previous vaccine receipt, but not refusal it was not possible to further evaluate this. The over 65 age group was the smallest in the study and the study was not powered for subgroup analysis. This must be borne in mind when interpreting these findings. However, this warrants further evaluation as it may highlight a specific age group in whom intent is low and targeted intervention may be effective.

4.12.4 Health Beliefs

Psychological models are considered useful predictors of vaccination intent, and a sound basis for developing intervention geared towards increasing intent and uptake(291). Two models were analysed in this study: The HBM and E-HBM. Analysis was also performed on a subset of HBM constructs formulated in relation to others of the same age, and comparing current with pre-HSCT status.

4.12.4.1 Health Belief Model

In univariable analysis, there was an association between all the HBM constructs and vaccination intent. HSCT recipients who agreed more strongly with statements about their susceptibility to influenza, their likelihood of contracting influenza and the severity of influenza in absence of vaccination, were more likely to have high vaccine intent. Conversely, those who agreed more strongly with statements about vaccine side effects and lack of efficacy were less likely to have high vaccine intent. In multivariable analysis, the HBM explained approximately 70% of the variance in vaccination intent. After adjusting for age and pre HSCT vaccination, stronger perceived barriers to vaccination were associated with low intent, and stronger perceived benefits with high intent. Barriers to vaccination statements explored concern around vaccine side effects including flu and flu-like illness, negative impact of vaccination on recovery from HSCT and clinical efficacy of vaccine. The benefits of vaccination statements explored prevention of influenza, attenuation of disease if infected, and limiting spread of virus to others. In a recent systematic review, perceived benefits and barriers to vaccination were identified as among the strongest psychological determinants of seasonal influenza vaccination intent and uptake amongst the general population, and our findings confirm that these factors are similarly important in high-risk HSCT recipients(293).

In multivariable sub-analysis of HBM constructs, HSCT recipients who agreed more strongly with statements framed in relation to other people of the same age were more

likely to have high intent. These statements explored increased susceptibility to influenza, likelihood of contracting influenza, severity of illness and vaccine side-effects.

4.12.4.2 Extended Health Belief Model

In univariable analysis, HSCT recipients who agreed more strongly with self-efficacy statements (*I have enough information and am able to decide if the seasonal flu vaccine is right for me*, and *I would find it easy to attend my GP surgery to receive the seasonal flu vaccine*) were more likely to have high intent. This important finding indicates that those patients who felt informed had higher vaccine intent, and conversely feeling less well informed is associated with low intent and vaccine hesitancy in this population. Equally, those being able to easily access vaccination services had higher vaccine intent. So, if healthcare providers can enable HSCT recipients in their decision making by providing information, this may enhance vaccine uptake. Likewise, if ease of access to vaccination services can be improved, logically this may also enhance uptake.

Those HSCT recipients who agreed with the emotional response statement (*If I receive the seasonal flu vaccine next winter I will worry less about catching the seasonal flu*) and anticipated regret statement (*I would regret it if I decided not to receive the seasonal flu vaccine next winter and became unwell with seasonal flu*) were more likely to have high vaccine intent. While it would not be appropriate to promote vaccine uptake by

provoking anxiety, discussion of potential benefits of vaccination with HSCT recipients may impact on these areas of health belief, and so promote SIIV intent and uptake. The anticipated regret statement '*I would regret it if I decided to receive the seasonal flu vaccine next winter and became unwell with side effects'* was not associated with vaccination intent and the mean agreement score for both groups was close to the neutral value. In multivariable analysis, after adjustment for age and pre HSCT influenza vaccination, stronger agreement with the emotional response statement, was significantly associated with high intent.

The emotional response construct when added to the HBM significantly improved the predictive value of the model. Both age over 65, and no vaccination prior to HSCT were significantly associated with low intent in both models. In a sub-analysis of those aged under 65, no vaccination prior to HSCT was not a significant predictor of intent.

4.12.5 GP and HSCT Team Cues to Vaccination

Cues to vaccination statements explored whether participants believe that their GP and HSCT team understand their condition well enough to know if the influenza vaccine is right for them, and if they were advised to have the vaccine whether they would do so. Most HSCT recipients with high intent scores gave high cue scores for both their HSCT team (100%) and GP (84.3%). Of those with low intent scores, the majority gave high

cue scores for their HSCT team (81.8%), but only a minority (31.8%) for their GP. This suggests that for those patients with high intent, recommendations from either their HSCT team or GP would reinforce planned behaviour, whereas for those with low intent, discussion with and advice from their HSCT team may be particularly important in helping to change intent. Cues from healthcare providers are considered a key factor in promoting vaccine uptake(291) and a study of cancer patients identified recommendation from an oncologist as a key predictor of vaccine uptake although did not compare this with advice from a general physician or practitioner(292). Our findings suggest that HSCT recipients particularly value the advice of their specialist team, and this reinforce the need for HSCT specialists to engage in discussion with patients about influenza vaccination.

4.12.6 Preferred Vaccination Location

Preference for vaccination at HSCT centres rather than GP practices was similar at 43.7% and 54.5% in low and high intent groups respectively. In the high intent group, more patients expressed a preference for vaccination at their GP practice than in the low intent group. This suggests that for approximately 50% of those with both low and high intent, access to a seasonal influenza vaccination service at HSCT centres may facilitate vaccination uptake.

4.12.7 Study Strengths and Limitations

The study adapted an established theoretical framework to investigate factors influencing vaccination intent in the high-risk HSCT population. Recruitment fell short of calculated sample size and therefore significant predictors may not have been detected in multivariable analysis. The study did not evaluate vaccine uptake, and so could not establish how this relates to intent. Although, intent is considered a reliable predictor of behaviour (74), other factors may exert influence and therefore the two may not correlate. This may be the case in this study population in whom changes in health status can occur relative rapidly due to post HSCT complications. The questionnaire was designed to be as specific as possible with regard to vaccine type (seasonal influenza vaccine) and timing of vaccination (the 2016-2017 influenza season). Questions were given a 'behavioural context' (ie if the vaccine is received or not) as otherwise intent may have influenced responses; for example someone with high vaccination intent may disagree with the statement '*the flu would be a severe illness for me*' as they believe the vaccine they intend to receive will offer protection(294). The questionnaire did not enquire about detailed aspects of HSCT procedure and complications including donor type, stem cell source, level of HLA matching, post-HSCT immunosuppressive therapy and GvHD and it remains possible that these variables may independently impact on intent. Patients were advised that their healthcare team would not have access to responses, and questionnaires were returned in sealed envelopes. However, despite this it remains the case that responses may be biased, and not accurately reflect individuals' healthcare beliefs. The study provides a snapshot at a

single time point within the 4 months prior to the influenza season and variation in intent over the year would not be captured by this study design.

4.12.8 Conclusion

This is the first study to investigate promoters and barriers to influenza vaccine intent in HSCT recipients. These findings offer a valuable insight into the demographic and behavioural factors, and health beliefs that contribute to vaccine intent amongst this high-risk patient group. Overall vaccine intent was high in the four months before the 2016-2017 UK influenza season. Lowest rates of intent were in the over 65 age group, and particularly amongst those who had not previously received the influenza vaccine. Whether this was related to prior vaccine refusal in this age group could not be determined from the study and warrants further investigation. Lower rates of intent were also found in the 16-34 age group. While we would advocate efforts to optimise vaccine intent and uptake across all HSCT recipients, it may be that specific interventions could usefully target these two age groups.

Based on our findings, the HBM is a useful framework for structuring interventions (whether face-to-face, or patient information literature) to increase SIIV uptake. Drawing attention to HSCT recipients increased risk of influenza, both in terms of susceptibility and severity, discussing the potential benefits of vaccination, and

exploring concerns around side effects may help to promote vaccine intent and uptake. Equally, planned behaviour may be reinforced through such discussion. Exploring and discussing reasons for refusal prior to HSCT may also be important.

For those with high vaccination intent, a recommendation from either the HSCT team or recipients' GP may help to reinforce planned behaviour. However, for those with low intent, recommendation from the HSCT team are particularly important. Currently only a minority of alloHSCT centres offer vaccination, and where practical and financially viable, the option for HSCT recipients to receive the SIIV locally could be explored as a potential means to encourage and optimise uptake.

Based on these findings, we would recommend the development of specific interventions aimed at reinforcing planned behaviour, and increasing intent amongst those who do not plan to receive the SIIV. The effectiveness of such interventions should be prospectively assessed. However, we would recommend that as a simple and potentially effective measure, HSCT specialists explore SIIV intent with their patients prior to the annual influenza season, address concerns about side effects, and discuss the rationale for and potential benefits of seasonal influenza vaccination.

5 Autoimmune cytopenias (AIC) following allogeneic haematopoietic stem cell transplant for acquired aplastic anaemia: A joint study of the Autoimmune Diseases and Severe Aplastic Anaemia Working Parties (ADWP/SAAWP) of the European Society for Blood and Marrow Transplantation (EBMT).

The following study was conducted as part of this MD (Res) with the support of the Autoimmune Diseases and Severe Aplastic Anaemia working parties of the European Society for Blood and Marrow Transplantation (EBMT). The study protocol was developed with assistance of Simona Iacobelli, EBMT Statistical Unit. Data collection and handling was overseen by Cora Knol-Bout, Sofie Terwell and Paul Bosman, EBMT Leiden Data Office. Statistical analysis was performed by Dirk-Jan Eikema, EBMT Statistical Unit.

5.1 Introduction

5.1.1 Immune mediated complications following HSCT

The structure and function of the healthy human immune system were reviewed in chapter 1, along with the necessary yet deleterious effect of HSCT on immunity and

subsequent patterns of immune reconstitution. Most of the major complications of HSCT are linked to this balance of immune function: Infection, as already outlined, results from quantitative and qualitative immune defects; GvHD describes alloreactivity between a transplanted donor immune system and host major or minor histocompatibility antigens, and may manifest as acute and/or chronic organ-specific or multi-system disease; Insufficient graft versus host interaction may result in limited GvL effect and an increased risk of relapse, which may be overcome by infusion of donor lymphocytes(25). A less common, but increasingly recognized complication of HSCT is autoimmune disease, thought to result from pathological immune autoreactivity targeting non-histocompatibility antigen common to donor and recipient. Autoimmune diseases have been described in a range of HSCT settings including autologous HSCT(295,296), allogeneic(297,298) and syngeneic HSCT(299). Transfer of autoimmune disease from donor to recipient has also been reported(300). Collectively this indicates that post-HSCT autoimmune disease is a pathological entity distinct from GvHD. Whether autoreactivity develops exclusively from the graft, or also in host immune cells that have survived conditioning, has not been determined.

5.1.2 Mechanisms of self-tolerance and pathogenesis of autoimmunity

As discussed in Chapter 1, immune-regulation is vitally important and limits autoimmune reactions. This immune-regulation, or self-tolerance, may be divided into central and peripheral mechanisms. Central tolerance describes the negative selection

of immature, autoreactive lymphocytes in central lymphoid tissue. While strong ligation of mature naïve T or B cell receptors by MHC:peptide complex on an APC in peripheral lymphoid tissue results in cell proliferation, strong activation of immature lymphoid cells in central tissue leads to a halting of maturation, receptor editing, and if receptor specificity and affinity remains strong after 1-2 days, cell apoptosis(5). It is thought that through positive selection of reactive lymphocytes, and this negative selection of strongly reactive lymphocytes, that populations of cells responsive to pathogen but not self, emerge from central lymphoid tissue(1). However, this central negative selection is not absolute, and mechanisms of peripheral tolerance provide a further layer of control. Peripheral tolerance may be divided into regulation intrinsic and extrinsic to the autoreactive cell. Intrinsic mechanisms include downregulation of B cell receptors on autoreactive clones(301), and upregulation of inhibitory receptors such as CD5 which limits immunoglobulin receptor signalling on B Cells(302), and Cytotoxic T-Lymphocyte antigen 4 (CTLA4) which is an inhibitor of the T Cell co-stimulatory pathway(303). Extrinsic mechanisms of regulation include: B Cell activating factor (BAFF) and Interleukin 7 (IL7) mediated competitive survival, whereby strongly autoreactive clones require high levels of these cytokines for survival, and are at a competitive disadvantage compared with more weakly reactive clones (304); induction of cell anergy in which autoreactive clones stimulated by self-antigen in the absence of the co-stimulatory signals associated with infection or inflammation become quiescent; and finally control of autoreactive clones by T Reg cells which limit differentiation to effector cells, and restrict effector function(6).

The pathogenesis of autoimmune disease remains poorly understood but seemingly relate to breakdown in these mechanisms of self-tolerance. First, there may be a genetic role, with documented familial aggregation of autoimmune conditions(305), the association of certain HLA types with autoimmune diseases (306), and autoimmunity manifesting in inherited primary immunodeficiencies (307). Secondly, environmental factors may contribute; in murine models inflammatory states have been show to trigger and promote autoreactive T cell populations (308,309), and infection may be associated with lymphodepletion and homeostatic expansion favouring autoreactive clones(310). Finally, iatrogenic dysregulation of the central and peripheral immune tolerance, through chemo-or immunotherapy leading to thymic damage and impaired thymic function, loss of regulatory T Cell control (6) and, again, homeostatic expansion post lymphodepleting therapies. Clearly, all of these factors may play a role in pathogenesis of autoimmune disease following HSCT.

5.1.3 Risk factors for autoimmune disease post HSCT

A number of possible risk factors for development of autoimmune disease post HSCT have been identified. These are non-malignant primary disease(311,312), an unrelated donor(313), profoundly lymphodepleting conditioning with alemtuzumab and ATG(295), and active chronic graft-versus-host-disease (cGvHD) (313,314). A large multi-centre retrospective study has identified use of ATG and CD34+ graft selection as a risk factor for new autoimmune disease, in the setting of autologous HSCT for primary

autoimmune disease(296). A multicentre study of umbilical cord blood transplants (UCBT) has identified younger recipient age and non-malignant primary disease as a risk factor for developing autoimmune disease(297).

5.1.4 Manifestations of autoimmune disease post HSCT

A spectrum of autoreactivity has been described following HSCT. This includes reports of autoreactivity without overt disease, for example detection of positive direct antiglobulin test (DAT) without overt haemolysis(315); transient and self-limiting autoimmunity in the form of positive thyroid peroxidase antibodies with associated hyperthyroidism(316); and finally overt, chronic, organ specific or multisystem autoimmune disease(317). The range of documented autoimmune diseases post-HSCT are summarised in Table 21.

- Haematological
  - Autoimmune Cytopenias
  - Acquired Haemophilia*
- Endocrine
  - Thyroiditis
  - TSH-Receptor blocking Ab*
- Neurological
  - Transverse myelitis
  - Graves' Disease
  - Myasthenia Gravis*
  - Peripheral Neuropathy
- Gastrointestinal
  - Ulcerative Colitis
  - Crohn's Disease
- Rheumatological
  - SLE
  - Antiphospholipid Syndrome*
  - RA
  - Sarcoidosis*
- Dermatological
  - Vitiligo
  - Psoriasis

*Table 21: Summary of autoimmune diseases documented post autologous and allogeneic HSCT. *=reported after autologous HSCT only.*(295–298,313)

The most commonly encountered AID post HSCT are autoimmune cytopenias (AIC) (317). AICs have previously been described in a number of HSCT contexts, but large registry studies hitherto have not explored AIC following HSCT for a single disease entity (298,313,315,318–320).

5.1.5 Acquired Aplastic Anaemia

Acquired aplastic anaemia (aAA) is defined as 'pancytopenia with a hypocellular marrow in the absence of an abnormal infiltrate or marrow fibrosis'(321). aAA is thought to be an autoimmune disorder in most cases with immune-mediated destruction of HSCs the final step in the pathological pathway(322) (323). Disease severity is stratified according to modified Camitta criteria, (324) and decision to proceed to allogeneic HSCT is based on patient age and comorbidity, response to first-line treatment and availability of a suitable donor (see Figure 33)

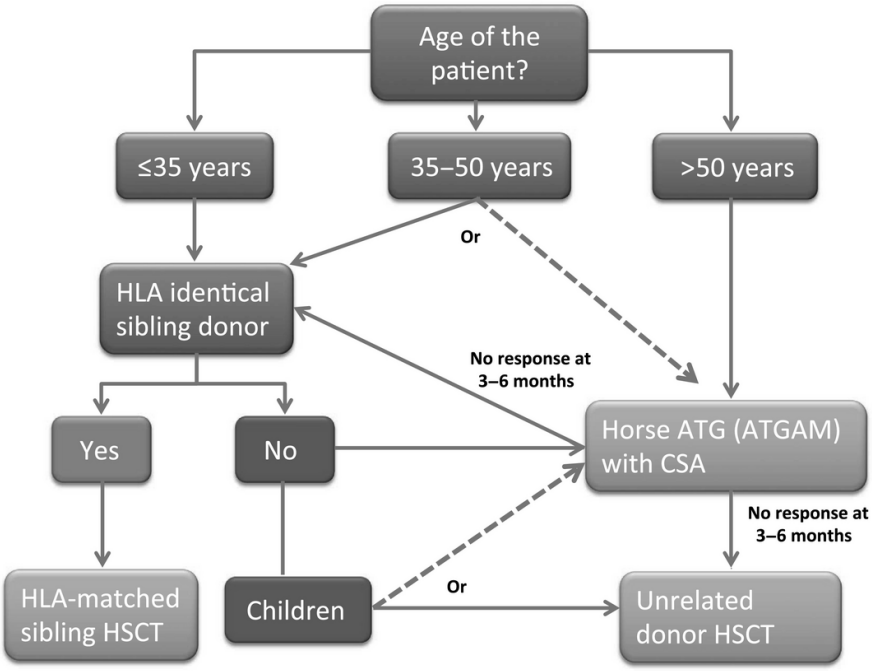

Figure 33: British Society of Haematology Management algorithm for patients with severe aplastic anaemia (321)*. Adapted from* (325)*. Used with permission.*

5.2 Study Overview

5.2.1 Study hypothesis, objectives and endpoints

Given documented risk factors for development of autoimmune disease post HSCT, and based on clinical suspicion, we hypothesised that the incidence of the most common autoimmune phenomenon post-HSCT, namely autoimmune cytopenias, are higher than described in other patient populations. We also sought to explore AIC diagnostic strategies, treatment approaches and patient outcomes across EBMT centres.

Primary Objective

To estimate the incidence of autoimmune cytopenias in adult and paediatric patients treated with allogeneic HSCT for aplastic anaemia between 1st January 2002 and December 31st, 2012 at EBMT centres and registered in the EBMT database.

Secondary Objectives

i)To identify risk factors associated with development of autoimmune cytopenias in the above population

ii)To document AIC diagnostic criteria employed across EBMT centres

iii)To document treatment strategies and responses

iv) To estimate overall survival following onset of AIC

Corresponding study endpoints were as follows:-

Primary End-point

The incidence of patients treated for aplastic anaemia with first allogeneic HSCT at EBMT centres between 2002 and 2012 who developed one or more autoimmune cytopenias

Secondary End-points

risk factors for developing autoimmune cytopenias by multivariate analysis

description of diagnostic criteria (Clinical and laboratory) applied to patients with reported AIC

Description of treatment strategies and response to each line of treatment

Survival time following onset of AIC

5.2.2 Inclusion and exclusion criteria

Inclusion Criteria

Adult and paediatric patients treated with first allogeneic HSCT for aAA at EBMT centres between 1 January 2002 and 31 December 2012.

Exclusion criteria

Autoimmune cytopenia diagnosed prior to HSCT

Second or subsequent allogeneic HSCT for aplastic anaemia

5.2.3 Methods and Statistical Analysis

This study was conducted in accordance with the Declaration of Helsinki, and approved by the European Society for Blood and Marrow Transplantation (EBMT) Autoimmune Disease Working Party (ADWP) and Severe Aplastic Anaemia Working Party (SAAWP). All EBMT centres performing allogeneic HSCT for aAA were invited to participate in the study. Centres agreeing to participate identified paediatric and adult patients with aAA treated between January 2002 and December 2012 with first allogeneic HSCT. Centres confirmed whether AIC was diagnosed post-HSCT. Patients diagnosed with AIC at any time point prior to HSCT were excluded. Data for patients without AIC were extracted from the EBMT registry. Centres identifying cases of AIC provided additional data on diagnostic criteria and investigations undertaken, therapies administered, and patient outcome using a study specific minimal essential data C (MEDC) form (See Appendix 9)

Incidence of AIC at 1,3,5 and 10 years post allogeneic HSCT was estimated by cumulative incidence curves, and death without AIC was considered as the competing event. In univariable analysis, impact of categorized risk factors on AIC incidence was evaluated using Gray's test. The independent impact on risk of variables significant in univariable analysis was assessed using a Cox cause-specific hazard model. Diagnostic criteria, treatment and outcomes are described. Where data points were missing this is indicated and cases were omitted from analyses pertaining to the missing variable only.

5.3 Results

530 patients (37.2% paediatric, 62.8% adult) patients from 41 participating centres were eligible for inclusion in the study. Median age at HSCT was 21.4 years (range 1.7-69.8). The median follow-up time was 6.4 years (interquartile range 4.2 to 9.0), and 1, 5 and 10-year overall survival for the cohort was 85.3% (95% CI 82.3-88.4%), 80.8% (77.4-84.2) and 78.8% (75.0-82.6%).

Characteristics of patients, grafts, and conditioning regimens are provided in Table 22. 25 patients at a median of 10.6 (range 2.6-91.5) months post allogeneic HSCT were diagnosed with AIC as follows: 32.0% (n=8) immune thrombocytopenia (ITP), 28.0% (n=7) autoimmune haemolytic anaemia (AIHA), 24.0% (n=6) Evans Syndrome (5 cases with AIHA and ITP, 1 case with AIHA and AIN), and 16.0% (n=4) autoimmune neutropenia (AIN) (Table 22).

The cumulative incidence of AIC at 1, 3, 5 and 10 years post HSCT was 2.5% (95% CI 1.2-3.9%), 4.4% (2.6-6.2%), 4.6% (2.8-6.5%) and 5.1% (3.1-7.2) respectively. Overall survival at 5 years after diagnosis of AIC was 85.9% (95% CI 71-100%), with all deaths occurring within the first 12 months.

Variable, n(%)	Cumulative Incidence of AIC at 10 years	95%CI	p value
Patient Sex, n=530			
Male, 317 (59.8)	5.6	2.7-8.5	
Female	4.6	1.6-7.5	0.779
Patient Age Group, n=530			
<18, 197 (37.2)	3.2	0.7-5.8	
≥18	6.4	3.3-9.4	0.209
Interval aAA diagnosis to treatment, n=530			
<12 months, 381 (71.9)	4.5	2.4-6.7	
>12 months	6.6	1.8-11.3	0.589
Patient CMV status, n=469			
Negative, 157 (33.4)	8.3	2.8-13.9	
Positive	3.7	1.6-5.9	0.142
Donor Type, n=521			
Related, 328 (62.4%)	4.0	1.0-6.0	
Unrelated	7.0	3.0-10.0	0.077
Stem Cell Source, n=521			
Bone Marrow, 358 (68.7)	3.3	1.2-5.6	
Peripheral Blood, 143 (27.4)	9.7	4.7-14.8	
Cord Blood 20 (3.8)	5.0	0.0-14.6	0.01*
Patient / Donor CMV Match, n=460			
Matched, 325 (70.7)	5.4	2.6-8.3	
Mismatched	4.7	1.0-8.3	0.959
Patient / Donor Sex Match n=515			
Matched, 294 (57.1)	4.9	2.0-7.9	
Mismatched - Male Donor, 111 (21.6)	5.8	1.3-10.3	
Mismatched - Female Donor, 110 (21.4)	4.7	0.7-8.7	0.854
Conditioning Type, n=497			
Myeloablative, 298(60)	2.9	0.6-5.2	
Reduced Intensity	8.1	4.2-12.1	0.005*
TBI, n=516			
Yes, 53 (10.3)	4.9	2.7-7.1	
No	8.1	4.8-15.7	0.305
Fludarabine containing regimen, n=530			
Yes, 309 (58.3)	6.5	3.7-9.4	
No	3.0	1.9-5.8	0.037*
Antithymocyte Globulin containing Regimen, n=530			
Yes, 202 (38.1)	2.7	0-0.56	
No	7.1	4.1-10.1	0.007*
ATG and Fludarabine	3.4	0.0-8.5	
ATG without Fludarabine	1.9	0.0-4.5	0.979
Alemtuzumab Containing Regimen, n=530			
Yes, 298 (60)	10.3	4.5-16.0	
No	3.6	1.6-5.7	0.003*
Alemtuzmab and Fludarabine	4.8	0.0-13.9	
Alemtuzumab without Fludarabine	11.6	4.8-18.4	0.118

*Table 22: Characteristics of n=530 recipients of allogeneic HSCT for aAA between 2002-2012. *=statistically significant at 95% confidence level*

5.3.1 Diagnostic Criteria

All AIC cases were suspected due to a new or worsening cytopenia. Direct antiglobulin test (DAT) was performed in 6/7 AIHA patients with 1 IgG positive, and 5 IgG and C3d positive (Table 23). All centres identified at least one biochemical marker of haemolysis (raised lactate dehydrogenase, unconjugated hyper-bilirubinaemia, or reduced haptoglobin) and 3 identified a peripheral blood reticulocytosis. Bone marrow examination (BME) was performed in 6/8 ITP patients, with 4 displaying normo- or hyper-regenerative megakaryopoiesis. All cases of Evans syndrome were DAT positive (4 IgG, 2 IgG and C3d) and all cases involving AIHA and ITP had BME with 4 displaying normo- or hyper-regenerative megakaryopoiesis. Biochemical markers of haemolysis were present in 5/6 cases and 2 patients had a peripheral blood reticulocytosis. Anti-neutrophil antibodies (ANAb) were screened for in all AIN cases, and the Evans syndrome case presenting with AIHA and AIN case, but were only detected in 1 patient.

5.3.2 Treatment and Response

Of 25 patients diagnosed with AIC, 21 (84%) were treated, while 2 (8%) ITP (case 1 and 2) and 2 (8%) AIN patients (case 22 and 23) received no AIC specific therapy. Treatment approach was heterogenous regarding both choice of therapeutic agents, and number of agents used at each treatment line (Table 23). The mainstays of therapy for ITP, AIHA and Evans syndrome were corticosteroids and intravenous immunoglobulin (IVIg). Corticosteroids were administered to 50%, 100% and 83% of treated ITP, AIHA and

Case No.	Diagnosis	Gender	Age at Allograft (Years)	Years from AlloHSCT to AIC diagnosis	DAT / Autoantibody	Treatmet Line and Response								AIC status at last follow-up
						First		Second		Third		Fourth		
1	ITP	Female	21	2.4	NA	No treatment								Untreated
2	ITP	Female	50	2.7	NA	No treatment								CR
3	ITP	Male	23	7.6	NA	Corticosteroid, IVIg	CR							CR (died)
4	ITP	Male	58	1.4	NA	CSA	PR*							PR
5	ITP	Male	21	0.6	NA	CSA, MMF, Rituximab	NR	Splenectomy	CR					CR
6	ITP	Female	37	1.2	NA	IVIg	CR	IVIg	CR					CR
7	ITP	Male	26	0.2	NA	Corticosteroid	NR	IVIg	NR	Splenectomy	CR			CR
8	ITP	Female	68	1.6	NA	Corticosteroid	NR	IVIg	NR	Rituximab	NR			NR (died)
9	AIHA	Male	38	0.5	IgG and C3d	Corticosteroid	CR							CR
10	AIHA	Female	17	0.4	IgG and C3d	Corticosteroid, IVIg	NR	Rituximab, PEX	CR					CR
11	AIHA	Male	17	1.4	IgG and C3d	Corticosteroid	NR	Rituximab	PR					PR
12	AIHA	Male	20	0.4	IgG	Corticosteroid, IVIg	NR	Rituximab, CSA	CR					CR
13	AIHA	Male	42	0.7	Not performed	Corticosteroid	CR*	Corticosteroid	CR*					CR
14	AIHA	Male	7	0.6	IgG and C3d	Corticosteroid	CR	Rituximab	CR	Rituximab, MMF	CR	Rituximab	CR	CR
15	AIHA	Female	20	0.8	IgG and C3d	Corticosteroid	NR	Rituximab	NR	IVIG	NR	Splenectomy	CR	CR
16	Evans Syndome (AIHA and ITP)	Female	5	2.4	IgG	Corticosteroid, IVIg	NR	Rituximab	NR					NR
17	Evans Syndome (AIHA and ITP)	Female	18	0.4	IgG and C3d	Corticosteroid, IVIg	CR*	Corticoseroid, IVIg, Rituximab	CR*					CR
18	Evans Syndome (AIHA and ITP)	Male	45	0.3	IgG	Corticosteroid, Rituximab	CR	Corticoseroid, IVIg	PR					PR
19	Evans Syndome (AIHA and ITP)	Male	19	0.4	IgG	Corticosteriod, IVIg	PR*	Corticosteroid, IVIg	NR	IVIg	PR*			PR
20	Evans Syndrome (AIHA and AIN)	Male	22	0.5	IgG and C3d	Corticosteroid, Rituximab	NR	Cyclophosphamide, PEX	NR	Splenectomy	NR			NR (died)
21	Evans Syndome (AIHA and ITP)	Female	11	1.0	IgG	IVIG	NR	Rituximab	NR	MMF	NR	Splenectomy	PR	PR
22	AIN	Female	4	Unknown	ANAb detected	No treatment								Untreated
23	AIN	Male	25	4.4	ANAb not detected	No treatment								Untreated
24	AIN	Male	63	1.2	ANAb not detected	GCSF	PR*							PR
25	AIN	Male	55	1.0	ANAb not detected	GCSF	PR*	GCSF, CD20mAb	CR*					CR

*Table 23: Treatment and outcome of 25 patients diagnosed with AIC following allogeneic. *Maintenance therapy required*

Evans syndrome patients respectively. Intravenous immunoglobulin (IVIg) was administered concurrently with corticosteroids, or as first or second-line monotherapy, in 66.7%, 42.9% and 83.3% of ITP, AIHA and Evans syndrome patients. The anti-CD20 monoclonal antibody Rituximab was administered to 71.4% and 83.3% of patients with AIHA and Evans syndrome respectively, but only 16.7% of ITP patients. Other immunomodulatory therapies for this group of patients were mycophenolate mofetil (MMF) 15%, and Cyclosporine A (CSA) 15%. 1 patient with Evans syndrome presenting with AIHA and AIN (Case 20) received cyclophosphamide and underwent plasma exchange (PEX). 4 (19.0%) patients did not respond or relapsed following immunomodulatory therapy and underwent splenectomy, with 2 achieving CR (1 ITP – Case 7, 2 AIHA Case 15), 1 PR (Evans Syndrome – Case 21) and 1 patient (AIHA and AIN – Case 20) dying without responding. Of the four patients with AIN, 2 (Case 24 and 25) were treated with granulocyte-colony stimulating factor (GSCF) to PR, one of whom required second-line therapy and achieved CR with Rituximab (case 25).

Overall 47.6% (10/21) of patients did not respond to first-line therapy, with 41.2% (7/17) and 57% (4/7) not responding to second or third-line therapy respectively. Of the 52.4 % (11/21) of treated patients who achieved either complete or partial response to first-line therapy, nearly two-thirds (63.6%, 7/11) relapsed and required further treatment. AIC status at last follow-up was 12% (3/25) untreated, 52% (13/25) CR, 24% (6/25) in PR, and 12% (3/25) NR. 24% (6/25) of patients required ongoing maintenance therapy (Table 23). Highest CR rate at last follow-up was seen in AIHA (85.7%) and lowest in Evans Syndrome (16.7%) (Figure 34). 3 patients died during the follow-up period, 1 in CR and 2 without responding to therapy.

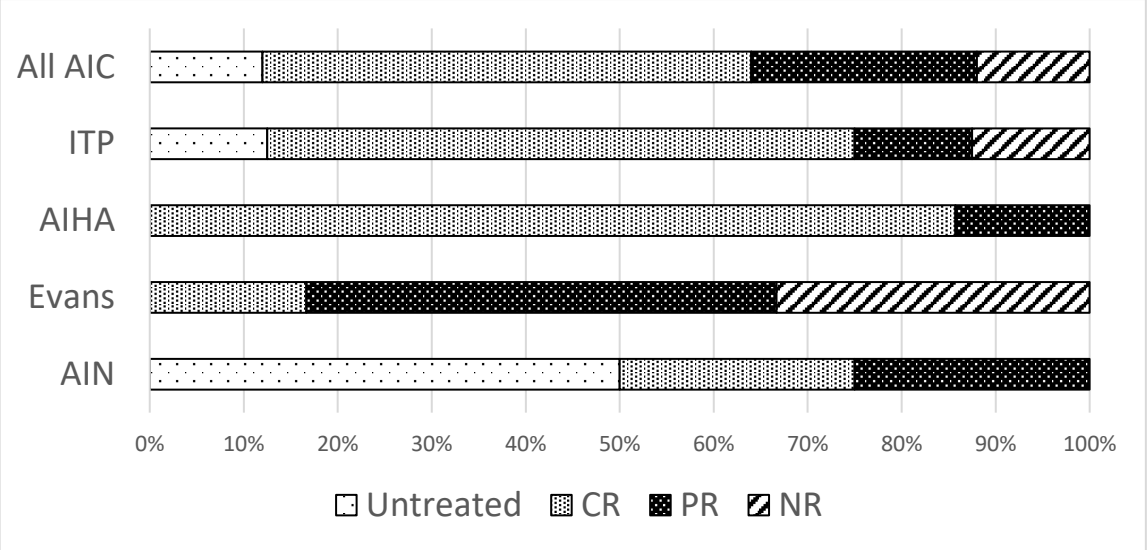

Figure 34: Status at last follow-up of 25 patients diagnosed with AIC following allogeneic HSCT for aAA between 2002-2012

Risk Factors for Development of AIC

In univariable analysis, cumulative incidence of AIC at 10 years post HSCT did not vary with patient sex (p=0.779), patient age group (<18 vs ≥18, p=0.209), interval from aAA diagnosis to HSCT (<12 vs ≥12 months, p=0.589), patient CMV status (p=0.142) donor type (related v unrelated, p=0.077), patient/donor CMV match (p=0.959), patient/donor sex match (p=0.854), or conditioning with TBI (p=0.305) (Table 22). However, 10-year cumulative incidence of AIC was higher amongst patients treated with non-myeloablative conditioning (8.1% [4.2-12.1] vs 2.9% [0.6-5.2], p=0.005), and conditioning regimens containing Fludarabine (6.5% [3.7-9.4] vs 3.0% [1.9-5.8], p=0.037) or Alemtuzumab (10.3% [4.5-16.0] vs 3.6% [1.6-5.7], p=0.003). Conditioning with anti-thymocyte globulin(ATG) was associated with a lower incidence of AIC (2.7 [0.0-5.6] vs 7.1% [4.1-10.1], p=0.007). Incidence of AIC was also significantly higher in

patients receiving PBSC (9.7% [4.7=14.8]) compared with UCB (5.0% [0.0-14.6]) and BM (3.3% [1.2-5.6]) (p=0.01). Incidence of AIC was not higher in regimens containing alemtuzumab and fludarabine compared with alemtuzumab alone (4.8 [0.0-13.9] vs. 11.6 [4.8-18.4]; p=0.118), nor in regimens containing ATG and fludarabine compared with ATG alone (3.4 [0.0-8.5] vs. 1.9 [0.0 – 4.5]; p=0.979).

A subgroup of 475 patients who received either BM or PBSC grafts, and had complete information on conditioning regimen were included in a multivariable analysis. Two models explored the independent association of conditioning intensity, stem cell source, use of alemtuzumab and/or fludarabine, or use of ATG with risk of AIC (Table 24). In the fludarabine and/or alemtuzumab model, these agents were not associated with an increased risk of AIC (1.24 [0.37-4.19]; p=0.723). However, after adjustment for alemtuzumab and/or fludarabine, transplant with PBSC remained independently associated with a higher risk of AIC (2.81 [1.06-7.49]; p=0.038), and MAC with lower risk (0.35 [0.12-0.98]; p=0.046). Likewise, in the multivariable ATG model, use of this serotherapy in conditioning was not associated with higher risk of AIC (0.35 [0.12-1.04]; p=0.058) but PBSC (2.74 [1.06-7.05]; p=0.037) and MAC (0.34 [0.12-0.95]; p=0.04) remained independently associated with higher and lower risk respectively. There was no increase in the competing risk, death without AIC, associated with any of these variables. Owing to small number of AIC cases it was not possible to adjust for additional characteristics and so these findings are not generalizable beyond the study population.

Covariates	AIC	Death without AIC
Alem and/or Flu vs. Neither Alem nor Flu	1.24 (0.37-4.19);p=0.723	1.40 (0.85-2.31);p=0.181
PBSC vs. BM	2.81 (1.06-7.49);p=0.038	1.15 (0.71-1.85);p=0.572
MAC v RIC	0.35 (0.12-0.98);p=0.046	0.78 (0.49-1.22);p=0.272
ATG vs. no ATG	0.35 (0.12-1.04);p=0.058	1.13 (0.73-1.69);p=0.616
PBSC vs. BM	2.74 (1.06-7.05);p=0.037	1.26 (0.79-2.00);p=0.338
MAC v RIC	0.34 (0.12-0.95);p=0.04	0.72 (0.46-1.12);p=0.146

Table 24: Estimated cause specific hazard ratios for AIC and death without AIC using multivariable regression model for n=475 recipients of allogeneic HSCT for aAA between 2002-2012.

5.4 Discussion

In our study population, 10-year cumulative incidence of AIC after allogeneic HSCT for aAA was 5.1% (3.1-7.2). ITP was the most frequent AIC diagnosis, followed by AIHA, Evans and AIN. AIC complicated both the early and late post-HSCT period, with time of diagnosis ranging from 2.6 months to 7.6 years. Incidence of AIC in our cohort is similar to that reported in previous studies of HSCT populations. In paediatric patients transplanted with a range of allogeneic graft types for malignant and non-malignant conditions, a 2.4-2.5% 10 year incidence of AIC, and a 2.4% incidence of AIHA are reported (298,320). Among adult recipients of UCB HSCT for haematological malignancy, 3 year incidence of AIHA and ITP were 5.4% and 1.4% respectively(318). Incidence of AIHA was similar at 4.1-4.4% amongst adult recipients transplanted with a range of graft types. (311,313). Median time of diagnosis in these studies ranged from 0.8 months to 8.4 years. The retrospective design of our study and those cited may underestimate true AIC incidence.

Cases of AIC in our study were diagnosed in accordance with recognized criteria. In the absence of haematological or biochemical markers of platelet destruction, ITP is a

diagnosis of exclusion requiring thorough assessment. However BME is recommended only in selected paediatric and adult patients, generally those in whom secondary ITP is suspected, or adults over 60 (326,327). In our present study, most patients diagnosed with ITP and all with Evans syndrome underwent BME, which may reflect the need to exclude HSCT specific differentials including disease relapse and graft failure. The diagnostic features of AIHA are well described, however in secondary cases not all typical laboratory findings may be present(328). While all cases of AIHA, and all but one case of Evans syndrome presented with at least one abnormal biochemical marker of haemolysis, reticulocytosis was not a dominant feature present in one-third of AIHA and Evans syndrome patients. Testing for ANAb is recommended as part of neutropenia work-up, however these are typically of low titre and low avidity (329). In this cohort all diagnosed cases of AIN were tested for ANAb but the majority were negative.

Most ITP patients were treated with corticosteroid or IVIg, while Rituximab was infrequently used. 50% of ITP patients achieved either CR or PR with each line of therapy. In the general population a response to first-line steroids of up to 80% has been reported (327,330), and the combination of IVIg with corticosteroid therapy may hasten platelet recovery (331). In randomized studies, the combination of Rituximab with dexamethasone compared with dexamethasone alone has been shown to improve response rates from 36-37% to 58-63% (332,333). Case reports and series of patients with ITP post HSCT have reported response rates of 30-50% following first and second-line treatment, with the former largely consisting of corticosteroid with or without IVIg, and the latter IVIg, Anti-CD20mAb or splenectomy (297,334–337). In cases of post-HSCT

ITP refractory to standard therapy, TPO-RA's have proven effective(338–340). In our cohort no patients were treated with TPO-RAs, which may reflect European Medicines Agency licensing of Romiplostim in 2009 and Eltrombopag in 2010, during the last 3 years of our study eligibility period.

Although randomized data are lacking, corticosteroids are an established first-line therapy for AIHA with retrospective studies reporting responses of approximately 75% (341). Again. there is limited evidence for second-line therapy, but Rituximab or splenectomy are the most commonly described (342). Similar strategies are reported in the management of paediatric and adult Evans syndrome(343,344). Patients in our study were largely treated according to this paradigm: All patients with AIHA received first-line corticosteroid with or without IVIg; one patient requiring second line therapy was re-treated with corticosteroids; and all others received Rituximab with all patients achieving a CR or PR. Evans syndrome appeared the most problematic AIC to treat, with the greatest proportion of non-responders. However, there was no excess of deaths in this group compared with other AIC diagnoses. In cases series of patients with AIHA post-HSCT, 14-33% response rates are reported with prednisolone alone, and 40-45% with prednisolone in combination with rituximab or IVIg (297,298,313–315,320). In these series, up to 33% of patients were reported refractory to therapy, whereas all patients in our series were in CR or PR at last follow-up.

Primary AIN is typically a disease of infancy, while secondary AIN occurs mostly in adults in the context of systemic autoimmune disease (345). Neutropenia may respond to treatment of the underlying autoimmune disease with GCSF administered cautiously

owing to concern about disease flare (329). Case reports of AIN post HSCT have documented disease ranging from transient positive ANA Ab and neutropenia not requiring treatment (346), to persistent neutropenia requiring GCSF support alone (319) or in combination with additional immunomodulatory agents (298). This pattern of disease is borne out in our study with half of diagnosed patients requiring no specific therapy. Two were treated with GCSF, one of whom required additional therapy with Anti-CD20mAb. Both treated patents achieved a PR.

In allogeneic HSCT for treatment of aplastic anaemia, conditioning regimens are modified according to recipient age and typical regimens are comprised of cyclophosphamide or fludarabine, ATG or Alemtuzumab, and total body irradiation in the setting of alternative donors. (347–350). In univariable analysis we identified an increased incidence of AIC amongst patients treated with alemtuzumab or fludarabine. Alemtuzumab used for treatment of multiple sclerosis has been associated with a range of autoimmune phenomenon including ITP (351,352). In patients with primary autoimmune disease treated with autologous HSCT, alemtuzumab exposure was identified as risk factors for secondary autoimmune disease (295,296). AICs have been described following fludarabine therapy for CLL (353–356), other haematological malignancies (357) and in a drug surveillance study (358), although this association has not been reported consistently (359,360). In contrast with our findings, conditioning with ATG has been associated with an increased risk of secondary autoimmune phenomenon following autologous HSCT for primary autoimmune disease (295,296), with lymphocyte depletion the putative mechanism. *In vitro,* ATG may promote expansion of CD4+CD25+ regulatory T (T Reg) cells from peripheral mononuclear cells

(361,362) although this is limited to rabbit (rATG) rather than horse ATG (hATG)(363). In a murine autoimmune encephalomyelitis model, rATG depleted effector T Cells, enhanced expansion of T Reg cells, prevented disease occurrence and attenuated early manifestations of the induced autoimmune disease (364). rATG has been reported to be beneficial in cases series of patients refractory to or unable to tolerate standard therapies for autoimmune disease (365–369). hATG is recommended as first-line immunosuppressive therapy for AA (321), however both hATG and rATG may be used in HSCT conditioning (349). The proportion of patients receiving rATG and hATG in our current study is not known. In the context of allogeneic HSCT, ATG may reduce autoreactivity and contribute to a reduction in risk of AIC. However, this finding did not remain significant in multivariable analysis, possibly due to small number of events, and further investigation is warranted.

In allogeneic HSCT for AA, BM conveys a lower risk of chronic GvHD and a survival advantage compared with PBSC, and is the recommended stem cell source in all age groups (370–372). Nonetheless, approximately one-third of all patients were transplanted with either PBSC or UCB in our study. Incidence of AIC was significantly higher amongst recipients of PBSC compared with UCB and BM. In multivariable analysis, after adjustment for concurrent ATG, and Fludarabine and/or alemtuzumab, transplant with PBSC and RIC was independently associated with a higher risk of AIC. A higher number of immunocompetent cells are harvested and transplanted with PBSC compared with BMH, (373) and immune recovery is more rapid (41). Early T Cell

recovery occurs predominantly through peripheral expansion, rather than de novo thymic ontogenesis in both T replete and deplete grafts (374,375). In RIC, host T cells that survive the conditioning regimen may also expand through this mechanism (376). Such homeostatic expansion contributes to a narrow T cell repertoire, qualitative immune deficiencies (376) and may favour auto-reactive clones(310), contributing to the emergence of autoimmune disease (45). It is possible that the larger inoculum of immunocompetent cells and more rapid immune reconstitution in PBSC compared with BM, and the potential for homeostatic expansion of host lymphocytes in RIC promotes development of autoimmunity; immune reconstitution studies in patients with AIC may provide further detail.

5.5 Conclusions

In conclusion, the incidence of AIC post allogeneic HSCT for aAA is similar to that reported among recipients of HSCT for other primary diseases. A diagnosis of AIC should be considered and investigated in cases of new or worsening cytopenia at any stage post alloHSCT for aAA. Diagnosis may be challenging and BME should be considered. Response rates reported here are similar to previous data, but treatment strategies are heterogenous and data registration (via the EBMT and other databases), is warranted to inform harmonized recommendations for management. Overall survival in the AIC group was not reduced compared with the overall cohort, although small number of events limited further analysis. Consistent with previous studies, exposure to fludarabine and alemtuzumab may contribute to post HSCT AIC, although these findings did not remain significant in multivariable analysis. Our finding that ATG may

ameliorate risk of AIC post allogeneic HSCT for aAA warrants further investigation as part of future prospective studies. We identified an increased risk of AIC following PBSC and RIC HSCT in this cohort. If validated in other data sets, this may provide further evidence that BM is the preferred stem cell source for transplant of patients with aAA.

6 Concluding Discussion

6.1 Routine Vaccination Programme (RVP) Practice after Adult and Paediatric Allogeneic Haematopoietic Stem Cell Transplant: A British Society of Blood and Marrow Transplantation Survey of UK NHS-Based Programmes.

6.1.1 Key Findings

With an overall response rate of 95% of UK adult and paediatric alloHSCT programmes, this BSBMT national study offers an up-to-date, detailed and comprehensive analysis of current RVP practice.

While all responding alloHSCT programmes recommend a RVP to recipients of alloHSCT, the specifics of practice varied across all survey themes, with heterogeneity in service organization, vaccine selection, commencement and delay of vaccination, and monitoring of response to vaccination. Factors contributing to this may include weak evidence for RVP practice, variation between guidelines, restrictions of national vaccine licensing and in some cases lack of familiarity with current recommendations.

6.1.2 Future Directions

A previous BSBMT vaccination survey conducted in 2007 (111) identified variation in vaccine selection. Although several international organisations have published recommendations in the intervening years, disparate practices persist. This indicates that there remains the need for a concerted effort to draw these issues to the attention of the HSCT community, and offer approaches to harmonize practice and bring it into line with current evidence.

6.1.2.1 Dissemination of Findings

The results of the survey have been presented orally at an international infectious disease conference (Federation of Infection Societies Annual Conference 2015). The survey was recently published in *Bone Marrow Transplantation*(132). An abstract has been submitted for the BSBMT 2017 Scientific Day so the findings can be presented directly to the UK HSCT community, promoting interest, engagement and discussion in this area.

6.1.2.2 Further Work

6.1.2.2.1 Engagement with Primary Care

The potential gap between HSCT programme recommendations and vaccine delivery in primary care has been highlighted by this survey. Potential barriers to effective RVP practice in Primary Care include: i) in some cases lack of specific vaccine recommendations from HSCT programmes. ii) Difficulty in or concern about using vaccines off-license (e.g. PCV13 or DTaP in adult HSCT recipients). iii) Poor information flow from primary care to the HSCT centre and vice-versa, and iv) Possibly inadequate oversight, quality assurance and audit of RVP practice. Furthermore, as most HSCT recipients (and certainly adult recipients) are receiving vaccines outside the national vaccination schedule, GP practices may encounter difficulties in record keeping and reimbursement for vaccines administered. An essential step in developing effective post HSCT RVP practice is to understand how HSCT programme recommendations translate into vaccine delivery in primary care, and further elucidate promoters and barriers to practice experienced by primary care practitioners.

A practical initial step would be to convene a focus group of GPs who care or have cared for HSCT recipients to discuss and explore these issues. After identifying common themes, a GP survey could be developed to scope this at a national level. Findings would help to ensure future guidelines or recommendation addressed practical issues.

6.1.2.2.2 Expanding the Evidence Base

While recognising the contribution of numerous researchers whose work is referenced throughout this thesis, it must be acknowledged that the evidence base for post HSCT vaccination is limited. Current evidence is insufficient to provide detailed practical guidance, or to allow rational modification of vaccination schedules according to HSCT variables such as donor type, stem cell source, conditioning intensity or lymphocyte depletion, or in response to post-HSCT clinical scenarios. The optimum timing of vaccine administration is in many cases also unclear. It has recently been argued that a 'one-size fits all' approach to vaccination sits uncomfortably with HSCT physicians (377), and this may contribute to both the variation in practice evident from the national survey, and also the inconsistent practice *within* HSCT programmes reported by single centre studies(102,105). Opportunities for future vaccine research are discussed in more detail in 6.2.2.

6.1.2.2.3 Developing National Guidance

While acknowledging the limited evidence base and resulting issues discussed above, a national HSCT specific vaccination guideline that synthesizes best practice recommendations and national licensing restrictions would be a practical step towards

harmonization of care. As above, part of the development process should involve engagement with GP colleagues to ensure the guideline addresses barriers to effective RVP practice in primary care. The aim of a national guideline would be to provide a single, high-quality, high-profile adult and paediatric information source that is readily accessible to both specialist and primary care teams, and provides recommendations specifically adapted to UK practice. Clearly, where evidence is most limited (e.g. using markers of immune reconstitution to guide RVP commencement, or effective tailoring of RVP schedule according to HSCT type) variation in practice will persist according to local experience and expertise. However, such a guideline may help to address some of the more problematic variation in practice reported at national and HSCT programme level.

6.2 A pilot study comparing the microneutralization assay and haemagglutination inhibition assay as measures of the immunogenicity of seasonal inactive influenza vaccine in recipients of reduced intensity conditioning allogeneic haematopoietic stem cell transplant during the first-year post-transplant

6.2.1 Key Findings

In this study of the immunogenicity of the SIIV administered in the first-year post RIC alloHSCT, humoral immune response was virtually undetectable by both the standard

HAI assay and the more sensitive VMN assay. Seroconversion rates were lower than reported in previous studies. In the current cohort, all patients were conditioned with in-vivo lymphocyte depleting agents, and the impact of this on vaccine response warrants further investigation.

This study highlights the need for novel immunogenic influenza vaccines and/or vaccination schedules. More broadly, these findings emphasise the challenges of determining the optimum vaccination strategy in this immunocompromised patient group. Certainly, a tension exists between the clinical need to offer early prophylaxis for VPDs, and the reconstituting immune system's capacity to respond to vaccination.

6.2.2 Future Directions

6.2.2.1 Dissemination of Findings

This study was presented as a poster at the EBMT 2017 annual conference. A manuscript for publication is currently in preparation.

6.2.2.2 Further Work

6.2.2.2.1 The VMN in future studies

The European Medicines Agency recommends that virus neutralization assays are conducted in all IIV immunogenicity studies. The current pilot study has shown that VMN, with ELISA endpoint at least, may detect seroconversions below the HAI threshold in HSCT recipients. In this study, the absence of seroresponse seems to reflect the poor immunogenicity of a single IIV dose in the first-year post HSCT, and the VMN assay my offer useful immunological data in studies of novel vaccine schedules or formulations. Although established COPs will be required to interpret such data. This issue is discussed further in 6.2.2.2.4.

6.2.2.2.2 Novel Vaccination Schedules

In the absence of clinical efficacy studies in this population, the correlation between Ab titre and level of protection from influenza is not known. While acknowledging this, it seems logical that if influenza Abs wane post-HSCT, strategies to maximise titres immediately before HSCT may confer some benefit during the early high-risk period when IIV immunogenicity is without doubt impaired. There are two possible approaches: i) Vaccination of donor to achieve donor derived immunity or ii) pre-HSCT

vaccination of recipient. While there is evidence that the former may be an effective approach for conjugate vaccines at least (378,379), there are ethical and practical barriers whether the donor is a sibling or VUD, and patently this is not possible in UCB alloHSCT. A recent study randomized donor-recipient pairs to no pre-HSCT vaccination, recipient pre-HSCT vaccination, or donor vaccination. All recipients were vaccinated at 6 months post HSCT. GMTs were statistically significantly higher for A(H1N1) in the recipient vaccination group compared with the no vaccination group, and for A(H3N2) the recipient group compared with both the no vaccination and donor vaccination group. However, seroresponse rates to the 6-month vaccination did not vary by randomization group. It may be that an early post-HSCT booster vaccination at 1-3 months may have enhanced immune response and this warrants further evaluation, including determination of Ab titre by VMN.

6.2.2.2.3 Novel Vaccines

Recent influenza vaccine developments include the licensing of a high-dose, and an adjuvanted vaccine. In a pilot study reported at the Infectious Disease Society of America 2016 conference, adult alloHSCT recipients were randomized to receive either standard seasonal trivalent IIV or an adjuvanted (MF-59) seasonal trivalent IIV. Recipients were vaccinated from 6 months post HSCT and seroconversion rates (range 21.9 – 57.1%) and GMTs were similar across both groups. Rates of local and systemic reactions were similar between the two groups(380). The investigators reported higher

seroconversion rates among recipients vaccinated during the previous influenza season. It is possible therefore that vaccination, although minimally immunogenic in the first year post-HSCT, may prime the immune system for subsequent seasonal vaccination and this could be evaluated in future observational studies. In a phase I study Halasa and colleagues compared single doses of the standard seasonal IIV to high-dose IIV containing 60ug HA units/component administered from 6 months post-HSCT. A(H3N2) GMTs and rates of HAI titre >40 (81% versus 36%, p=0.04) were greater for high dose vaccine, but not for A(H1N1) or influenza B. More combined solicited injection-site reactions were reported in the high dose group (67% versus 31%, p=0.033) but there was no difference between reported systemic reactions (381). Future studies could investigate earlier vaccination timepoints, and alternative vaccination regimens including pre-HSCT vaccination. Again, VMN assay may yield useful information in potentially more immunogenic schedules than single, standard dose IIV.

A major goal in influenza vaccination is the development of a universal influenza vaccine provoking Ab response to conserved virus antigen. This would offer prolonged immunity against a range of influenza subtypes including potential pandemic strains without the need for annual vaccination and changes in composition (382). Although in early stages of development, such vaccines may offer new strategies for the immunization of immunocompromised patients including HSCT recipients. Given the Ab target would be non-HA Ag, the HAI assay would be of little use as virus may be

neutralized yet HA binding sites would remain available for erythrocyte agglutination. Modified virus neutralization assays may be an alternative.

6.2.2.2.4 Evaluating Correlates of Protection, Clinical Efficacy and Effectiveness

A major limitation in IIV immunogenicity studies is the lack of validated correlates of protection. As previously discussed this is part of a broader problem, which has culminated in revision of CMHP IIV licensing criteria. Given the size of the HSCT population in the UK, establishing any meaningful COPs, or evaluating clinical efficacy will remain a significant challenge for the foreseeable future. Sufficiently powered prospective studies would require engagement at the national level.

A 'case test-negative control' modification of the case-control study design has been used to estimate post-licensing clinical effectiveness (383). In this design, the study population is drawn from patients presenting with clinical symptoms of upper respiratory tract infection and/or influenza. PCR confirmed positive influenza cases are matched with a PCR negative comparison group. Vaccination status is established and from this effectiveness can be estimated. This offers a relatively low-cost approach to estimating effectiveness and removes the need to intensively recruit a control group. This design has not been used to estimate vaccine effectiveness in immunocompromised subgroups, and warrants further consideration.

6.3 The impact of seasonal influenza infection and vaccination health beliefs on vaccination intent amongst adult recipients of autologous and allogeneic haematopoietic stem cell transplant

6.3.1 Key Findings

In this first study to explore influenza vaccination intent amongst HSCT recipients, potential promoters and barriers were identified. A modified Health Belief Model was a good predictor of vaccine intent, and offers a basis for developing targeted interventions. Specifically, HSCT recipients who perceived greater potential benefits from influenza vaccination, who felt vaccination would mean they worry less about catching influenza, and were less concerned about potential side-effects and lack of efficacy had stronger vaccination intent. Adults aged over 65 who had not previously received the influenza vaccine were likely to have lower vaccine intent and are a group in who targeted interventions to promote influenza vaccine uptake may be particularly important. HSCT recipients, and particularly those with low vaccine intent, seem to value a recommendation from their HSCT specialist over their General Practitioner. This emphasizes the importance of the HSCT team engaging in clear discussion with recipients about the influenza vaccine and addressing concerns. In practice, the evidence of poor influenza vaccine immunogenicity in the first year post-HSCT, and

uncertainty about optimum vaccination time-point should be discussed, but this balanced against excellent safety profile in the general population, and no evidence for increased rate of side effects or risk of worsening of GvHD in HSCT recipients.

6.3.2 Future Directions

6.3.2.1 Dissemination of Findings

An abstract will be submitted to forthcoming HSCT conference and a manuscript for publication is in preparation.

6.3.2.2 Further Work

Influenza vaccine information targeted at HSCT recipients may help to improve vaccination intent and uptake. This should specifically address HSCT recipient risk from influenza infection, potential benefits of vaccination and concerns about vaccination side-effects post HSCT. While the study in Chapter 4 specifically focused on the influenza vaccine, similar strategies may help to promote vaccination uptake more broadly. The effectiveness of such strategies should be assessed prospectively, and may also seek to explore the gap between vaccination intent and behaviour in this

patient group. The possibility of developing patient literature by Anthony Nolan is currently being explored.

6.4 Autoimmune cytopenias (AIC) following allogeneic haematopoietic stem cell transplant for acquired aplastic anaemia: A joint study of the Autoimmune Diseases and Severe Aplastic Anaemia Working Parties (ADWP/SAAWP) of the European Society for Blood and Marrow Transplantation (EBMT).

6.4.1 Key Findings

In this study of AIC developing after allogeneic HSCT for aAA, the incidence identified was similar to that reported in previous studies of other HSCT populations. Response to treatment was also similar to previously reported data, although heterogenous treatment strategies, study populations and definitions of response across the cited studies limit interpretation. Overall survival of those patients developing AIC was not worse than the overall cohort, however small number of cases limited further analysis. In keeping with previous findings, and understanding of the pathogenesis of autoimmune disease and AIC, conditioning regimens containing alemtuzumab and fludarabine were associated with increased incidence of AIC in univariable. This may be

related to profound lymphodepletion and subsequent homeostatic expansion associated with these agents. We found a lower incidence of AIC in patients who received ATG in the conditioning regimen. A previous study has reported lower AIC risk with ATG, however our results are more in keeping with basic scientific findings regarding ATG's effect on regulatory T cell homeostasis. This finding warrants further investigation. In multivariable analysis after adjusting for conditioning regimen, PBSC source and RIC were associated with an increased risk of AIC compared with BM and MAC respectively. This is a novel finding, and may relate to increased number of mature lymphocytes in PBSC HSCT, and differences in pattern and rate of immune reconstitution between RIC and MAC.

6.4.2 Future Directions

6.4.2.1 Dissemination of Findings

An abstract was presented in poster format at the American Society of Haematology (ASH) 2017 annual meeting in Atlanta, Georgia. A manuscript for publication is in preparation

6.4.2.2 Further Work

The relatively low incidence, and necessarily long follow-up time makes prospective studies of AIC post HSCT challenging. However, further retrospective studies may help to expand on our findings. Our finding that ATG is associated, in univariable analysis, with lower risk of AIC could be further explored in other patient populations, and it is important to establish whether this effect is related to rATG or hATG. In the future the analysis could be repeated over a subsequent 10-year period to determine whether findings remain consistent with time. In the general population, low incidence of AIC has made randomised controlled trials of therapy challenging, and this is even more the case in the post-HSCT setting. Centralized reporting of data is fundamental to establishing treatment strategies.

Immune reconstitution data sets could be reviewed, to scope whether patients developed autoimmune complications post HSCT. A comparison of rate and pattern of reconstitution could then be made between patients who did and did not develop AIC, although this would be limited by confounding factors.

6.5 Final Remarks

In conclusion, vaccines are considered one of the great modern achievements in global health (384), and while a small part of a complex medical treatment, we must nonetheless work to ensure that high-risk, immunocompromised HSCT recipients derive maximum benefit from this simple, low-risk, low-cost strategy to prevent morbidity and mortality from VPDs.

References

1. Murphy K. Janeway's Immunobiology. 8th editio. Garland Science, Taylor and Francis Group; 2012.

2. Crisan M, Dzierzak E. The many faces of hematopoietic stem cell heterogeneity. Development [Internet]. 2016 Dec 15 [cited 2017 Jan 6];143(24):4571–81. Available from: http://dev.biologists.org/content/143/24/4571.long

3. Beutler B, Goodnow CC. How host defense is encoded in the mammalian genome. Mamm Genome [Internet]. Springer-Verlag; 2011 Feb 25 [cited 2017 Apr 12];22(1–2):1–5. Available from: http://link.springer.com/10.1007/s00335-010-9312-4

4. Dowling JK, Mansell A. Toll-like receptors: the swiss army knife of immunity and vaccine development. Clin Transl Immunol [Internet]. 2016 May [cited 2017 Jan 6];5(5):e85. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4910119&tool=pmcentrez&rendertype=abstract

5. Goodnow CC, Sprent J, de St Groth BF, Vinuesa CG. Cellular and genetic mechanisms of self tolerance and autoimmunity. Nature [Internet]. 2005;435(7042):590–7. Available from: http://www.nature.com/nature/journal/v435/n7042/full/nature03724.html%5Cnhttp://www.nature.com/nature/journal/v435/n7042/pdf/nature03724.pdf

6. Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M. Immunologic self-tolerance

maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. J Immunol [Internet]. 1995;155(3):1151–64. Available from: http://www.ncbi.nlm.nih.gov/pubmed/7636184

7. World Health Organization. World Health Organization - Immunization [Internet]. 2017 [cited 2017 Jan 6]. Available from: http://www.who.int/topics/immunization/en/

8. Chang M-H. Decreasing incidence of hepatocellular carcinoma among children following universal hepatitis B immunization. Liver Int [Internet]. 2003 Oct [cited 2017 Jan 6];23(5):309–14. Available from: http://doi.wiley.com/10.1034/j.1478-3231.2003.00865.x

9. Garland SM, Hernandez-Avila M, Wheeler CM, Perez G, Harper DM, Leodolter S, et al. Quadrivalent vaccine against human papillomavirus to prevent anogenital diseases. N Engl J Med [Internet]. 2007 May 10 [cited 2016 Apr 16];356(19):1928–43. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17494926

10. Andre FE, Booy R, Bock HL, Clemens J, Datta SK, John TJ, et al. Vaccination greatly reduces disease, disability, death and inequity worldwide [Internet]. 2008. Available from: http://www.who.int/bulletin/volumes/86/2/07-040089/en/

11. World Health Organization. World Health Organization - Smallpox [Internet]. 2017 [cited 2017 Jan 6]. Available from:

http://www.who.int/csr/disease/smallpox/en/

12. Kallerup RS, Foged C. Subunit Vaccine Delivery [Internet]. First Edit. Foged C, Rades T, Perrie Y, Hook S, editors. Springer; 2015. 15-29 p. Available from: http://link.springer.com/10.1007/978-1-4939-1417-3

13. Nicholls TR, Leach AJ, Morris PS. The short-term impact of each primary dose of pneumococcal conjugate vaccine on nasopharyngeal carriage: Systematic review and meta-analyses of randomised controlled trials. Vaccine [Internet]. 2016 Feb 3 [cited 2017 Jan 8];34(6):703–13. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26742947

14. Tetsutani K, Ishii KJ. Adjuvants in influenza vaccines. Vaccine [Internet]. Elsevier Ltd; 2012;30(52):7658–61. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0264410X12014533

15. Minutello M, Senatore F, Cecchinelli G, Bianchi M, Andreani T, Podda A, et al. Safety and immunogenicity of an inactivated subunit influenza virus vaccine combined with MF59 adjuvant emulsion in elderly subjects, immunized for three consecutive influenza seasons. Vaccine [Internet]. 1999;17(2):99–104. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9987141

16. Weinberg G a, Szilagyi PG. Vaccine epidemiology: efficacy, effectiveness, and the translational research roadmap. J Infect Dis. 2010;201(11):1607–10.

17. Siegrist C-A. Vaccine Immunology [Internet]. Elsevier. Elsevier; 2008 [cited 2015 Aug 12]. 17-36 p. Available from:

http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=19335722

18. Hobson D, Curry RL, Beare a S, Ward-Gardner a. The role of serum haemagglutination-inhibiting antibody in protection against challenge infection with influenza A2 and B viruses. J Hyg (Lond). 1972;70:767–77.

19. European Medicines Agency. Note for guidance on harmonisation of requirements for influenza vaccines [Internet]. 1997. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003945.pdf

20. National Health Service. NHS vaccination schedule [Internet]. 2017 [cited 2017 Jan 9]. Available from: http://www.nhs.uk/conditions/vaccinations/pages/vaccination-schedule-age-checklist.aspx

21. Crawley C, Kirkland K, Pearce R. BSBMT Report to Specialist Commissioners. 2016.

22. British Society of Blood and Marrow Transplantation. 2015 Activity [Internet]. 2015. 2016. Available from: http://bsbmt.org/2015-activity/

23. Bacigalupo A, Ballen K, Rizzo D, Giralt S, Lazarus H, Ho V, et al. Defining the intensity of conditioning regimens : working definitions. 2009;15(12):1628–33.

24. British Society for Histocompatibility and Immunogenetics. Guidelines for selection and HLA matching of related, adult unrelated donors and umbilical cord

units for haematopoietic progenitor cell transplantation. 2013;1–36. Available from: http://www.bshi.org.uk/assets/pdf/HSCTGuide.pdf

25. Kolb H-J. Graft-versus-leukemia effects of transplantation and donor lymphocytes. Hematology. 2011;112(12):4371–83.

26. Jagasia MH, Greinix HT, Arora M, Williams KM, Wolff D, Cowen EW, et al. National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: I. The 2014 Diagnosis and Staging Working Group report. Biol Blood Marrow Transplant [Internet]. NIH Public Access; 2015 Mar [cited 2017 Jan 5];21(3):389–401.e1. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25529383

27. Wingard JR, Majhail NS, Brazauskas R, Wang Z, Sobocinski K a., Jacobsohn D, et al. Long-Term Survival and Late Deaths After Allogeneic Hematopoietic Cell Transplantation. J Clin Oncol [Internet]. 2011 Jun 1 [cited 2014 Oct 13];29(16):2230–9. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3107742&tool=pmcentrez&rendertype=abstract

28. Lee SJ, Flowers MED. Recognizing and Managing Chronic Graft-Versus-Host Disease. Hematology [Internet]. 2008 Jan 1 [cited 2017 Jan 5];2008(1):134–41. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19074071

29. Zhang L, Chu J, Yu J, Wei W. Cellular and molecular mechanisms in graft-versus-host disease. J Leukoc Biol [Internet]. 2015;99(February):279–86. Available from:

http://www.jleukbio.org/cgi/doi/10.1189/jlb.4RU0615-254RR

30. Dertschnig S, Hauri-Hohl MM, Vollmer M, Hollander G a., Krenger W. Impaired thymic expression of tissue-restricted antigens licenses the de novo generation of autoreactive CD4+ T cells during murine acute GVHD. Blood. 2015;125(17):2720–3.

31. Krenger W, Rossi S, Piali L, Hollä GA. Thymic atrophy in murine acute graft-versus-host disease is effected by impaired cell cycle progression of host pro-T and pre-T cells. Blood [Internet]. 2000;90(1):347–54. Available from: www.ncbi.nlm.nih.gov/pubmed/10891472

32. Cuthbert RJ, Iqbal A, Gates A, Toghill PJ, Russell NH. Functional hyposplenism following allogeneic bone marrow transplantation. J Clin Pathol [Internet]. 1995;48(3):257–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/7730489

33. Mohty M, Apperley JF. Long-Term Physiological Side Effects After Allogeneic Bone Marrow Transplantation. Hematol Am Soc Hematol Educ Program. 2010;229–36.

34. Ogonek J, Kralj Juric M, Ghimire S, Varanasi PR, Holler E, Greinix H, et al. Immune Reconstitution after Allogeneic Hematopoietic Stem Cell Transplantation. Front Immunol [Internet]. 2016;7(November):507. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27909435%5Cnhttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC5112259

35. Toubert A. Chapter 14 - Immune reconstitution after allogeneic HSCT. EBMT-ESH Handb. 2012;

36. Storek J. Immunological reconstitution after hematopoietic cell transplantation - its relation to the contents of the graft. Expert Opin Biol Ther [Internet]. Taylor & Francis; 2008 May 13 [cited 2017 Jan 10];8(5):583–97. Available from: http://www.tandfonline.com/doi/full/10.1517/14712598.8.5.583?instName=UCL+%28University+College+London%29

37. Ojielo CI, Cooke K, Mancuso P, Standiford TJ, Olkiewicz KM, Clouthier S, et al. Defective phagocytosis and clearance of Pseudomonas aeruginosa in the lung following bone marrow transplantation. J Immunol [Internet]. 2003 Oct 15;171(8):4416–24. Available from: http://www.ncbi.nlm.nih.gov/pubmed/14530368

38. Auletta JJ, Lazarus HM. Immune restoration following hematopoietic stem cell transplantation: an evolving target. Bone Marrow Transplant. 2005;35(9):835–57.

39. Mohty M, Gaugler B, Faucher C, Sainty D, Lafage-Pochitaloff M, Vey N, et al. Recovery of Lymphocyte and Dendritic Cell Subsets Following Reduced Intensity Allogeneic Bone Marrow Transplantation. Hematology [Internet]. 2002 Jan 4 [cited 2017 Jan 9];7(3):157–64. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12243978

40. Park BG, Park C-J, Jang S, Chi H-S, Kim D-Y, Lee J-H, et al. Reconstitution of

lymphocyte subpopulations after hematopoietic stem cell transplantation: comparison of hematologic malignancies and donor types in event-free patients. Leuk Res. 2015;39(12):1334–41.

41. Storek J, Dawson M a, Storer B, Stevens-Ayers T, Maloney DG, Marr K a, et al. Immune reconstitution after allogeneic marrow transplantation compared with blood stem cell transplantation. Blood. 2001;97(11):3380–9.

42. Thomson BG, Robertson KA, Gowan D, Heilman D, Broxmeyer HE, Emanuel D, et al. Analysis of engraftment, graft-versus-host disease, and immune recovery following unrelated donor cord blood transplantation. Blood [Internet]. 2000 Oct 15 [cited 2017 Jan 9];96(8):2703–11. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11023501

43. Shenoy S, Mohanakumar T, Todd G, Westhoff W, Dunnigan K, Adkins DR, et al. Immune reconstitution following allogeneic peripheral blood stem cell transplants. Bone Marrow Transplant [Internet]. 1999 Feb 12 [cited 2017 Jan 9];23(4):335–46. Available from: http://www.nature.com/doifinder/10.1038/sj.bmt.1701581

44. Mackall CL, Bare C V, Granger LA, Sharrow SO, Titus JA, Gress RE. Thymic-independent T cell regeneration occurs via antigen-driven expansion of peripheral T cells resulting in a repertoire that is limited in diversity and prone to skewing. J Immunol [Internet]. American Association of Immunologists; 1996 Jun 15 [cited 2017 Jan 9];156(12):4609–16. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8648103

45. Le Campion A, Gagnerault M-C, Auffray CD, Bé C, Poitrasson-Riviè Re M, Lallemand E, et al. Lymphopenia-induced spontaneous T-cell proliferation as a cofactor for autoimmune disease development. Blood. 2009;114(9):1784–93.

46. Heining C, Spyridonidis A, Bernhardt E, Schulte-Mönting J, Behringer D, Grüllich C, et al. Lymphocyte reconstitution following allogeneic hematopoietic stem cell transplantation: a retrospective study including 148 patients. Bone Marrow Transplant [Internet]. Nature Publishing Group; 2007 May 26 [cited 2017 Jan 9];39(10):613–22. Available from: http://www.nature.com/doifinder/10.1038/sj.bmt.1705648

47. Ljungman P, Lewensohn-fuchs I, Hammarström V, Aschan J, Brandt L, Bolme P, et al. Long-term immunity to measles, mumps, and rubella after allogeneic bone marrow transplantation. Blood [Internet]. 1994 Jul 15;84(2):657–63. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8025290

48. Pauksen K, Duraj V, Ljungman P, Sjölin J, Oberg G, Lönnerholm G, et al. Immunity to and immunization against measles, rubella and mumps in patients after autologous bone marrow transplantation. Bone Marrow Transplant [Internet]. 1992 Jun [cited 2015 Apr 9];9(6):427–32. Available from: http://www.ncbi.nlm.nih.gov/pubmed/1628126

49. Ljungman P, Fridell E, Lönnqvist B, Bolme P, Böttiger M, Gahrton G, et al. Efficacy and safety of vaccination of marrow transplant recipients with a live attenuated measles, mumps, and rubella vaccine. J Infect Dis [Internet]. 1989 Apr;159(4):610–5. Available from:

http://www.ncbi.nlm.nih.gov/pubmed/2647859

50. Ljungman P, Wiklund-Hammarsten M, Duraj V, Hammarström L, Lönnqvist B, Paulin T, et al. Response to tetanus toxoid immunization after allogeneic bone marrow transplantation. J Infect Dis. 1990;162(2):496–500.

51. Ljungman P, Duraj V, Magnius L. Response to immunization against polio after allogeneic marrow transplantation. Bone Marrow Transplant [Internet]. 1991 Feb;7(2):89–93. Available from: http://www.ncbi.nlm.nih.gov/pubmed/1646664

52. Engelhard D, Handsher R, Naparstek E, Hardan I, Strauss N, Aker M, et al. Immune response to polio vaccination in bone marrow transplant recipients. Bone Marrow Transplant [Internet]. 1991 Oct [cited 2015 Sep 9];8(4):295–300. Available from: http://www.ncbi.nlm.nih.gov/pubmed/1661633

53. Parkkali T, Stenvik M, Ruutu T, Hovi T, Volin L, Ruutu P. Randomized comparison of early and late vaccination with inactivated poliovirus vaccine after allogeneic BMT. Bone Marrow Transplant. Nature Publishing Group; 1997;20(8):663–8.

54. Winston DJ, Ho WG, Schiffman G, Champlin RE, Feig SA, Gale RP. Pneumococcal vaccination of recipients of bone marrow transplants. Arch Intern Med [Internet]. 1983 Sep;143(9):1735–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/6351777

55. Giebink GS, Warkentin PI, Ramsay NK, Kersey JH. Titers of antibody to pneumococci in allogeneic bone marrow transplant recipients before and after vaccination with pneumococcal vaccine. J Infect Dis. 1986;154(4):590–6.

56. Hammarström V, Pauksen K, Björkstrand B, Simonsson B, Oberg G, Ljungman P. Tetanus immunity in autologous bone marrow and blood stem cell transplant recipients. Bone Marrow Transplant. 1998;22(1):67–71.

57. Pauksen K, Hammarström V, Ljungman P, Sjölin J, Oberg G, Lönnerholm G, et al. Immunity to poliovirus and immunization with inactivated poliovirus vaccine after autologous bone marrow transplantation. Clin Infect Dis [Internet]. 1994 Apr [cited 2015 Apr 9];18(4):547–52. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8038308

58. Hammarström V, Pauksen K, Azinge J, Oberg G, Ljungman P. Pneumococcal immunity and response to immunization with pneumococcal vaccine in bone marrow transplant patients: the influence of graft versus host reaction. Support Care Cancer [Internet]. 1993 Jul [cited 2015 Aug 14];1(4):195–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8193881

59. Gratwohl A, Brand R, Frassoni F, Rocha V, Niederwieser D, Reusser P, et al. Cause of death after allogeneic haematopoietic stem cell transplantation (HSCT) in early leukaemias: an EBMT analysis of lethal infectious complications and changes over calendar time. Bone Marrow Transplant [Internet]. 2005 Nov [cited 2017 Jan 10];36(9):757–69. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16151426

60. Miller HK, Braun TM, Stillwell T, Harris AC, Choi S, Connelly J, et al. Infectious Risk Following Allogeneic Hematopoietic Cell Transplant Complicated by Acute Graft-Versus-Host Disease. Biol Blood Marrow Transplant [Internet]. 2016 Dec 22 [cited

2017 Jan 10]; Available from:

http://www.sciencedirect.com/science/article/pii/S1083879116311892

61. Pranab Sharma Acharya SK. Infectious Complications of Hematopoietic Stem Cell Transplantation. J Stem Cell Res Ther [Internet]. OMICS International; 2013 Jun 1 [cited 2017 Jan 10];s3(01):1–8. Available from: http://www.omicsonline.org/infectious-complications-of-hematopoietic-stem-cell-transplantation-2157-7633.S3-002.php?aid=14005

62. Hassan I a, Chopra R, Swindell R, Mutton KJ. Respiratory viral infections after bone marrow/peripheral stem-cell transplantation: the Christie hospital experience. Bone Marrow Transplant. 2003;32(1):73–7.

63. Ljungman P. Respiratory Virus Infections in Stem Cell Transplant Patients : The European Experience. Biol Blood Marrow Transplant. 2001;7:5s–7s.

64. Nichols WG, Guthrie K a, Corey L, Boeckh M. Influenza infections after hematopoietic stem cell transplantation: risk factors, mortality, and the effect of antiviral therapy. Clin Infect Dis. 2004;39(9):1300–6.

65. Ljungman P, Ward KN, Crooks BN a, Parker A, Martino R, Shaw PJ, et al. Respiratory virus infections after stem cell transplantation : a prospective study from the Infectious Diseases Working Party of the European Group for Blood and Marrow Transplantation Summary : Bone Marrow Transplant. 2001;28:479–84.

66. Whimbey E, Elting LS, Couch RB, Lo W, Williams L, Champlin RE, et al. Influenza A virus infections among hospitalized adult bone marrow transplant recipients.

Bone Marrow Transplant [Internet]. 1994 Apr;13(4):437–40. Available from: www.ncbi.nlm.nih.gov/pubmed/8019468

67. Ljungman P, de la Camara R, Perez-Bercoff L, Abecasis M, Campuzano JBN, Cannata-Ortiz MJ, et al. Outcome of pandemic H1N1 infections in hematopoietic stem cell transplant recipients. Haematologica. 2011;96(8):1231–5.

68. Mohty B, Thomas Y, Vukicevic M, Nagy M, Levrat E, Bernimoulin M, et al. Clinical features and outcome of 2009-influenza A (H1N1) after allogeneic hematopoietic SCT. Bone Marrow Transplant [Internet]. Nature Publishing Group; 2012;47(2):236–42. Available from: http://dx.doi.org/10.1038/bmt.2011.57

69. Espinosa-Aguilar L, Green JS, Forrest GN, Ball ED, Maziarz RT, Strasfeld L, et al. Novel H1N1 Influenza in Hematopoietic Stem Cell Transplantation Recipients: Two Centers' Experiences. Biol Blood Marrow Transplant [Internet]. Elsevier Ltd; 2011;17(4):566–73. Available from: http://dx.doi.org/10.1016/j.bbmt.2010.07.018

70. George B, Ferguson P, Kerridge I, Gilroy N, Gottlieb D, Hertzberg M. The clinical impact of infection with swine flu (H1N109) strain of influenza virus in hematopoietic stem cell transplant recipients. Biol Blood Marrow Transplant [Internet]. Elsevier Ltd; 2011;17(1):147–53. Available from: http://dx.doi.org/10.1016/j.bbmt.2010.07.004

71. Protheroe RE, Kirkland KE, Pearce RM, Kaminaris K, Bloor A, Potter MN, et al. The clinical features and outcome of 2009 H1N1 influenza infection in allo-SCT

patients: a British Society of Blood and Marrow Transplantation study. Bone Marrow Transplant [Internet]. 2012 Jan;47(1):88–94. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21358686

72. Reid G, Huprikar S, Patel G, Razonable RR, Mossad S, Levi M, et al. A multicenter evaluation of pandemic influenza A/H1N1 in hematopoietic stem cell transplant recipients. Transpl Infect Dis [Internet]. 2013 Oct [cited 2015 Sep 9];15(5):487–92. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23890293

73. Rihani R, Hayajneh W, Sultan I, Ghatasheh L, Abdel-Rahman F, Hussein N, et al. Infections with the 2009 H1N1 influenza virus among hematopoietic SCT recipients: a single center experience. Bone Marrow Transplant [Internet]. Nature Publishing Group; 2011;46(11):1430–6. Available from: http://dx.doi.org/10.1038/bmt.2010.329

74. Donaldson LJ, Rutter PD, Ellis BM, Greaves FEC, Mytton OT, Pebody RG, et al. Mortality from pandemic A/H1N1 2009 influenza in England: public health surveillance study. BMJ. 2009;339:b5213.

75. Choi S-M, Boudreault A a, Xie H, Englund J a, Corey L, Boeckh M. Differences in clinical outcomes after 2009 influenza A/H1N1 and seasonal influenza among hematopoietic cell transplant recipients. Blood. 2011;117(19):5050–6.

76. Lemaitre M, Carrat F. Comparative age distribution of influenza morbidity and mortality during seasonal influenza epidemics and the 2009 H1N1 pandemic. BMC Infect Dis. 2010;10:162–6.

77. Babor F, Grund S, Siepermann M, Oommen PT, Kuhlen M, Schuster FR, et al. Epidemiology and clinical characteristics of pandemic (H1N1) 2009 influenza infection in pediatric hemato-oncology and hematopoietic stem cell transplantation patients. Transpl Infect Dis. 2012;14(6):589–94.

78. Tran D, Science M, Dix D, Portwine C, Zelcer S, Johnston DL, et al. Pandemic (H1N1) 2009 influenza in Canadian pediatric cancer and hematopoietic stem cell transplant patients. Influenza Other Respi Viruses. 2012;6(6):12–5.

79. Engelhard D, Cordonnier C, Shaw PJ, Parkalli T, Guenther C, Martino R, et al. Early and late invasive pneumococcal infection following stem cell transplantation: a European Bone Marrow Transplantation survey. Br J Haematol [Internet]. 2002 May [cited 2015 Aug 14];117(2):444–50. Available from: http://doi.wiley.com/10.1046/j.1365-2141.2002.03457.x

80. Torda a., Chong Q, Lee a., Chen S, Dodds a., Greenwood M, et al. Invasive pneumococcal disease following adult allogeneic hematopoietic stem cell transplantation. Transpl Infect Dis [Internet]. 2014;16(5):751–9. Available from: http://doi.wiley.com/10.1111/tid.12268

81. Kumar D, Humar a, Plevneshi a, Siegal D, Franke N, Green K, et al. Invasive pneumococcal disease in adult hematopoietic stem cell transplant recipients: a decade of prospective population-based surveillance. Bone Marrow Transplant. 2008;41(8):743–7.

82. Youssef S, Rodriguez G, Rolston K V, Champlin RE, Raad II, Safdar A.

Streptococcus pneumoniae infections in 47 hematopoietic stem cell transplantation recipients: clinical characteristics of infections and vaccine-breakthrough infections, 1989-2005. Medicine (Baltimore). 2007;86(2):69–77.

83. Atkinson K, Storb R, Prentice RL, Weiden PL, Witherspoon RP, Sullivan K, et al. Analysis of late infections in 89 long-term survivors of bone marrow transplantation. Blood. 1979;53(4):720–31.

84. Cordonnier C, Bernaudin JF, Bierling P, Huet Y, Vernant JP. Pulmonary complications occurring after allogeneic bone marrow transplantation. A study of 130 consecutive transplanted patients. Cancer. 1986;58(5):1047–54.

85. Aucouturier P, Barra A, Intrator L, Cordonnier C, Schulz D, Duarte F, et al. Long lasting IgG subclass and antibacterial polysaccharide antibody deficiency after allogeneic bone marrow transplantation. Blood. 1987;70(3):779–85.

86. Lossos IS, Breuer R, Or R, Strauss N, Elishoov H, Naparstek E, et al. Bacterial Pneumonia in Recipients of Bone Marrow Transplantation. Transplantation. 1995;60(7):672–8.

87. Nakano T, Shimono Y, Sugiyama K, Nishihara H, Higashigawa M, Komada Y, et al. Clinical features of measles in immunocompromised children. Acta Paediatr Jpn [Internet]. 1996 Jun [cited 2015 Sep 11];38(3):212–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8741308

88. Machado CM, Goncalves FB, Pannuti CS, Dulley FL, de Souza V. Measles in bone marrow transplant recipients. Blood. 2002;99(8):83–7.

89. Ljungman P, Wiklund-Hammarsten M, Duraj V, Hammarström L, Lönnqvist B, Paulin T, et al. Response to tetanus toxoid immunization after allogeneic bone marrow transplantation. J Infect Dis. 1990;162(2):496–500.

90. Cordonnier C, Ljungman P, Juergens C, Maertens J, Selleslag D, Sundaraiyer V, et al. Immunogenicity, Safety, and Tolerability of 13-Valent Pneumococcal Conjugate Vaccine Followed by 23-Valent Pneumococcal Polysaccharide Vaccine in Recipients of Allogeneic Hematopoietic Stem Cell Transplant Aged >/= 2 Years: An Open-Label Study. Clin Infect Dis. 2015;61:313–23.

91. Engelhard D, Nagler A, Hardan I, Morag A, Aker M, Baciu H, et al. Antibody response to a two-dose regimen of influenza vaccine in allogeneic T cell-depleted and autologous BMT recipients. Bone Marrow Transplant. 1993;11(1):1–5.

92. Karras N a., Weeres M, Sessions W, Xu X, DeFor TE, Young J-AAH, et al. A Randomized Trial of One versus Two Doses of Influenza Vaccine after Allogeneic Transplantation. Biol Blood Marrow Transplant. 2013;19(1):109–16.

93. Cordonnier C, Labopin M, Chesnel V, Ribaud P, Camara RD La, Martino R, et al. Immune response to the 23-valent polysaccharide pneumococcal vaccine after the 7-valent conjugate vaccine in allogeneic stem cell transplant recipients: Results from the EBMT IDWP01 trial. Vaccine. 2010;28(15):2730–4.

94. Cordonnier C, Labopin M, Chesnel V, Ribaud P, De La Camara R, Martino R, et al. Randomized study of early versus late immunization with pneumococcal conjugate vaccine after allogeneic stem cell transplantation. Clin Infect Dis.

2009;48(10):1392–401.

95. FACT-JACIE. FACT-JACIE International Standards for Hematopoietic Cellular Transplantation Product Collection, Processing and Administration [Internet]. 2015. Available from: http://www.jacie.org/standards

96. Tomblyn M, Chiller T, Einsele H, Gress R, Sepkowitz K, Storek J, et al. Guidelines for preventing infectious complications among hematopoietic cell transplantation recipients: a global perspective. Preface. Bone Marrow Transplant. 2009;15(8):1143–238.

97. Rubin LG, Levin MJ, Ljungman P, Davies EG, Avery R, Tomblyn M, et al. 2013 IDSA clinical practice guideline for vaccination of the immunocompromised host. Clin Infect Dis. 2014 Feb;58(3):e44-100.

98. Hilgendorf I, Wolff D, Meisel R. Vaccination of allogeneic haematopoietic stem cell transplant recipients: Report from the International Consensus Conference on Clinical Practice in chronic GVHD. Vaccine. 2011;29:2825–33.

99. Children's Cancer and Leukaemia Group. Vaccinations for Paediatric Patients Treated With Standard-Dose Chemotherapy and Haematopoietic Stem Cell Transplantation (HSCT) Recipients [Internet]. 2014. Available from: http://www.cclg.org.uk/write/MediaUploads/Member area/Treatment guidelines/Vaccinations_for_Children_treated_with_Standard-dose_Chemotherapy_and_HSCT_Recipients-Sept_2014-FINAL_CCLG.pdf

100. Rubin LG, Levin MJ, Ljungman P, Davies EG, Avery R, Tomblyn M, et al. 2013 IDSA

clinical practice guideline for vaccination of the immunocompromised host. Clin Infect Dis. 2014;58:1–57.

101. Royal College of Physicians of Ireland. Immunisation Guidelines for Ireland 2013 [Internet]. 2013. Available from: http://www.hse.ie/eng/health/immunisation/hcpinfo/guidelines/

102. Ariza-Heredia EJ, Gulbis AM, Stolar KR, Kebriaei P, Shah DP, McConn KK, et al. Vaccination guidelines after hematopoietic stem cell transplantation: practitioners' knowledge, attitudes, and gap between guidelines and clinical practice. Transpl Infect Dis. 2014 Nov 18;16(6):878–86.

103. Lerchenfeldt SM, Cronin SM, Chandrasekar PH. Vaccination adherence in hematopoietic stem cell transplant patients: a pilot study on the impact of vaccination cards and reminder telephone calls. Transpl Infect Dis [Internet]. 2013 Dec [cited 2015 Apr 8];15(6):634–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23890163

104. Meiring J, de Silva TI, Snowden JA. A study of adherence to a vaccination schedule following adult allogeneic haematopoietic stem cell transplants in UK transplant centre. Bone Marrow Transplant. 2015;50(S1):s203–4.

105. Leonard H, Anthias C. Improving the re-vaccination programme for patients post allograft. Bone Marrow Transplant. 2015;50(S1):216–7.

106. Pauksen K, Hammarström V, Ljungman P, Sjölin J, Oberg G, Lönnerholm G, et al. Immunity to poliovirus and immunization with inactivated poliovirus vaccine

after autologous bone marrow transplantation. Clin Infect Dis [Internet]. 1994;18(4):547–52. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8038308

107. Ambati A, Boas LS V, Ljungman P, Testa L, De Oliveira JF, Aoun M, et al. Evaluation of pretransplant influenza vaccination in hematopoietic SCT: A randomized prospective study. Bone Marrow Transplant [Internet]. Nature Publishing Group; 2015;50(6):858–64. Available from: http://www.embase.com/search/results?subaction=viewrecord&from=export&id=L603275306; http://dx.doi.org/10.1038/bmt.2015.47; http://findit.library.jhu.edu/resolve?sid=EMBASE&issn=14765365&id=doi:10.1038/bmt.2015.47&atitle=Evaluation+of+pretransplant+infl

108. Department of Health. Immunisation against infectious disease 2006 - The Green Book [Internet]. Department of Health, Richmond House, 79 Whitehall, London SW1A 2NJ, UK, dhmail@dh.gsi.gov.uk; 2015 [cited 2014 Nov 23]. Available from: https://www.gov.uk/government/collections/immunisation-against-infectious-disease-the-green-book#the-green-book

109. Royal College of Paediatrics and Child Health. Immunisation of the immunocompromised child. 2002.

110. Halasa N, Green M. Optimizing vaccination in the pediatric stem cell transplant population: The need for prospective data. Pediatr Transplant [Internet]. 2014;18(8):788–9. Available from: http://doi.wiley.com/10.1111/petr.12372

111. Gilleece MH, Towlson K, Wilson M, Littlewood T, Cook G, Marks DI. Vaccination against infection after haemopoietic stem cell transplant. Biol Blood Marrow Transplant [Internet]. Elsevier; 2007 Feb 2 [cited 2014 Nov 18];13(2):75. Available from: http://www.bbmt.org/article/S1083879106010597/fulltext

112. Kumar D, Chen MH, Welsh B, Siegal D, Cobos I, Messner H a, et al. A randomized, double-blind trial of pneumococcal vaccination in adult allogeneic stem cell transplant donors and recipients. Clin Infect Dis. 2007;45(12):1576–82.

113. Public Health England. Introduction of MenB immunisation for infants. 2015.

114. Campbell H, Saliba V, Borrow R, Ramsay M, Ladhani SN. Targeted vaccination of teenagers following continued rapid endemic expansion of a single meningococcal group W clone (sequence type 11 clonal complex), United Kingdom 2015. Eurosurveillance. 2015;20(28):1–5.

115. Gilleece MH, Towlson K, Wilson M, Littlewood T, Cook G, Marks D. Vaccination against Infection after Haematopoietic Stem Cell Transplant : A Survey of Practice in the UK and Ireland. Biol Blood Marrow Transplant [Internet]. Elsevier; 2007 Sep 2 [cited 2014 Nov 18];21(S15):S614–708. Available from: http://www.bbmt.org/article/S1083879106010597/fulltext

116. Meisel R, Kuypers L, Dirksen U, Schubert R, Gruhn B, Strauss G, et al. Pneumococcal conjugate vaccine provides early protective antibody responses in children after related and unrelated allogeneic hematopoietic stem cell transplantation. 2007;109(6):2322–6.

117. Guinan EC, Molrine DC, Antin JH, Lee MC, Weinstein HJ, Sallan SE, et al. Polysaccharide conjugate vaccine responses in bone marrow transplant patients. Transplantation [Internet]. 1994 Mar 15 [cited 2015 Apr 9];57(5):677–84. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8140632

118. British Medical Association and Royal Pharmaceutical Society. British National Formulary [Internet]. 2017 [cited 2017 Feb 15]. Available from: https://www.medicinescomplete.com/about/publications.htm

119. Public Health England. Meningococcal ACWY conjugate vaccination (MenACWY). 2015.

120. Patel SR, Ortín M, Cohen BJ, Borrow R, Irving D, Sheldon J, et al. Revaccination with measles, tetanus, poliovirus, Haemophilus influenzae type B, meningococcus C, and pneumococcus vaccines in children after hematopoietic stem cell transplantation. Clin Infect Dis. 2007;44(5):625–34.

121. Parkkali T, Käyhty H, Lehtonen H, Ruutu T, Volin L, Eskola J, et al. Tetravalent meningococcal polysaccharide vaccine is immunogenic in adult allogeneic BMT recipients. Bone Marrow Transplant. 2001;27:79–84.

122. Mahler MB, Taur Y, Jean R, Kernan NA, Prockop SE, Small TN. Safety and immunogenicity of the tetravelent protein-conjugated meningococcal vaccine (MCV4) in recipients of related and unrelated allogeneic stem cell transplantation (alloHSCT). Biol Blood Marrow Transplant. 2012;18(1):145–9.

123. Small TN, Zelenetz AD, Noy A, Rice RD, Trippett TM, Abrey L, et al. Pertussis

immunity and response to tetanus-reduced diphtheria-reduced pertussis vaccine (Tdap) after autologous peripheral blood stem cell transplantation. Biol Blood Marrow Transplant [Internet]. 2009 Dec [cited 2016 Feb 11];15(12):1538–42. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19896077

124. Shanis DL, Anandi P, Grant C, Pophali PA, Koklanaris E, Savani BN, et al. Extensive Chronic Graft-Versus-Host-Disease Significantly Increases the Risk of Severe and Multifocal Genital Tract HPV Disease in Long-Term Survivors of Allogeneic Stem Cell Transplantation. Blood [Internet]. American Society of Hematology; 2015 Dec 3 [cited 2016 Jun 2];126(23):1956. Available from: http://www.bloodjournal.org/content/126/23/1956.abstract

125. Battiwalla M, Shanis D, Anandi P, Grant C, Bachi A, Vyas N, et al. Genital Human Papillomavirus (HPV) Reactivation patterns in Female Allotransplant Survivors Support HPV Vaccination. Bone Marrow Transplant. 2016;51(S2):S93.

126. FUTURE II study Group. Quadrivalent vaccine against human papillomavirus to prevent high-grade cervical lesions. N Engl J Med [Internet]. 2007 May 10 [cited 2016 Apr 16];356(19):1915–27. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17494925

127. Hill K, Milnthorpe J, Hurlock C, Jarvis L, Newman J, McKeag N, et al. Measles, Mumps, Rubella (MMR) and Varicella Immunity Post-Allogeneic HSCT. Bone Marrow Transplant. 2016;51(S1):S96.

128. Engelhard D, Zakay-Rones Z, Shapira MY, Resnick I, Averbuch D, Grisariu S, et al.

The humoral immune response of hematopoietic stem cell transplantation recipients to AS03-adjuvanted A/California/7/2009 (H1N1)v-like virus vaccine during the 2009 pandemic. Vaccine. 2011;29(9):1777–82.

129. Boles EE, Chiuzan C, Ragucci D, Hudspeth MP. Analysis of factors affecting immune recovery and initial response to tetanus after DTaP vaccination in pediatric allogeneic HSCT patients. Pediatr Transplant [Internet]. 2014;18(8):882–8. Available from: http://doi.wiley.com/10.1111/petr.12361

130. Parkkali T, Käyhty H, Hovi T, Olander R-M, Roivainen M, Volin L, et al. A randomized study on donor immunization with tetanus-diphtheria, Haemophilus influenzae type b and inactivated poliovirus vaccines to improve the recipient responses to the same vaccines after allogeneic bone marrow transplantation. Bone Marrow Transplant. 2007;39(3):179–88.

131. Barra a, Cordonnier C, Preziosi MP, Intrator L, Hessel L, Fritzell B, et al. Immunogenicity of Haemophilus influenzae type b conjugate vaccine in allogeneic bone marrow recipients. J Infect Dis. 1992;166(5):1021–8.

132. Miller PDE, de Silva TI, Skinner R, Gilleece M, Peniket A, Hamblin A, et al. Routine vaccination practice after adult and paediatric allogeneic haematopoietic stem cell transplant: a survey of UK NHS programmes. Bone Marrow Transplant [Internet]. Macmillan Publishers Limited, part of Springer Nature.; 2017 May;52(January 2017):775–7. Available from: http://dx.doi.org/10.1038/bmt.2016.362

133. International Committee on Taxonomy of Viruses. Virus Taxonomy: 2015 Release [Internet]. 2015 [cited 2017 Jan 12]. Available from: http://ictvonline.org/virusTaxonomy.asp

134. Hause BM, Collin EA, Liu R, Huang B, Sheng Z, Lu W, et al. Characterization of a novel influenza virus in cattle and Swine: proposal for a new genus in the Orthomyxoviridae family. MBio [Internet]. 2014 Mar 4 [cited 2017 Jan 12];5(2):e00031-14. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3958797&tool=pmcentrez&rendertype=abstract

135. Osterhaus AD, Rimmelzwaan GF, Martina BE, Bestebroer TM, Fouchier RA. Influenza B virus in seals. Science [Internet]. 2000 May 12 [cited 2017 Jan 12];288(5468):1051–3. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10807575

136. Noah DL, Noah JW. Adapting global influenza management strategies to address emerging viruses. Am J Physiol Lung Cell Mol Physiol [Internet]. 2013 Jul 15 [cited 2017 Jan 12];305(2):L108-17. Available from: http://ajplung.physiology.org/content/305/2/L108.abstract

137. Barberis I, Myles P, Ault SK, Bragazzi NL, Martini M. History and evolution of influenza control through vaccination: from the first monovalent vaccine to universal vaccines. J Prev Med Hyg [Internet]. 2016 Sep [cited 2017 Jan 12];57(3):E115–20. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=5139605&tool=pmc

entrez&rendertype=abstract

138. Samji T. Influenza A: Understanding the viral life cycle. Yale Journal of Biology and Medicine. 2009.

139. Elsevier. Rapid Reference to Influenza [Internet]. 2012. Available from: http://www.rapidreferenceinfluenza.com/resource-center

140. Nelson MI, Holmes EC. The evolution of epidemic influenza. Nat Rev Genet [Internet]. Nature Publishing Group; 2007 Mar 30 [cited 2017 Apr 21];8(3):196–205. Available from: http://www.nature.com/doifinder/10.1038/nrg2053

141. Hamilton BS, Whittaker GR, Daniel S. Influenza virus-mediated membrane fusion: determinants of hemagglutinin fusogenic activity and experimental approaches for assessing virus fusion. Viruses [Internet]. 2012 Jul [cited 2017 Jan 12];4(7):1144–68. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3407899&tool=pmcentrez&rendertype=abstract

142. Böttcher-Friebertshäuser E, Garten W, Matrosovich M, Klenk HD. The hemagglutinin: a determinant of pathogenicity. Curr Top Microbiol Immunol [Internet]. 2014 Jan [cited 2017 Jan 12];385:3–34. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25031010

143. García-Sastre A. Influenza virus receptor specificity: disease and transmission. Am J Pathol [Internet]. 2010 Apr [cited 2017 Jan 12];176(4):1584–5. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2843447&tool=pmc

entrez&rendertype=abstract

144. Palese P, Tobita K, Ueda M, Compans RW. Characterization of temperature sensitive influenza virus mutants defective in neuraminidase. Virology [Internet]. 1974 Oct [cited 2017 Jan 12];61(2):397–410. Available from: http://www.ncbi.nlm.nih.gov/pubmed/4472498

145. World Health Organization. Influenza Factsheet [Internet]. 2016 [cited 2017 Jan 12]. Available from: http://www.who.int/mediacentre/factsheets/fs211/en/

146. Thompson WW, Weintraub E, Dhankhar P, Cheng P-Y, Brammer L, Meltzer MI, et al. Estimates of US influenza-associated deaths made using four different methods. Influenza Other Respi Viruses [Internet]. 2009 Jan [cited 2017 Jan 12];3(1):37–49. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4986622&tool=pmcentrez&rendertype=abstract

147. Public Health England. Surveillance of influenza and other respiratory viruses in the United Kingdom: Winter 2015 to 2016 [Internet]. 2016. Available from: https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/526405/Flu_Annual_Report_2015_2016.pdf

148. Lau LLH, Cowling BJ, Fang VJ, Chan K-H, Lau EHY, Lipsitch M, et al. Viral shedding and clinical illness in naturally acquired influenza virus infections. J Infect Dis [Internet]. 2010 May 15 [cited 2017 Jan 12];201(10):1509–16. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3060408&tool=pmc

entrez&rendertype=abstract

149. Ghebrehewet S, MacPherson P, Ho A. Influenza. BMJ [Internet]. 2016 Dec 7 [cited 2017 Jan 12];355:i6258. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=5141587&tool=pmcentrez&rendertype=abstract

150. Tamerius J, Nelson MI, Zhou SZ, Viboud C, Miller MA, Alonso WJ. Global influenza seasonality: reconciling patterns across temperate and tropical regions. Environ Health Perspect [Internet]. 2011 Apr [cited 2017 Jan 13];119(4):439–45. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3080923&tool=pmcentrez&rendertype=abstract

151. Finkelman BS, Viboud C, Koelle K, Ferrari MJ, Bharti N, Grenfell BT. Global patterns in seasonal activity of influenza A/H3N2, A/H1N1, and B from 1997 to 2005: viral coexistence and latitudinal gradients. PLoS One [Internet]. 2007 Dec 12 [cited 2017 Jan 13];2(12):e1296. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2117904&tool=pmcentrez&rendertype=abstract

152. World Health Organization. WHO recommendations on the composition of influenza virus vaccines [Internet]. 2017 [cited 2017 Jan 13]. Available from: http://www.who.int/influenza/vaccines/virus/recommendations/en/

153. Couch RB. An overview of serum antibody responses to influenza virus antigens.

Dev Biol (Basel) [Internet]. 2003 Jan [cited 2017 Jan 13];115:25–30. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15088772

154. Lambert ND, Ovsyannikova IG, Pankratz S, Jacobson RM, Poland GA. Understanding the immune response to seasonal influenza vaccination in older adults: a systems biology approach. Expert Rev Vaccines. 2012;11(9):985–94.

155. Webster RG, Laver WG. Preparation and properties of antibody directed specifically against the neuraminidase of influenza virus. J Immunol [Internet]. 1967 Jul [cited 2017 Jan 13];99(1):49–55. Available from: http://www.ncbi.nlm.nih.gov/pubmed/6029282

156. Monto AS, Kendal AP. Effect of neuraminidase antibody on Hong Kong influenza. Lancet (London, England). 1973 Mar;1(7804):623–5.

157. Coudeville L, Bailleux F, Riche B, Megas F, Andre P, Ecochard R. Relationship between haemagglutination-inhibiting antibody titres and clinical protection against influenza: development and application of a bayesian random-effects model. BMC Med Res Methodol. 2010 Mar;10(1):18.

158. Barrett PN, Berezuk G, Fritsch S, Aichinger G, Hart MK, El-Amin W, et al. Efficacy, safety, and immunogenicity of a Vero-cell-culture-derived trivalent influenza vaccine: a multicentre, double-blind, randomised, placebo-controlled trial. Lancet (London, England). 2011;377(9767):751–9.

159. Black S, Nicolay U, Vesikari T, Knuf M, Del Giudice G, Della Cioppa G, et al. Hemagglutination Inhibition Antibody Titers as a Correlate of Protection for

Inactivated Influenza Vaccines in Children. Pediatr Infect Dis J. 2011;30(12):1081–5.

160. European Medicines Agency. Guideline on Influenza Vaccines. 2014.

161. Pauksen K, Linde a, Hammarström V, Sjölin J, Carneskog J, Jonsson G, et al. Granulocyte-macrophage colony-stimulating factor as immunomodulating factor together with influenza vaccination in stem cell transplant patients. Clin Infect Dis. 2000;30:342–8.

162. Gandhi MK, Egner W, Sizer L, Inman I, Zambon M, Craig JI, et al. Antibody responses to vaccinations given within the first two years after transplant are similar between autologous peripheral blood stem cell and bone marrow transplant recipients. Bone Marrow Transplant. 2001;28(June):775–81.

163. Avetisyan G, Aschan J, Hassan M, Ljungman P. Evaluation of immune responses to seasonal influenza vaccination in healthy volunteers and in patients after stem cell transplantation. Transplantation. 2008;86(2):257–63.

164. Issa NC, Marty FM, Gagne LS, Koo S, Verrill K a., Alyea EP, et al. Seroprotective Titers against 2009 H1N1 Influenza A Virus after Vaccination in Allogeneic Hematopoietic Stem Cell Transplantation Recipients. Biol Blood Marrow Transplant. 2011;17:434–8.

165. Gueller S, Allwinn R, Mousset S, Martin H, Wieters I, Herrmann E, et al. Enhanced Immune Response after a Second Dose of an AS03-Adjuvanted H1N1 Influenza A Vaccine in Patients after Hematopoietic Stem Cell Transplantation. Biol Blood

Marrow Transplant [Internet]. Elsevier Inc; 2011;17(10):1546–50. Available from: http://dx.doi.org/10.1016/j.bbmt.2011.02.004

166. Karras N, Weeres M, Sessions W, Xu X, DeFor TE, Young J-AH, et al. A Randomized Trial of One Vs. Two Doses of Influenza Vaccine Following Allogeneic Transplantation. Biol Blood Marrow Transplant. 2013;19(1):109–16.

167. de Lavallade H, Garland P, Sekine T, Hoschler K, Marin D, Stringaris K, et al. Repeated vaccination is required to optimize seroprotection against H1N1 in the immunocompromised host. Haematologica. 2011;96(2):307–14.

168. Mohty B, Bel M, Vukicevic M, Nagy M, Levrat E, Meier S, et al. Graft-versus-host disease is the major determinant of humoral responses to the AS03-adjuvanted influenza A/09/H1N1 vaccine in allogeneic hematopoietic stem cell transplant recipients. Haematologica [Internet]. 2011 Jun [cited 2014 Dec 15];96(6):896–904. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3105652&tool=pmcentrez&rendertype=abstract

169. Dhédin N, Krivine A, Le N, Mallet A, Lioure B, Bay J, et al. Comparable humoral response after two doses of adjuvanted influenza A / H1N1pdm2009 vaccine or natural infection in allogeneic stem cell transplant recipients. Vaccine [Internet]. 2014;32(5):585–91. Available from: http://dx.doi.org/10.1016/j.vaccine.2013.11.073

170. Ljungman P, Nahi H, Linde A. Vaccination of patients with haematological

malignancies with one or two doses of influenza vaccine: A randomised study. Br J Haematol. 2005;130(1):96–8.

171. Machado CM, Cardoso MR a, da Rocha IF, Boas LS V, Dulley FL, Pannuti CS. The benefit of influenza vaccination after bone marrow transplantation. Bone Marrow Transplant [Internet]. 2005 Nov [cited 2014 Dec 3];36(10):897–900. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16170332

172. Orenstein W a, Bernier RH, Hinman a R. Assessing vaccine efficacy in the field. Further observations. Epidemiol Rev. 1988;10(18):212–41.

173. Fedson DS. Measuring protection: efficacy versus effectiveness. Dev Biol Stand [Internet]. 1998 Jan [cited 2015 Mar 26];95:195–201. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9855432

174. World Health Organization. Observed Rates of Vaccine Reactions - Influenza Vaccine. 2012.

175. Govaert TM, Dinant GJ, Aretz K, Masurel N, Sprenger MJ, Knottnerus JA. Adverse reactions to influenza vaccine in elderly people: randomised double blind placebo controlled trial. BMJ [Internet]. BMJ Group; 1993 Oct 16 [cited 2017 Feb 24];307(6910):988–90. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8241913

176. Margolis KL, Nichol KL, Poland GA, Pluhar RE. Frequency of Adverse Reactions to Influenza Vaccine in the Elderly. JAMA [Internet]. American Medical Association; 1990 Sep 5 [cited 2017 Feb 24];264(9):1139. Available from:

http://jama.jamanetwork.com/article.aspx?doi=10.1001/jama.1990.03450090075029

177. Bridges CB, Thompson WW, Meltzer MI, Reeve GR, Talamonti WJ, Cox NJ, et al. Effectiveness and cost-benefit of influenza vaccination of healthy working adults: A randomized controlled trial. JAMA. 2000;284(13):1655–63.

178. Nichol KL, Margolis KL, Lind A, Murdoch M, McFadden R, Hauge M, et al. Side Effects Associated With Influenza Vaccination in Healthy Working Adults. Arch Intern Med [Internet]. American Medical Association; 1996 Jul 22 [cited 2017 Feb 24];156(14):1546. Available from: http://archinte.jamanetwork.com/article.aspx?doi=10.1001/archinte.1996.00440130090009

179. Delore V, Salamand C, Marsh G, Arnoux S, Pepin S, Saliou P. Long-term clinical trial safety experience with the inactivated split influenza vaccine, Vaxigrip?? Vaccine. 2006;24(10):1586–92.

180. Van de Witte S V., Nauta J, Giezeman-Smits KM, de Voogd JM. Trivalent inactivated subunit influenza vaccine Influvac: 25-year experience of safety and immunogenicity. Vaccine. 2008;1:2414–7.

181. World Health Organization. Information Sheet - Observed Rate of Vaccine Reactions: Influenza Vaccine. 2012.

182. Juurlink DN, Stukel TA, Kwong J, Kopp A, McGeer A, Upshur RE, et al. Guillain-Barré Syndrome After Influenza Vaccination in Adults: a population-based study.

Arch Intern Med [Internet]. 2006 Nov 13 [cited 2017 Feb 24];166(20):2217–21. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17101939

183. De Serres G, Toth E, Ménard S, Grenier JL, Roussel R, Tremblay M, et al. Oculo-respiratory syndrome after influenza vaccination: trends over four influenza seasons. Vaccine. 2005;23(28):3726–32.

184. Skowronski DM, Strauss B, De Serres G, MacDonald D, Marion SA, Naus M, et al. Oculo-respiratory Syndrome: A New Influenza Vaccine–Associated Adverse Event? Clin Infect Dis [Internet]. 2003 Mar 15 [cited 2017 Feb 24];36(6):705–13. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12627354

185. Al-Dabbagh M, Lapphra K, Scheifele DW, Halperin SA, Langley JM, Cho P, et al. Elevated inflammatory mediators in adults with oculorespiratory syndrome following influenza immunization: a public health agency of Canada/Canadian Institutes of Health Research Influenza Research Network Study. Clin Vaccine Immunol [Internet]. American Society for Microbiology; 2013 Aug [cited 2017 Feb 24];20(8):1108–14. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23697573

186. Olkinuora H, Käyhty H, Davidkin I, Roivainen M, Olander R-M, Kantele JM, et al. Immunity after (re)vaccination of paediatric patients following haematopoietic stem cell transplantation. Acta Paediatr [Internet]. 2012 Aug [cited 2014 Nov 23];101(8):e373-7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22537137

187. Ljungman P, Avetisyan G. Influenza vaccination in hematopoietic SCT recipients. Bone Marrow Transplant [Internet]. 2008 Nov [cited 2014 Dec 9];42(10):637–41. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18724396

188. Zbinden D, Manuel O. Influenza vaccination in immunocompromised patients: efficacy and safety. Immunotherapy [Internet]. 2014;6(2):131–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24491087

189. Katz JM, Hancock K, Xu X. Serologic assays for influenza surveillance, diagnosis and vaccine evaluation. Expert Rev Anti Infect Ther. 2011;9(6):669–83.

190. Kendal a. P, Cate TR. Increased sensitivity and reduced specificity of hemagglutination inhibition tests with ether-treated influenza B/Singapore/222/79. J Clin Microbiol. 1983;18(4):930–4.

191. Frank a. L, Puck J, Hughes BJ, Cate TR. Microneutralization test for influenza A and B and parainfluenza 1 and 2 viruses that uses continuous cell lines and fresh serum enhancement. J Clin Microbiol. 1980;12(3):426–32.

192. Veguilla V, Hancock K, Schiffer J, Gargiullo P, Lu X, Aranio D, et al. Sensitivity and specificity of serologic assays for detection of human infection with 2009 pandemic H1N1 virus in U.S. populations. J Clin Microbiol. 2011;49(6):2210–5.

193. Rowe T, Abernathy R a, Hu-Primmer J, Thompson WW, Lu X, Lim W, et al. Detection of antibody to avian influenza A (H5N1) virus in human serum by using a combination of serologic assays. J Clin Microbiol. 1999;37(4):937–43.

194. Gross P, Davis A. Neutralization Test in Influenza: Use in Individuals Without

Haemagglutination Inhibition Antibody. J Clin Microbiol. 1979;10(3):382–4.

195. Ljungman P, Cordonnier C, Einsele H, Englund J, Machado CM, Storek J, et al. Vaccination of hematopoietic cell transplant recipients. Bone Marrow Transplant [Internet]. Nature Publishing Group; 2009 Oct [cited 2014 Oct 30];44(8):521–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19861986

196. Passweg JR, Baldomero H, Bregni M, Cesaro S, Dreger P, Duarte RF, et al. Hematopoietic SCT in Europe: data and trends in 2011. Bone Marrow Transplant [Internet]. 2013;48(9):1161–7. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3763517&tool=pmcentrez&rendertype=abstract

197. World Health Organization. Manual for the laboratory diagnosis and virological surveillance of influenza. World Heal Organ 2011. 2011;153.

198. Oh DY, Barr IG, Mosse JA, Laurie KL. MDCK-SIAT1 cells show improved isolation rates for recent human influenza viruses compared to conventional MDCK cells. J Clin Microbiol [Internet]. 2008 Jul [cited 2017 Jan 18];46(7):2189–94. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2446904&tool=pmcentrez&rendertype=abstract

199. Matrosovich M, Matrosovich T, Carr J, Roberts NA, Klenk H-D. Overexpression of the alpha-2,6-sialyltransferase in MDCK cells increases influenza virus sensitivity to neuraminidase inhibitors. J Virol [Internet]. 2003 Aug [cited 2017 Jan

18];77(15):8418–25. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=165236&tool=pmcentrez&rendertype=abstract

200. Who. Manual for the laboratory diagnosis and virological surveillance of influenza. World Heal Organ 2011. 2011;153.

201. Field A. Discovering Statistics Using IBM SPSS Statistics. Fourth. Sage; 2014.

202. Reed GF, Meade BD, Steinhoff MC. The reverse cumulative distribution plot: a graphic method for exploratory analysis of antibody data. Pediatrics [Internet]. 1995;96(3 Pt 2):600–3. Available from: http://www.ncbi.nlm.nih.gov/pubmed/7659485

203. Verschoor CP, Singh P, Russell ML, Bowdish DME, Brewer A, Cyr L, et al. Microneutralization Assay Titres Correlate with Protection against Seasonal Influenza H1N1 and H3N2 in Children. PLoS One. 2015;10(6):e0131531.

204. World Health Organization. Recommendations for Influenza Vaccine Composition [Internet]. 1998. Available from: http://www.who.int/influenza/vaccines/vaccinerecommendations1/en/index23.html

205. Pyhala R, Kleemola M, Viakorpi R. The HI Test Modified by Ether Treatment in the Sero-Epidemiological Surveillance of Influenza B. J Hyg (Lond). 1985;94(3):341–8.

206. Goodwin K, Viboud C, Simonsen L. Antibody response to influenza vaccination in the elderly: A quantitative review. Vaccine. 2006;24(8):1159–69.

207. Torda a, Chong Q, Lee a, Chen S, Dodds a, Greenwood M, et al. Invasive pneumococcal disease following adult allogeneic hematopoietic stem cell transplantation. Transpl Infect Dis [Internet]. 2014 Oct [cited 2014 Dec 3];16(5):751–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25040633

208. Shah DP, Ghantoji SS, Mulanovich VE, Ariza-heredia EJ, Chemaly RF. Management of respiratory viral infections in hematopoietic cell transplant recipients. 2012;2(4):203–18. Available from: www.ncbi.nlm.nih.gov/pubmed/23226621%0Awww.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC3512176

209. Tomblyn M, Chiller T, Einsele H, Gress R, Sepkowitz K, Storek J, et al. Guidelines for preventing infectious complications among hematopoietic cell transplantation recipients: a global perspective. Biol Blood Marrow Transplant [Internet]. 2009 Oct [cited 2014 Jul 10];15(10):1143–238. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3103296&tool=pmcentrez&rendertype=abstract

210. Mosher CE, Redd WH, Rini CM, Burkhalter JE, DuHamel KN. Physical, psychological, and social sequelae following hematopoietic stem cell transplantation: a review of the literature. Psychooncology. 2009 Feb;18(2):113–27.

211. Cooke L, Gemmill R, Kravits K, Grant M. Psychological issues of stem cell transplant. Semin Oncol Nurs [Internet]. NIH Public Access; 2009 May [cited 2017 Jan 30];25(2):139–50. Available from:

http://www.ncbi.nlm.nih.gov/pubmed/19411017

212. Orenstein WA, Despres S, Trusts PC. The National Vaccine Advisory Committee : Reducing Patient and Provider Barriers to Maternal Immunizations. 2015;

213. Bertoncini P, Schoenauer R, Agarkova I, Hegner M. Vaccine hesitancy: Definition, scope and determinants. Vaccine. 2005;33(34):4161–4.

214. Public Health England. Influenza immunisation programme for England. Data collection survey Season 2015 to 2016 [Internet]. 2016 [cited 2017 Feb 5]. Available from: https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/544552/Seasonal_flu_GP_patient_groups_annual_report_2015_2016.pdf

215. NHS England. The National Flu Immunisation Programme 2016/2017 [Internet]. 2016 [cited 2017 Feb 22]. Available from: https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/529954/Annual_flu_letter_2016_2017.pdf

216. McIntyre A, Zecevic A, Diachun L. Influenza Vaccinations: Older Adults' Decision-Making Process. Can J Aging. 2013;33(1):92–8.

217. Yu DSF, Low LPL, Lee IFK, Lee DTF, Ng WM. Predicting Influenza Vaccination Intent Among At-Risk Chinese Older Adults in Hong Kong. Nurs Res [Internet]. 2014;63(4):270–7. Available from: http://content.wkhealth.com/linkback/openurl?sid=WKPTLP:landingpage&an=00006199-201407000-00007

218. Wooten KG, Wortley PM, Singleton J a., Euler GL. Perceptions matter: Beliefs about influenza vaccine and vaccination behavior among elderly white, black and Hispanic Americans. Vaccine [Internet]. Elsevier Ltd; 2012;30(48):6927–34. Available from: http://dx.doi.org/10.1016/j.vaccine.2012.08.036

219. Nagata JM, Hernández-Ramos I, Kurup a S, Albrecht D, Vivas-Torrealba C, Franco-Paredes C. Social determinants of health and seasonal influenza vaccination in adults ≥65 years: A systematic review of qualitative and quantitative data. BMC Public Health. 2013;13(1):1–25.

220. Kroneman M, Van Essen G a., John Paget W. Influenza vaccination coverage and reasons to refrain among high-risk persons in four European countries. Vaccine. 2006;24(5):622–8.

221. Jessop a. B, Dumas H, Moser C a. Delivering Influenza Vaccine to High-Risk Adults: Subspecialty Physician Practices. Am J Med Qual. 2012;

222. Vinograd I, Baslo R, Eliakim-Raz N, Farbman L, Taha A, Sakhnini A, et al. Factors associated with influenza vaccination among adult cancer patients: a case-control study. Clin Microbiol Infect. 2014;20(9):899–905.

223. Loubet P, Kernéis S, Groh M, Loulergue P, Blanche P, Verger P, et al. Attitude, knowledge and factors associated with influenza and pneumococcal vaccine uptake in a large cohort of patients with secondary immune deficiency. Vaccine. 2015 Jul 17;33(31):3703–8.

224. Wheelock A, Thomson A, Sevdalis N. Social and psychological factors underlying

adult vaccination behavior: lessons from seasonal influenza vaccination in the US and the UK. Expert Rev Vaccines [Internet]. 2013;12(8):893–901. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23944683

225. Chapman GB, Elliot J, Coups BA. Predictors of influenza vaccine. Acceptance among healthy adult workers. AAOHN J. 1999;29:249–62.

226. Ernsting A, Gellert P, Schneider M, Lippke S. A mediator model to predict workplace influenza vaccination behaviour--an application of the health action process approach. Psychol Health [Internet]. 2013;28(5):579–92. Available from: http://www.scopus.com/inward/record.url?eid=2-s2.0-84876343300&partnerID=tZOtx3y1

227. Liao Q, Wong WS, Fielding R. How do anticipated worry and regret predict seasonal influenza vaccination uptake among Chinese adults? Vaccine [Internet]. Elsevier Ltd; 2013;31(38):4084–90. Available from: http://dx.doi.org/10.1016/j.vaccine.2013.07.009

228. Malosh R, Ohmit SE, Petrie JG, Thompson MG, Aiello AE, Monto AS. Factors associated with influenza vaccine receipt in community dwelling adults and their children. Vaccine. 2014;32(16):1841–7.

229. Nowrouzi-Kia B, McGeer A. External cues to action and influenza vaccination among post-graduate trainee physicians in Toronto, Canada. Vaccine [Internet]. 2014;32(30):3830–4. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24837775

230. Naleway AL, Henkle EM, Ball S, Bozeman S, Gaglani MJ, Kennedy ED, et al. Barriers and facilitators to influenza vaccination and vaccine coverage in a cohort of health care personnel. Am J Infect Control [Internet]. 2014 Apr [cited 2015 Apr 13];42(4):371–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24679562

231. Paparone P. Supporting Influenza Vaccination Intent Among Nurses. JONA J Nurs Adm [Internet]. 2015;45(3):133–8. Available from: http://content.wkhealth.com/linkback/openurl?sid=WKPTLP:landingpage&an=00005110-201503000-00005

232. Corace K, Prematunge C, McCarthy A, Nair RC, Roth V, Hayes T, et al. Predicting influenza vaccination uptake among health care workers: What are the key motivators? Am J Infect Control [Internet]. Elsevier Inc; 2013;41(8):679–84. Available from: http://dx.doi.org/10.1016/j.ajic.2013.01.014

233. FitzSimons D, Hendrickx G, Lernout T, Badur S, Vorsters A, Van Damme P. Incentives and barriers regarding immunization against influenza and hepatitis of health care workers. Vaccine [Internet]. 2014;32(38):4849–54. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0264410X14008731

234. Mytton OT, O'Moore EM, Sparkes T, Baxi R, Abid M. Knowledge, attitudes and beliefs of health care workers towards influenza vaccination. Occup Med (Lond) [Internet]. 2013;63(3):189–95. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23447033

235. Henninger M, Naleway A, Crane B, Donahue J, Irving S. Predictors of seasonal influenza vaccination during pregnancy. Obstet Gynecol [Internet]. 2013;121(4):741–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23635673

236. Gorman JR, Brewer NT, Wang JB, Chambers CD. Theory-based predictors of influenza vaccination among pregnant women. Vaccine [Internet]. Elsevier Ltd; 2012;31(1):213–8. Available from: http://dx.doi.org/10.1016/j.vaccine.2012.10.064

237. Eppes C, Wu A, You W, Cameron K a., Garcia P, Grobman W. Barriers to influenza vaccination among pregnant women. Vaccine [Internet]. Elsevier Ltd; 2013;31(27):2874–8. Available from: http://dx.doi.org/10.1016/j.vaccine.2013.04.031

238. Yuet Yuen C, Yee Fong D, Lai Lee I, Chu S, Sau-mei E, Tarrant M. Prevalence and predictors of maternal seasonal influenza vaccination in Hong Kong. Vaccine [Internet]. Elsevier Ltd; 2013;31(45):5281–8. Available from: http://dx.doi.org/10.1016/j.vaccine.2013.08.063

239. Bödeker B, Walter D, Reiter S, Wichmann O. Cross-sectional study on factors associated with influenza vaccine uptake and pertussis vaccination status among pregnant women in Germany. Vaccine [Internet]. Elsevier Ltd; 2014;32(33):4131–9. Available from: http://dx.doi.org/10.1016/j.vaccine.2014.06.007

240. Frew PM, Saint-Victor DS, Owens LE, Omer SB. Socioecological and message

framing factors influencing maternal influenza immunization among minority women. Vaccine [Internet]. Elsevier Ltd; 2014;32(15):1736–44. Available from: http://dx.doi.org/10.1016/j.vaccine.2014.01.030

241. Myers LB, Goodwin R. Using a theoretical framework to determine adults' intention to vaccinate against pandemic swine flu in priority groups in the UK. Public Health. Elsevier Ltd; 2012;126(SUPPL.1):S53–6.

242. Bíró A. Determinants of H1N1 vaccination uptake in England. Prev Med (Baltim) [Internet]. Elsevier Inc.; 2013;57(2):140–2. Available from: http://dx.doi.org/10.1016/j.ypmed.2013.04.017

243. Valour F, Bénet T, Chidiac C. Pandemic A(H1N1)2009 influenza vaccination in Lyon University Hospitals, France: Perception and attitudes of hospital workers. Vaccine. 2013;31(4):592–5.

244. Nan X, Kim J. Predicting H1N1 vaccine uptake and H1N1-related health beliefs: the role of individual difference in consideration of future consequences. J Health Commun [Internet]. 2014;19(3):376–88. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24354858

245. Anne-Laure CB, Jocelyn R, Nathanaël L, De-Lambal X, Fabrice C, Michel S. Predictors of IV behaviors during and after the 2009 influenza pandemic in France. Vaccine [Internet]. Elsevier Ltd; 2014;32(17):2007–15. Available from: http://dx.doi.org/10.1016/j.vaccine.2013.12.045

246. Lewthwaite P, Campion K, Blackburn B, Kemp E, Major D, Sarangi K. Healthcare

workers' attitude towards influenza vaccination after the 2009 pandemic. Occup Med (Chic Ill). 2014;64(5):348–51.

247. Börjesson M, Enander A. Perceptions and sociodemographic factors influencing vaccination uptake and precautionary behaviours in response to the A/H1N1 influenza in Sweden. Scand J Public Health [Internet]. 2013;(October 2013):215–22. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24259541

248. Winston CA, Wortley PM, Lees KA. Factors associated with vaccination of medicare beneficiaries in five U.S. communities: Results from the racial and ethnic adult disparities in immunization initiative survey, 2003. J Am Geriatr Soc [Internet]. 2006 Feb [cited 2015 Jul 9];54(2):303–10. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16460383

249. Tabbarah M, Zimmerman RK, Nowalk MP, Janosky JE, Troy JA, Raymund M, et al. What predicts influenza vaccination status in older Americans over several years? J Am Geriatr Soc [Internet]. 2005 Aug [cited 2015 Jul 9];53(8):1354–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16078961

250. Ganguly R, Webster TB, Chmel H, Yangco BV. Influence of physician's recommendation on influenza immunization: perception and acceptance among a group of institutionalized elderly. Serodiagn Immunother Infect Dis [Internet]. 1990 Jun [cited 2015 Jul 8];4(3):167–71. Available from: http://www.sciencedirect.com/science/article/pii/0888078690900015

251. Armstrong K, Berlin M, Schwartz JS, Propert K, Ubel PA. Barriers to influenza

immunization in a low-income urban population. Am J Prev Med [Internet]. 2001 Jan [cited 2015 Jul 8];20(1):21–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11137770

252. Lewis-Parmar H, McCann R. Achieving national influenza vaccine targets--an investigation of the factors affecting influenza vaccine uptake in older people and people with diabetes. Commun Dis Public Health [Internet]. 2002 Jun [cited 2015 Jul 8];5(2):119–26. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12166297

253. Bekker HL, Gough D, Williams M. Attendance choices about the Influenza Immunization Programme: Evidence for targeting patients' beliefs. Psychol Health Med [Internet]. Taylor & Francis Group; 2003 Aug 19 [cited 2015 Jul 8];8(3):279–88. Available from: http://www.tandfonline.com/doi/abs/10.1080/1354850031000135722?journalCode=cphm20#.VZ1FNuvF_dk

254. Mangtani P, Breeze E, Stirling S, Hanciles S, Kovats S, Fletcher A. Cross-sectional survey of older peoples' views related to influenza vaccine uptake. BMC Public Health [Internet]. 2006 Jan [cited 2015 Jul 8];6(1):249. Available from: http://www.biomedcentral.com/1471-2458/6/249

255. Gallagher S, Povey R. Determinants of older adults' intentions to vaccinate against influenza: a theoretical application. J Public Health (Oxf) [Internet]. 2006 Jun [cited 2015 Jul 8];28(2):139–44. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16641171

256. Keenan H, Campbell J, Evans PH. Influenza vaccination in patients with asthma: why is the uptake so low? Br J Gen Pract [Internet]. 2007 May [cited 2015 May 29];57(538):359–63. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2047009&tool=pmcentrez&rendertype=abstract

257. Colley E. Influenza vaccination in adults with a long-term condition. Community Pract [Internet]. 2008 Apr [cited 2015 Jul 8];81(4):25–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18497225

258. Carter WB, Beach LR, Inui TS, Kirscht JP, Prodzinski JC. Developing and testing a decision model for predicting influenza vaccination compliance. Health Serv Res [Internet]. 1986 Feb [cited 2015 Jul 8];20(6 Pt 2):897–932. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1068913&tool=pmcentrez&rendertype=abstract

259. Honkanen PO, Keistinen T, Kivela SL. Factors associated with influenza vaccination coverage among the elderly: role of health care personnel. Public Health [Internet]. 1996 May [cited 2015 Jul 8];110(3):163–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8668762

260. Zimmerman RK, Nowalk MP, Santibanez TA, Jewell IK, Raymond M. Shortage of influenza vaccine in 2000-2001: did it change patient beliefs? Am J Prev Med [Internet]. 2003 May [cited 2015 Jul 8];24(4):349–53. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12726873

261. Abel RL, McGaha P, Young R, Bing M, Foran T, Oaks B. Understanding differences in influenza immunization rates: a survey of African-American and caucasian medicare beneficiaries in Texas. J Health Hum Serv Adm [Internet]. 2003 Jan [cited 2015 Jul 8];26(2):174–98. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15330489

262. Mayo AM, Cobler S. Flu vaccines and patient decision making: what we need to know. J Am Acad Nurse Pract [Internet]. 2004 Sep [cited 2015 Jul 8];16(9):402–10. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15495694

263. Nowalk MP, Zimmerman RK, Shen S, Jewell IK, Raymund M. Barriers to pneumococcal and influenza vaccination in older community-dwelling adults (2000-2001). J Am Geriatr Soc [Internet]. 2004 Jan [cited 2015 Jul 8];52(1):25–30. Available from: http://www.ncbi.nlm.nih.gov/pubmed/14687311

264. Brewer NT, Hallman WK. Subjective and objective risk as predictors of influenza vaccination during the vaccine shortage of 2004-2005. Clin Infect Dis [Internet]. 2006 Dec 1 [cited 2015 Jul 8];43(11):1379–86. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17083008

265. Frew PM, Painter JE, Hixson B, Kulb C, Moore K, del Rio C, et al. Factors mediating seasonal and influenza A (H1N1) vaccine acceptance among ethnically diverse populations in the urban south. Vaccine [Internet]. 2012 Jun 13 [cited 2015 Jul 8];30(28):4200–8. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3522428&tool=pmcentrez&rendertype=abstract

266. Weinstein ND, Kwitel A, McCaul KD, Magnan RE, Gerrard M, Gibbons FX. Risk perceptions: assessment and relationship to influenza vaccination. Health Psychol [Internet]. 2007 Mar [cited 2015 Jul 8];26(2):146–51. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17385965

267. Shim E, Chapman GB, Townsend JP, Galvani AP. The influence of altruism on influenza vaccination decisions. J R Soc Interface [Internet]. 2012 Sep 7 [cited 2015 Jul 8];9(74):2234–43. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3405754&tool=pmcentrez&rendertype=abstract

268. Zimmerman RK, Santibanez TA, Janosky JE, Fine MJ, Raymund M, Wilson SA, et al. What affects influenza vaccination rates among older patients? An analysis from inner-city, suburban, rural, and Veterans Affairs practices. Am J Med [Internet]. 2003 Jan [cited 2015 Jul 8];114(1):31–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12543287

269. Telford R, Rogers A. What influences elderly peoples' decisions about whether to accept the influenza vaccination? A qualitative study. Health Educ Res [Internet]. 2003 Dec [cited 2015 Jul 8];18(6):743–53. Available from: http://www.ncbi.nlm.nih.gov/pubmed/14654506

270. Madhavan SS, Rosenbluth SA, Amonkar M, Fernandes A, Borker R. Immunization predictors in rural adults under 65 years of age. J Health Care Poor Underserved [Internet]. 2003 Feb [cited 2015 Jul 8];14(1):100–21. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12613071

271. Chapman GB, Coups EJ. Emotions and preventive health behavior: worry, regret, and influenza vaccination. Health Psychol. 2006;25(1):82–90.

272. Wray RJ, Jupka K, Ross W, Dotson D, Whitworth AR, Jacobsen H. How can you improve vaccination rates among older African Americans? J Fam Pract [Internet]. 2007 Nov [cited 2015 Jul 8];56(11):925–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17976341

273. Johnson DR, Nichol KL, Lipczynski K. Barriers to Adult Immunization. Am J Med. 2008;121(28–35).

274. Harris KM, Maurer J, Lurie N. Is getting influenza vaccine coverage data out during mid-season feasible? Evidence from a national survey of U.S. adults. Vaccine [Internet]. 2009 Jun 8 [cited 2015 Jul 8];27(28):3697–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19588550

275. Ganguly R, Cameron D. Factors affecting immunization rate in a cohort of elderly veterans: a retrospective pilot study of influenza vaccine compliance. Vaccine [Internet]. 1989 Oct [cited 2015 Jul 8];7(5):462–4. Available from: http://www.ncbi.nlm.nih.gov/pubmed/2815980

276. Wrenn K, Zeldin M, Miller O. Influenza and pneumococcal vaccination in the emergency department: is it feasible? J Gen Intern Med [Internet]. 1994 Aug [cited 2015 Jul 8];9(8):425–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/7965235

277. Fiebach NH, Viscoli CM. Patient acceptance of influenza vaccination. Am J Med

[Internet]. 1991 Oct [cited 2015 Jul 8];91(4):393–400. Available from: http://www.ncbi.nlm.nih.gov/pubmed/1951383

278. Johnson DR, Nichol KL, Lipczynski K. Barriers to adult immunization. Am J Med [Internet]. 2008 Jul [cited 2015 Jul 8];121(7 Suppl 2):S28-35. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18589065

279. Gibbons FX. Behavioral Intentions, Expectations and Willingness [Internet]. National Cancer Institute Behavioural Research programme. 2008 [cited 2017 Jan 30]. Available from: https://cancercontrol.cancer.gov/brp/research/constructs/intent-expect-willingness.html

280. Sheeran P. Intention — Behavior Relations : A Conceptual and Empirical Review. Eur Rev Soc Psychol. 2002;12(1):1–36.

281. Rosenstock IM. Historical Origins of the Health Belief Model. Heal Educ Behav [Internet]. SAGE PublicationsSage CA: Los Angeles, CA; 1974 Dec 1 [cited 2017 Feb 22];2(4):328–35. Available from: http://heb.sagepub.com/lookup/doi/10.1177/109019817400200403

282. Rosenstock IM, Strecher VJ, Becker MH. Social learning theory and the health belief model. Health Educ J. 1988;15(2):175–83.

283. Brewer NT, Chapman GB, Gibbons FX, Gerrard M, McCaul KD, Weinstein ND. Meta-analysis of the relationship between risk perception and health behavior: the example of vaccination. Health Psychol. 2007;26(2):136–45.

284. Rosenstock IM, Strecher VJ, Becker MH. Social Learning Theory and the Health Belief Model. Heal Educ Behav [Internet]. Sage PublicationsSage CA: Thousand Oaks, CA; 1988 Jan 1 [cited 2017 Feb 21];15(2):175–83. Available from: http://heb.sagepub.com/cgi/doi/10.1177/109019818801500203

285. Abraham C, Sheeran P. Acting on intentions: The role of anticipated regret. Br J Soc Psychol. 2003;42:495–511.

286. Panayides P. Coefficient Alpha: Interpret With Caution. Eur J Psychol. 2013;9(4):687–96.

287. Box GEP, Tidwell PW. Transformation of the Independent Variables. Technometrics. Taylor & Francis Group; 1962 Apr 30;4(4):531–50.

288. Hassan I a, Chopra R, Swindell R, Mutton KJ. Respiratory viral infections after bone marrow/peripheral stem-cell transplantation: the Christie hospital experience. Bone Marrow Transplant. 2003;32(1):73–7.

289. Martino R, Porras RP, Rabella N, Williams J V., Ramila E, Margall N, et al. Prospective Study of the Incidence, Clinical Features, and Outcome of Symptomatic Upper and Lower Respiratory Tract Infections by Respiratory Viruses in Adult Recipients of Hematopoietic Stem Cell Transplants for Hematologic Malignancies. Biol Blood Marrow Transpl. 2005;11(10):781–96.

290. Cameron KA, Rintamaki LS, Kamanda-Kosseh M, Noskin GA, Baker DW, Makoul G. Using theoretical constructs to identify key issues for targeted message design: African American seniors' perceptions about influenza and influenza vaccination.

Health Commun [Internet]. Taylor & Francis Group; 2009 Jun 3 [cited 2017 Feb 10];24(4):316–26. Available from: http://www.tandfonline.com/doi/abs/10.1080/10410230902889258

291. Schmid P, Rauber D, Betsch C, Lidolt G, Denker M-L. Barriers of Influenza Vaccination Intention and Behavior - A Systematic Review of Influenza Vaccine Hesitancy, 2005 - 2016. PLoS One. 2017;12(1):1–46.

292. Vinograd I, Baslo R, Farbman L, Taha A, Sakhnini A, Lador A, et al. Factors associated with influenza vaccination among adult cancer patients : a case – control study. Clin Microbiol Infect. European Society of Clinical Infectious Diseases; 2014;20(9):899–905.

293. Yeung MPS, Lam FLY, Coker R. Factors associated with the uptake of seasonal influenza vaccination in adults: a systematic review. J Public Health (Bangkok). 2016 Jan 6;38(4):746–53.

294. Miles A. Perceived Severity [Internet]. National Cancer Institute Behavioural Research programme. 2008 [cited 2017 Feb 13]. Available from: https://cancercontrol.cancer.gov/brp/research/constructs/perceived_severity.html

295. Loh Y, Oyama Y, Statkute L, Quigley K, Yaung K, Gonda E, et al. Development of a secondary autoimmune disorder after hematopoietic stem cell transplantation for autoimmune diseases: Role of conditioning regimen used. Blood. 2007;109(6):2643–8.

296. Daikeler T, Labopin M, Di Gioia M, Abinun M, Alexander T, Miniati I, et al. Secondary autoimmune diseases occurring after HSCT for an autoimmune disease: a retrospective study of the EBMT Autoimmune Disease Working Party. Blood [Internet]. 2011 Aug 11 [cited 2014 Oct 14];118(6):1693–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21596847

297. Daikeler T, Labopin M, Ruggeri A, Crotta A, Abinun M, Hussein AA, et al. New autoimmune diseases after cord blood transplantation: a retrospective study of EUROCORD and the Autoimmune Disease Working Party of the European Group for Blood and Marrow Transplantation. Blood [Internet]. 2013 Feb 7 [cited 2014 Oct 26];121(6):1059–64. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23247725

298. Faraci M, Zecca M, Pillon M, Rovelli A, Menconi MC, Ripaldi M, et al. Autoimmune hematological diseases after allogeneic hematopoietic stem cell transplantation in children: an Italian multicenter experience. Biol Blood Marrow Transplant [Internet]. 2014 Feb [cited 2015 Jan 9];20(2):272–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24274983

299. Nishio M, Sawada K, Koizumi K, Endoh T, Takashima H, Hashimoto H, et al. Autoimmune thrombocytopenia following syngeneic peripheral blood stem cell transplantation. Rinsho Ketsueki [Internet]. 1998 Aug;39(8):580–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9785976

300. Niederwieser D, Gentilini C, Hegenbart U, Lange T, Moosmann P, Pönisch W, et al. Transmission of donor illness by stem cell transplantation: should screening be

different in older donors? Bone Marrow Transplant [Internet]. 2004 Oct 30 [cited 2017 Nov 20];34(8):657–65. Available from: http://www.nature.com/articles/1704588

301. Bell SE, Goodnow CC. A selective defect in IgM antigen receptor synthesis and transport causes loss of cell surface IgM expression on tolerant B lymphocytes. EMBO J [Internet]. European Molecular Biology Organization; 1994 Feb 15 [cited 2017 Nov 23];13(4):816–26. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8112296

302. Hippen KL, Tze LE, Behrens TW. CD5 maintains tolerance in anergic B cells. J Exp Med [Internet]. The Rockefeller University Press; 2000 Mar 6 [cited 2017 Nov 23];191(5):883–90. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10704468

303. Huurman VAL, Unger WWJ, Koeleman BPC, Oaks MK, Chandraker AK, Terpstra OT, et al. Differential inhibition of autoreactive memory- and alloreactive naive T cell responses by soluble cytotoxic T lymphocyte antigen 4 (sCTLA4), CTLA4Ig and LEA29Y. Clin Exp Immunol [Internet]. Wiley-Blackwell; 2007 Dec [cited 2017 Nov 23];150(3):487–93. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17924973

304. Thien M, Phan TG, Gardam S, Amesbury M, Basten A, Mackay F, et al. Excess BAFF Rescues Self-Reactive B Cells from Peripheral Deletion and Allows Them to Enter Forbidden Follicular and Marginal Zone Niches. Immunity [Internet]. 2004 Jun [cited 2017 Nov 23];20(6):785–98. Available from:

http://www.ncbi.nlm.nih.gov/pubmed/15189742

305. Cárdenas-Roldán J, Rojas-Villarraga A, Anaya J-M. How do autoimmune diseases cluster in families? A systematic review and meta-analysis. BMC Med [Internet]. BioMed Central; 2013 Mar 18 [cited 2017 Nov 12];11:1–22. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23497011

306. Gough SCL, Simmonds MJ. The HLA Region and Autoimmune Disease: Associations and Mechanisms of Action. Curr Genomics [Internet]. Bentham Science Publishers; 2007 Nov [cited 2017 Nov 12];8(7):453–65. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19412418

307. Consolini R, Renee Forbes L, Wahlstrom J, Pignata C, Giardino G, Gallo V, et al. Unbalanced immune System: immunodeficiencies and Autoimmunity. Front Paediatr. 2016;4:1–9.

308. Hirota K, Hashimoto M, Yoshitomi H, Tanaka S, Nomura T, Yamaguchi T, et al. T cell self-reactivity forms a cytokine milieu for spontaneous development of IL-17 $^{+}$ Th cells that cause autoimmune arthritis. J Exp Med [Internet]. 2007 Jan 22 [cited 2017 Nov 12];204(1):41–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17227914

309. Lang KS, Recher M, Junt T, Navarini AA, Harris NL, Freigang S, et al. Toll-like receptor engagement converts T-cell autoreactivity into overt autoimmune disease. Nat Med [Internet]. 2005 Feb 16 [cited 2017 Jul 30];11(2):138–45. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15654326

310. King C, Ilic A, Koelsch K, Sarvetnick N, Jolla L. Homeostatic Expansion of T Cells during Immune Insufficiency Generates Autoimmunity. Cell. 2004;117:265–77.

311. Zhen Y, Bangzhao W, Y.N. Z, W.J. W, S.N. C, A.N. S, et al. Clinical and serological characterization of autoimmune hemolytic anemia after allogeneic hematopoietic stem cell transplantation. Chin Med J (Engl) [Internet]. Chinese Medical Association; 2014;127(7):1235–8. Available from: http://www.cmj.org/ch/reader/create_pdf.aspx?file_no=20132823&year_id=2014&quarter_id=7&falg=1%5Cnhttp://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=emed11&NEWS=N&AN=2014240199

312. O'Brien T a, Eastlund T, Peters C, Neglia JP, Defor T, Ramsay NKC, et al. Autoimmune haemolytic anaemia complicating haematopoietic cell transplantation in paediatric patients: high incidence and significant mortality in unrelated donor transplants for non-malignant diseases. Br J Haematol [Internet]. 2004 Oct [cited 2014 Oct 29];127(1):67–75. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15384979

313. Sanz J, Arriaga F, Montesinos P, Orti G, Lorenzo I, Cantero S, et al. Autoimmune hemolytic anemia following allogeneic hematopoietic stem cell transplantation in adult patients. Bone Marrow Transpl [Internet]. 2007 May 12 [cited 2014 Oct 13];39(9):555–61. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17351645

314. Chen F, Owen I, Savage D. Late onset haemolysis and red cell autoimmunisation after allogeneic bone marrow transplant. Bone Marrow Transplant.

1997;19:491–5.

315. Wang M, Wang W, Abeywardane A, Adikarama M, McLornan D, Raj K, et al. Autoimmune Hemolytic Anemia after Allogeneic Hematopoietic Stem Cell Transplantation: Analysis of 533 Adult Patients Who Underwent Transplantation at King's College Hospital. Biol Blood Marrow Transplant [Internet]. Elsevier Inc; 2015;21(1):60–6. Available from: http://linkinghub.elsevier.com/retrieve/pii/S1083879114005643

316. Ishikawa F, Shigematsu H, Gondo H, Okamura T, Niho Y. Case report Autoreactive antibodies following autologous peripheral blood stem cell transplantation. 1998;(May):729–31.

317. Holbro A, Abinun M, Daikeler T. Management of autoimmune diseases after haematopoietic stem cell transplantation. Br J Haematol [Internet]. Blackwell Publishing Ltd (9600 Garsington Road, Oxford OX4 2XG, United Kingdom); 2012 May [cited 2014 Oct 13];157(3):281–90. Available from: http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=emed10&NEWS=N&AN=2012204407

318. Sanz J, Arango M, Carpio N, Montesinos P, Moscardó F, Martín G, et al. Autoimmune cytopenias after umbilical cord blood transplantation in adults with hematological malignancies: a single-center experience. Bone Marrow Transplant [Internet]. Nature Publishing Group; 2014 Aug 2 [cited 2015 Feb 26];49(8):1084–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24887383

319. Page KM, Mendizabal AM, Prasad VK, Martin PL, Parikh S, Wood S, et al. Posttransplant Autoimmune Hemolytic Anemia and Other Autoimmune Cytopenias are Increased in Very Young Infants Undergoing Unrelated Donor Umbilical Cord Blood Transplantation. Biol Blood Marrow Transplant [Internet]. 2008 Oct [cited 2014 Oct 13];14(10):1108–17. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3735356&tool=pmcentrez&rendertype=abstract

320. Ahmed I, Teruya J, Murray-Krezan C, Krance R. The incidence of autoimmune hemolytic anemia in pediatric hematopoietic stem cell recipients post first and second hematopoietic stem cell transplant HHS Public Access. Pediatr Transpl. 2015;19(4):391–8.

321. Killick SB, Bown N, Cavenagh J, Dokal I, Foukaneli T, Hill A, et al. Guidelines for the diagnosis and management of adult aplastic anaemia. Br J Haematol [Internet]. 2016 Jan [cited 2017 Nov 23];172(2):187–207. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26568159

322. Zeng Y, Katsanis E. The complex pathophysiology of acquired aplastic anaemia. Clin Exp Immunol [Internet]. 2015 Jun [cited 2017 Nov 23];180(3):361–70. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25683099

323. Goto M, Kuribayashi K, Takahashi Y, Kondoh T, Tanaka M, Kobayashi D, et al. Identification of autoantibodies expressed in acquired aplastic anaemia. Br J Haematol [Internet]. 2013 Feb [cited 2017 Nov 20];160(3):359–62. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23116149

324. Bacigalupo A, Hows J, Gluckman E, Nissen C, Marsh J, Van Lint MT, et al. Bone marrow transplantation (BMT) versus immunosuppression for the treatment of severe aplastic anaemia (SAA): a report of the EBMT SAA working party. Br J Haematol [Internet]. 1988 Oct [cited 2017 Nov 23];70(2):177–82. Available from: http://www.ncbi.nlm.nih.gov/pubmed/3056497

325. Sureda A, Bader P, Cesaro S, Dreger P, Duarte RF, Dufour C, et al. Indications for allo- and auto-SCT for haematological diseases, solid tumours and immune disorders: current practice in Europe, 2015. Bone Marrow Transplant [Internet]. 2015 Aug 23 [cited 2017 Nov 23];50(8):1037–56. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25798672

326. Neunert C, Lim W, Crowther M, Cohen A, Solberg L, Crowther MA. The American Society of Hematology 2011 evidence-based practice guideline for immune thrombocytopenia. Blood [Internet]. 2011 [cited 2017 Nov 10];117(11):4190–207. Available from: http://www.bloodjournal.org/content/bloodjournal/117/16/4190.full.pdf

327. Provan D, Stasi R, Newland AC, Blanchette VS, Bolton-Maggs P, Bussel JB, et al. International consensus report on the investigation and management of primary immune thrombocytopenia. Blood [Internet]. 2010 Jan 14 [cited 2014 Jul 11];115(2):168–86. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19846889

328. Valent P, Lechner K. Diagnosis and treatment of autoimmune haemolytic anaemias in adults: a clinical review. Wien Klin Wochenschr [Internet]. 2008 Mar

[cited 2017 Nov 10];120(5–6):136–51. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18365153

329. Youinou P, Jamin C, Le Pottier L, Renaudineau Y, Hillion S, Pers J-O. Diagnostic criteria for autoimmune neutropenia. Autoimmun Rev [Internet]. 2014 Jan [cited 2014 Nov 5];13(4–5):574–6. Available from: http://www.sciencedirect.com/science/article/pii/S1568997214000111

330. Cheng G. Eltrombopag, a thrombopoietin- receptor agonist in the treatment of adult chronic immune thrombocytopenia: a review of the efficacy and safety profile. Ther Adv Hematol [Internet]. SAGE Publications; 2012 Jun [cited 2017 Nov 12];3(3):155–64. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23556122

331. Godeau B, Chevret S, Varet B, Lefrère F, Zini JM, Bassompierre F, et al. Intravenous immunoglobulin or high-dose methylprednisolone, with or without oral prednisone, for adults with untreated severe autoimmune thrombocytopenic purpura: a randomised, multicentre trial. Lancet (London, England) [Internet]. 2002 Jan 5 [cited 2017 Nov 12];359(9300):23–9. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0140673602072756

332. Gudbrandsdottir S, Birgens HS, Frederiksen H, Jensen BA, Jensen MK, Kjeldsen L, et al. Rituximab and dexamethasone vs dexamethasone monotherapy in newly diagnosed patients with primary immune thrombocytopenia. Blood [Internet]. 2013 Mar 14 [cited 2017 Nov 12];121(11):1976–81. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23293082

333. Zaja F, Baccarani M, Mazza P, Bocchia M, Gugliotta L, Zaccaria A, et al. Dexamethasone plus rituximab yields higher sustained response rates than dexamethasone monotherapy in adults with primary immune thrombocytopenia. Blood [Internet]. American Society of Hematology; 2010 Apr 8 [cited 2017 Nov 12];115(14):2755–62. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20130241

334. Hequet O, Salles G, Ketterer N, Espinouse D, Dumontet C, Thieblemont C, et al. Autoimmune thrombocytopenic purpura after autologous stem cell transplantation. Bone Marrow Transplant [Internet]. 2003 Jul [cited 2014 Oct 13];32(1):89–95. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12815483

335. Ahmad I, Haider K, Kanthan R. Autoimmune thrombocytopenia following tandem autologous peripheral blood stem cell transplantation for refractory germ cell tumor. Bone Marrow Transplant [Internet]. 2004 Aug [cited 2014 Oct 13];34(3):279–80. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15170159

336. Raj K, Narayanan S, Augustson B, Ho a, Mehta P, Duncan N, et al. Rituximab is effective in the management of refractory autoimmune cytopenias occurring after allogeneic stem cell transplantation. Bone Marrow Transplant [Internet]. 2005 Feb [cited 2014 Oct 13];35(3):299–301. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15568036

337. Fujita H, Togami K, Mori M, Hashimoto H, Nagai K, Nagai Y, et al. Successful

treatment with azathioprine for autoimmune thrombocytopenia developing after autologous peripheral blood stem cell transplantation. Rinsho Ketsueki [Internet]. 2007 Aug [cited 2015 Jan 27];48(8):637–41. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17867300

338. Beck JC, Burke MJ, Tolar J. Response of refractory immune thrombocytopenia after bone marrow transplantation to romiplostim. Pediatr Blood Cancer [Internet]. 2010 Mar [cited 2015 Jan 27];54(3):490–1. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19908296

339. Poon LM, Di Stasi A, Popat U, Champlin RE, Ciurea SO. Romiplostim for delayed platelet recovery and secondary thrombocytopenia following allogeneic stem cell transplantation. Am J Blood Res [Internet]. 2013 Jan;3(3):260–4. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3755526&tool=pmcentrez&rendertype=abstract

340. O'Donovan EM, Rezvani K, Sargent J, Richardson D, Tharmalingam H, Veys P, et al. Thrombopoietic Agonists Show Efficacy in ITP Related to Allogeneic Stem Cell Transplantation,. Blood [Internet]. 2011;118(21):3292. Available from: http://www.bloodjournal.org/content/118/21/3292?sso-checked=true

341. Barcellini W, Fattizzo B, Zaninoni A, Radice T, Nichele I, Di Bona E, et al. Clinical heterogeneity and predictors of outcome in primary autoimmune hemolytic anemia: a GIMEMA study of 308 patients. Blood [Internet]. American Society of Hematology; 2014 Nov 6 [cited 2017 Nov 12];124(19):2930–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25232059

342. Crowther M, Chan YLT, Garbett IK, Lim W, Vickers MA, Crowther MA. Evidence-based focused review of the treatment of idiopathic warm immune hemolytic anemia in adults. Blood [Internet]. American Society of Hematology; 2011 Oct 13 [cited 2017 Nov 12];118(15):4036–40. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21778343

343. Michel M, Chanet VR, Dechartres AS, Morin A-S, Piette J-C, Cirasino L, et al. The spectrum of Evans syndrome in adults: new insight into the disease based on the analysis of 68 cases. Blood [Internet]. 2009 [cited 2017 Nov 20];114(15):3167–72. Available from: http://www.bloodjournal.org/content/bloodjournal/114/15/3167.full.pdf?sso-checked=true

344. Miano M. How I manage Evans Syndrome and AIHA cases in children. Br J Haematol [Internet]. 2016 Feb [cited 2017 Nov 20];172(4):524–34. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26625877

345. Gibson C, Berliner N. How we evaluate and treat neutropenia in adults. Blood. 2014;124(8):1251–8.

346. Tosi P, Bandini G, Tazzari P, Raspadori D, Cirio TM, Rosti G, et al. Autoimmune neutropenia after unrelated bone marrow transplantation. Bone Marrow Transplant [Internet]. 1994 Dec;14(6):1003–4. Available from: http://www.ncbi.nlm.nih.gov/pubmed/7711662

347. Rainer Storb B, Etzioni R, Anasetti C, Appelbaum FR, Dean Buckner C, Bensinger

W, et al. Cyclophosphamide Combined With Antithymocyte Globulin in Preparation for Allogeneic Marrow Transplants in Patients With Aplastic Anemia. Blood [Internet]. 1994;84(3):941–9. Available from: http://www.bloodjournal.org/content/bloodjournal/84/3/941.full.pdf

348. Maury S, Bacigalupo A, Anderlini P, Aljurf M, Marsh J, Socié G, et al. Improved outcome of patients older than 30 years receiving HLA-identical sibling hematopoietic stem cell transplantation for severe acquired aplastic anemia using fludarabine-based conditioning: a comparison with conventional conditioning regimen. Haematologica [Internet]. Haematologica; 2009 Sep 1 [cited 2017 Nov 18];94(9):1312–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19734425

349. Bacigalupo A, Socie' G, Lanino E, Prete A, Locatelli F, Locasciulli A, et al. Fludarabine, cyclophosphamide, antithymocyte globulin, with or without low dose total body irradiation, for alternative donor transplants, in acquired severe aplastic anemia: A retrospective study from the ebmt-saa working party. Haematologica. 2010;95(6):976–82.

350. Marsh JC, Gupta V, Lim Z, Ho AY, Ireland RM, Hayden J, et al. Alemtuzumab with fludarabine and cyclophosphamide reduces chronic graft-versus-host disease after allogeneic stem cell transplantation for acquired aplastic anemia. Blood [Internet]. 2011 [cited 2017 Nov 18];118(8):2351–7. Available from: http://www.bloodjournal.org/content/bloodjournal/118/8/2351.full.pdf

351. Kousin-Ezewu O, Coles A. Alemtuzumab in multiple sclerosis: latest evidence and

clinical prospects. Ther Adv Chronic Dis [Internet]. 2013 May;4(3):97–103. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23634277

352. Costelloe L, Jones J, Coles A. Secondary autoimmune diseases following alemtuzumab therapy for multiple sclerosis. Expert Rev Neurother [Internet]. 2012 Mar 9 [cited 2017 Nov 22];12(3):335–41. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22364332

353. Bastion Y, Coiffier B, Dumontet C, Espinouse D, Bryon PA. Severe autoimmune hemolytic anemia in two patients treated with fludarabine for chronic lymphocytic leukemia. Ann Oncol Off J Eur Soc Med Oncol [Internet]. 1992 Feb;3(2):171–2. Available from: http://www.ncbi.nlm.nih.gov/pubmed/1606091

354. Tosti S, Caruso R, D'Adamo F, Picardi A, Ali Ege M, Girelli G, et al. Severe autoimmune hemolytic anemia in a patient with chronic lymphocytic leukemia responsive to fludarabine-based treatment. Ann Hematol [Internet]. 1992 Nov;65(5):238–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/1457584

355. Myint H, Copplestone JA, Orchard J, Craig V, Curtis D, Prentice AG, et al. Fludarabine-related autoimmune haemolytic anaemia in patients with chronic lymphocytic leukaemia. Br J Haematol [Internet]. 1995 Oct;91(2):341–4. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8547072

356. Weiss RB, Freiman J, Kweder SL, Diehl LF, Byrd JC. Hemolytic anemia after fludarabine therapy for chronic lymphocytic leukemia. J Clin Oncol [Internet].

1998 May [cited 2017 Nov 19];16(5):1885–9. Available from: http://ascopubs.org/doi/10.1200/JCO.1998.16.5.1885

357. Jiang Y, Peng H, Cui X, Zhou Y, Yuan D, Sui X, et al. Autoimmune thrombocytopenia: a complication of fludarabine therapy in the treatment of Waldenstrom's macroglobulinemia. Int J Clin Exp Med [Internet]. e-Century Publishing Corporation; 2014;7(12):5937–42. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25664138

358. Garbe E, Andersohn F, Bronder E, Klimpel A, Thomae M, Schrezenmeier H, et al. Drug induced immune haemolytic anaemia in the Berlin Case-Control Surveillance Study. Br J Haematol [Internet]. Blackwell Publishing Ltd; 2011 Sep 1 [cited 2017 Nov 19];154(5):644–53. Available from: http://doi.wiley.com/10.1111/j.1365-2141.2011.08784.x

359. Mauro FR, Foa R, Cerretti R, Giannarelli D, Coluzzi S, Mandelli F, et al. Autoimmune hemolytic anemia in chronic lymphocytic leukemia: clinical, therapeutic, and prognostic features. Blood [Internet]. 2000 May 1;95(9):2786–92. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10779422

360. Dearden C, Wade R, Else M, Richards S, Milligan D, Hamblin T, et al. The prognostic significance of a positive direct antiglobulin test in chronic lymphocytic leukemia: a beneficial effect of the combination of fludarabine and cyclophosphamide on the incidence of hemolytic anemia. Blood [Internet]. 2008 Feb 15 [cited 2017 Nov 19];111(4):1820–6. Available from: http://www.bloodjournal.org/cgi/doi/10.1182/blood-2007-07-101303

361. Lopez M, Clarkson MR, Albin M, Sayegh MH, Najafian N. A Novel Mechanism of Action for Anti-Thymocyte Globulin: Induction of CD4+CD25+Foxp3+ Regulatory T Cells. J Am Soc Nephrol [Internet]. 2006 Sep 7 [cited 2017 Nov 19];17(10):2844–53. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16914538

362. Shimony O, Nagler A, Gellman YN, Refaeli E, Rosenblum N, Eshkar-Sebban L, et al. Anti-T lymphocyte globulin (ATG) induces generation of regulatory T cells, at least part of them express activated CD44. J Clin Immunol [Internet]. 2012 Feb 7 [cited 2017 Nov 22];32(1):173–88. Available from: http://link.springer.com/10.1007/s10875-011-9599-2

363. Feng X, Kajigaya S, Solomou EE, Keyvanfar K, Xu X, Raghavachari N, et al. Rabbit ATG but not horse ATG promotes expansion of functional CD4+CD25highFOXP3+ regulatory T cells in vitro. Blood [Internet]. American Society of Hematology; 2008 Apr 1 [cited 2017 Nov 24];111(7):3675–83. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18250226

364. Chung DT, Korn T, Richard J, Ruzek M, Kohm AP, Miller S, et al. Anti-thymocyte globulin (ATG) prevents autoimmune encephalomyelitis by expanding myelin antigen-specific Foxp3+ regulatory T cells. Int Immunol [Internet]. 2007 Aug 1 [cited 2017 Nov 19];19(8):1003–10. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17698561

365. Schmitt WH, Hagen EC, Neumann I, Nowack R, Flores-Suárez LF, van der Woude FJ, et al. Treatment of refractory Wegener's granulomatosis with antithymocyte globulin (ATG): an open study in 15 patients. Kidney Int [Internet]. 2004 Apr

[cited 2017 Nov 22];65(4):1440–8. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0085253815498564

366. Hagen EC, de Keizer RJ, Andrassy K, van Boven WP, Bruijn JA, van Es LA, et al. Compassionate treatment of Wegener's granulomatosis with rabbit anti-thymocyte globulin. Clin Nephrol [Internet]. 1995 Jun;43(6):351–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/7554518

367. Tarkowski A, Andersson-Gäre B, Aurell M. Use of anti-thymocyte globulin in the management of refractory systemic autoimmune diseases. Scand J Rheumatol [Internet]. 1993;22(6):261–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8266027

368. Stratton RJ, Wilson H, Black CM. Pilot study of anti-thymocyte globulin plus mycophenolate mofetil in recent-onset diffuse scleroderma. Rheumatology (Oxford) [Internet]. 2001 Jan;40(1):84–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11157146

369. Morishita Y, Matsukawa Y, Kura Y, Takei M, Tomita Y, Nishinarita S, et al. Antithymocyte globulin for a patient with systemic lupus erythematosus complicated by severe pancytopenia. J Int Med Res [Internet]. 1997 Jul 25 [cited 2017 Nov 22];25(4):219–23. Available from: http://journals.sagepub.com/doi/10.1177/030006059702500409

370. Bacigalupo A, Socié G, Schrezenmeier H, Tichelli A, Locasciulli A, Fuehrer M, et al. Bone marrow versus peripheral blood as the stem cell source for sibling

transplants in acquired aplastic anemia: survival advantage for bone marrow in all age groups. Haematologica [Internet]. Haematologica; 2012 Aug 1 [cited 2017 Nov 18];97(8):1142–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22315497

371. Schrezenmeier H, Passweg JR, Marsh JCW, Bacigalupo A, Bredeson CN, Bullorsky E, et al. Worse outcome and more chronic GVHD with peripheral blood progenitor cells than bone marrow in HLA-matched sibling donor transplants for young patients with severe acquired aplastic anemia. 2007 [cited 2017 Nov 18];110:1397–400. Available from: http://www.bloodjournal.org/content/bloodjournal/110/4/1397.full.pdf

372. Socié G. Allogeneic BM transplantation for the treatment of aplastic anemia: current results and expanding donor possibilities. Hematol Am Soc Hematol Educ Progr [Internet]. American Society of Hematology; 2013 Dec 6 [cited 2017 Nov 18];2013(1):82–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24319167

373. Singhal S, Powles R, Kulkarni S, Treleaven J, Sirohi B, Millar B, et al. Comparison of marrow and blood cell yields from the same donors in a double-blind, randomized study of allogeneic marrow vs blood stem cell transplantation. Bone Marrow Transplant [Internet]. Nature Publishing Group; 2000 Mar 9 [cited 2017 Nov 19];25(5):501–5. Available from: http://www.nature.com/articles/1702173

374. Bahceci E, Epperson D, Douek DC, Melenhorst JJ, Childs RC, Barrett AJ. Early reconstitution of the T-cell repertoire after non-myeloablative peripheral blood

stem cell transplantation is from post-thymic T-cell expansion and is unaffected by graft-versus-host disease or mixed chimaerism. Br J Haematol [Internet]. Blackwell Science Ltd; 2003 Sep 1 [cited 2017 Nov 19];122(6):934–43. Available from: http://doi.wiley.com/10.1046/j.1365-2141.2003.04522.x

375. Larosa F, Marmier C, Robinet E, Ferrand C, Saas P, Deconinck E, et al. Peripheral T-cell expansion and low infection rate after reduced-intensity conditioning and allogeneic blood stem cell transplantation. Bone Marrow Transplant [Internet]. 2005 May 14 [cited 2017 Nov 19];35(9):859–68. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15765116

376. Jiménez M, Ercilla G, Martínez C. SPOTLIGHT REVIEW Immune reconstitution after allogeneic stem cell transplantation with reduced-intensity conditioning regimens. Leukemia [Internet]. 2007 [cited 2017 Nov 18];21:1628–37. Available from: http://www.nature.com/articles/2404681.pdf

377. Carpenter PA, Englund JA. How I vaccinate blood and marrow transplant recipients. Blood. 2016;127(23):2825–32.

378. Molrine DC, Guinan EC, Antin JH, Parsons SK, Weinstein HJ, Wheeler C, et al. Donor immunization with Haemophilus influenzae type b (HIB)-conjugate vaccine in allogeneic bone marrow transplantation. Blood. 1996;87(7):3012–8.

379. Storek J, Dawson M a, Lim LC-L, Burman BE, Stevens-Ayers T, Viganego F, et al. Efficacy of donor vaccination before hematopoietic cell transplantation and recipient vaccination both before and early after transplantation. Bone Marrow

Transplant [Internet]. 2004 Feb [cited 2014 Dec 15];33(3):337–46. Available from: http://www.ncbi.nlm.nih.gov/pubmed/14647254

380. Natori Y, Humar A, Lipton J, Kim D, Hoschler K, Ashton P, et al. A Pilot Randomized Controlled Trial of Adjuvanted vs. Nonadjuvanted Influenza vaccine in Adult Allogeneic Hematopoietic Stem Cell Transplant Recipients. IDWeek. New Orleans; 2016.

381. Halasa NB, Savani BN, Asokan I, Kassim A, Simons R, Summers C, et al. Randomized, Double Blind, Study of the safety and Immunogenicity of Standard-Dose Trivalent Inactivated Influenza Vaccine versus High-Dose Trivalent Inactivated Influenza Vaccine in Adult Hematopoietic Stem Cell Transplant Patients. Biol Blood Marrow Transpl [Internet]. Elsevier Inc; 2015;22(3):528–35. Available from: http://dx.doi.org/10.1016/j.bbmt.2015.12.003

382. Impagliazzo A, Milder F, Kuipers H, Wagner M, Zhu X, Hoffman RMB, et al. A stable trimeric influenza hemagglutinin stem as a broadly protective immunogen. 2015;(August):1–12.

383. Foppa IM, Haber M, Ferdinands JM, Shay DK. The case test-negative design for studies of the effectiveness of influenza vaccine. Vaccine [Internet]. Elsevier Ltd; 2013;31(30):3104–9. Available from: http://dx.doi.org/10.1016/j.vaccine.2013.04.026

384. Bustreo F, Kieny M-P. WHO | Vaccines: A global health success story that keeps us on our toes [Internet]. World Health Organization; 2016 [cited 2017 Feb 26].

Available from: http://www.who.int/mediacentre/commentaries/vaccines/en/

Appendix 1 – Summary of Guidelines and Recommendations for Vaccination of Haematopoietic Stem Cell Transplant Recipients

	GUIDELINE					
	Infectious Diseases Society of America 2013	International Consensus Conference on Clinical Practice in GVHD 2011	American Society of Blood and Marrow Transplantation 2009	Immunisation Guidelines for Ireland 2015	Royal College of Paediatrics and Child Health 2002	Children's Cancer and Leukaemia Group 2014
Age Group	Adult/Paediatric	Adult/Paediatric	Adult/Paediatric	Adult/Paediatric	Paediatric	Paediatric
Commencement post alloHSCT (months)	3-6	>6 IIV from 4 months if community outbreak	3-6	3-6	12 – Sibling Donor. 18 –Unrelated donor	12 IIV every Autumn from 6 months
GvHD	No live vaccines	No Live vaccines Consider measuring response to inactive vaccines	No live vaccines Consider measuring response to inactive vaccines	No lives vaccines	No live vaccines Consider inactive vaccines	No Live vaccines No inactive vaccines if active cGvHD

Immunosuppressive Therapy	No Live vaccines Consider delay if high dose corticosteroids	No Live Vaccines Consider delaying inactive in:- Adults - if Pred>0.5mg/kg + second IST agent **OR** Any three IST agents **BUT** Do not postpone>3 months Paeds / Adolescent – No inactive vaccine delay for any IST agent	No Live vaccines	No live vaccines	No live vaccines Inactive vaccines – If no IST for 6 months **BUT** can consider earlier administration	No live vaccines Inactive vaccines – If no IST for 6 months

Assessing Response	HepB recommended	In patients with GVHD	Routinely test After vaccination:- PCV HepB Every 4-5 years:- Hep B Measles DT Polio	Consider every 5 years:- Hep B Measles DT Polio	Explicitly not recommended	Not discussed
Live Vaccine criteria for administration	2 yrs Post-HSCT No cGvHD No IST 8-11 months post IVIg	2 years post HSCT No cGvHD No IST for 3 months	2 years post HSCT No cGvHD No IST Consider pre and post Ab titres	2 yrs post Tx No cGvHD No IST CD4>400x10^6/L IgM>0.5g/L	18 months post HSCT (MMR) No cGvHD No IST 12 months No IVIg 3 months	18 months post HSCT (MMR) No cGvHD No IST 12 months No IVIg 3 months
Serotherapy	Consider delay if recent Rituximab or Alemtuzumab	See IST	Discusses Rituximab but no specific recommendations	No Recommendation	See IST	See IST

Inactivated Influenza Vaccine	6 months (4 if outbreak) If age 6 months to 8 years and first IIV give second dose after 4 weeks	6 months (4 if outbreak) If given at 4 months give second dose 1 month later If age 6 months to 8 years and first IIV give second dose after 4 weeks	6 months (4 if outbreak) If given earlier than 6 months consider second dose 1month later If age 6 months to 8 years and first IIV give second dose after 4 weeks	6 months Initially 2 doses for all age groups then 1 dose annually	12 months single dose	6 months Single dose
Pneumococcal Vaccine	Commence 3-6 months 3 x monthly PCV13 1 PSV at 12/12 **OR** 1 PCV13 at 12/12 if cGvHD	Commence 6 months 3x monthly PCV13 1 PSV at 12/12 **OR** 1 PCV13 at 12/12 if cGvHD	Commence 3-6 months 3x monthly PCV13 1 PSV at 12/12 **OR** 1 PCV13 at 12/12 if cGvHD Test response from 1 month after 3or4 doses	Commence 3-6 months 3x monthly PCV13 1 PSV at 12/12 **OR** 1 PCV13 at 12/12 if cGvHD	Commence 15 months (21 for UD) Age <24 months:- 3 x monthly PCV 1 x PSV at 24 months post HSCT Age>24 months	Commence 12 months 3x monthly PCV13 1 PSV at 12/12 **OR** 1 PCV13 at 12/12

					2 doses of PCV for 3 months 1 x PSV at 24 months post HSCT	
Haemophilus Influenzae B Vaccine	3 x monthly Hib from 6-12 months	3 x monthly Hib from 6 months Booster at 18 months	3 x monthly Hib from 6-12 months	3 x monthly Hib from 6-12 months	3 x monthly Hib from 12 months (18 for UD)	3 x monthly Hib from 12 months Booster at 48 months
Meningococcal Vaccine	Commence 6-12 months -2 x monthly MenACWY for those aged 11-18 -booster at age 6-18 if first dose given age 11-15	Commence 6-12 months - 3 x monthly MenC	Commence 6-12 months -Follow country recommendation for general population	Commence 7 months - 2 x monthly MenACWY + MenB - 1x MenACWY at 11 months	Commence 12 months (18 for UD) 3 x monthly MenC	Commence 12 months - 2 x MenC - 1 x MenACWY at 24 months - MenC booster at 48 months and school leaver

Diphtheria-Tetanus-Pertussis Vaccine	Commence 6 months -age<7 x 3DTaP -age>7 x3 DTaP **OR** dTap followed by x2 DT **OR** x2 dT	Commence 6 months 3xDTaP **AND** Booster at 18 months	Commence 6-12 months -3 x DTaP unless not licensed in which case dTap -If Td consider diphtheria Ab levels if increased risk envisaged	Commence 6 months -DTaP x 3 for all **THEN** age <10 DTaP after 3 years dTap 10 years later age>10 dTap after 3 years dTap 10 years later	Commence 12 months (18 for UD) 3xDTaP	Commence 12 months Age <10 -DTaP x 3 -DTaP booster 48 months -dT school leaver booster Age>10 -dTaP x 3 -dTaP booster 48 months -dT school leaver booster
Hepatitis B Vaccine	Commence 6 months All recipients x3 doses test response	Commence 6 months All recipients negative for HepB markers X3 monthly doses Booster at 18	For HBsAg or HBcAB Positive patients If HBsAg or HBcAb Negative follow country recommendations	Commence 7 months All recipients age >10 X3 doses at 7,9 and 11 months Test response	Consider on case by case basis	Consider on case by case basis

		months Test response	X3 monthly doses	after 3rd dose		
Injectable Polio Vaccine	X3 IPV 6-12 months post	X3 monthly IPV Booster at 18 months	X3 IPV 6-12 months post	X3 monthly IPV doses from 6 months Booster after 3 years	12 months (18 for UD) X3 monthly IPV	12 months X3 monthly IPV Booster at 48 months School leaver booster
Human Papillomavirus Vaccine	6-12 months CONSIDER X 3 HPV for F11-26 X3 HPV4 for M11-26	6-12 months CONSIDER X3 HPV for F12-17 Vaccination of Males (no specifics given)	Country specific recommendations	12 months HPV x 3 at 12,14 and 29 months F age 10-45 M age 10-26	Not discussed (vaccine not routinely available at time)	12 months X3 doses for F>12
Measles-Mumps-Rubella (Live attenuated) In measles seronegative recipients	Commence 2 years if criteria for lives vaccines met and 8-11 months after last IVIg dose X 2 doses	Commence 2 years if criteria for lives vaccines met X 2 doses	Commence 2 years if criteria for lives vaccines met X 2 doses	Commence 2 years if criteria for lives vaccines met X 2 doses	Commence 2 years if criteria for lives vaccines met and 3months after last IVIg dose	Commence 18 months if criteria for lives vaccines met X 2 doses

					(30 months UD) X 2 doses	
Varicella Vaccine (Live attenuated) In varicella seronegative recipients	Commence 2 years if criteria for lives vaccines met and 8-11 months after last IVIg dose Consider following criteria -CD4>200cell/mm^3 AND -Response to >/=1 other vaccine	CONSIDER Commence 24 months if criteria for live vaccines met AND Immunological response to prior inactive vaccines	CONSIDER If criteria for live vaccines met	CONSIDER Commence 24 months if criteria for lives vaccines met	Not discussed	Not recommended

Appendix 2 – Routine Vaccination Programme Practice Survey

Routine Vaccination Programme Practices after Allogeneic Haematopoietic Stem Cell Transplantation: A Survey of NHS-Based Adult and Paediatric Allogeneic Transplant Programmes

In this survey, we will be asking questions about routine vaccination practices after allogeneic haematopoietic stem cell transplant (HSCT) in your transplant programme.

For the purposes of this survey we are defining a Routine Vaccination Programme (RVP) as a series of scheduled vaccinations administered after allogeneic HSCT in the absence of any contraindications and as a part of standard post-transplant care.

We are collecting separate survey responses for adult and paediatric Routine Vaccination Programmes (RVP). If your hospital has both adult and paediatric allogeneic HSCT programmes, please could this survey be competed twice, once for adult and once for paediatric programmes. You will be asked to specify to which age group your response relates.

We would be grateful if this survey could be completed by the healthcare professional/s taking primary responsibility for the adult and/or paediatric Routine Vaccination Programmes (RVP).
It may help you to have your local guidelines or standard operating procedure (SOP) available for reference.

There are 25 questions in the survey and should take 10-15 minutes to complete.

All data collected will be reported anonymously.

Transplant Programme Details

The following questions ask for details about your transplant programme and your role

1. Please give the name of your transplant programme *

2. Please indicate whether you are completing this survey in relation to an adult or paediatric allogeneic HSCT programme? *

-Adult allogeneic HSCT
-Paediatric allogeneic HSCT

If paediatric please indicate the age range of patients treated in your programme

3. Which statement best describes your transplant programme? Please select one answer

-Fully JACIE accredited for allogeneic HSCT; completed one cycle
-Fully JACIE accredited for allogeneic HSCT; completed two cycles
-Fully JACIE accredited for allogeneic HSCT; completed three cycles
-Not yet accredited but have undergone first time inspection
-Working towards JACIE accreditation

4. Which of the following best describes your role? Please select one answer

-HSCT programme director
-HSCT physician (consultant / associate specialist)
-HSCT physician (HSCT Fellow)
-HSCT (Haematology trainee)
-HSCT clinical nurse specialist
-Other (please specify):

Organisational Questions
The following questions ask how RVP is organised in your transplant programme

5. Does your transplant programme recommend a Routine Vaccination Programme (RVP) after Allogeneic HSCT? Please note that if you select 'no' this will take you directly to the end of the survey *

-Yes
-No

6. Does your transplant programme maintain a document controlled standard operating procedure (SOP) detailing your RVP? *

- Yes
-No

If yes please give year of implementation or latest revision

7. What is the main guideline or policy document that is referenced in your SOP, and/or to which healthcare practitioners will refer to guide RVP decisions in your transplant programme? Please select the most appropriate description and give the title of the guideline or policy below. *

-International vaccination guideline / policy **specific** to HSCT recipients (e.g. ASBMT 2009, IDSA 2013)
-International vaccination guideline / policy **specific** to HSCT recipients modified for local use (e.g. modified ASBMT 2009, IDSA 2013)
-National vaccination guideline / policy **specific** to HSCT recipients (e.g. RCPCH 2002, CCLG 2014)
-National vaccination guideline / policy **not specific** to HSCT recipients (e.g. DOH

Green Book)
-Locally developed vaccination guideline / policy **specific** to HSCT recipients
-Locally developed vaccination guideline / policy **not specific** to HSCT recipients
Other (please specify):

Please give guideline or policy title

8. In what healthcare clinic or setting do HSCT recipients from your transplant programme receive vaccinations as part of RVP? Please select all that apply

-Transplant centre in the setting of a doctor-led post-transplant or late-effects clinic (vaccine administered by either nurse or doctor)
-Transplant centre in the setting of a nurse-led post-transplant or late-effects clinic
-Infectious diseases unit or clinic
-Secondary care (HSCT recipient's referring or local Hospital)
-Primary care (HSCT recipient's general practice surgery)
-Other (please specify):

9. Does your transplant programme have a vaccination guideline or policy that addresses the vaccination needs of HSCT recipients planning to travel outside of the UK post-HSCT?

-Yes
-No

10. Where is the administration of vaccines given as part of RVP usually recorded? Please select all that apply

-Within the transplant centre In the HSCT recipient's electronic medical records
-Within the transplant centre In the HSCT recipient's paper records
-Outside the transplant centre In the records of the healthcare provider that administers the vaccine
-Outside the transplant centre in a vaccine administration booklet or card that the
-HSCT recipient looks after
-Other (please specify):

11. When did your transplant programme last audit RVP practice against either an SOP or guideline? *

-12-24 months ago
->24 months ago
-Not audited

Routine Vaccination Programme (RVP) Specific Questions

The following questions ask for specific details about RVP in your transplant programme

It may help you to have your guidelines or SOP available for reference

12. At what time point post HSCT is RVP usually commenced in your transplant programme assuming no contraindications?

	< 3 months	3 months	6 months	9 months	12 months	18 months	>18 months
-Sibling donor allogeneic or syngeneic HSCT							
Unrelated donor allogeneic HSCT							

13. Does your transplant programme routinely use a laboratory marker of immune-reconstitution to guide initiation of RVP? Please select all that apply

-Absolute lymphocyte count
-Lymphocyte subsets
-Immunoglobulin levels
-None
-Other (please specify):

14. Which of the following situations would prompt a delay in administration of live attenuated and inactive vaccines given as part of RVP in your transplant programme? Live attenuated vaccines include - MMR, intranasal influenza, varicella (Varivax) Inactive vaccines include - pneumococcal conjugate, tetanus-diphtheria-pertussis, Haemophilus influenza conjugate, meningococcal conjugate, inactivated polio, recombinant hepatitis B, inactivated influenza Please select all that apply to each of live attenuated and inactive vaccines

	Live attenuated	Inactive
-Acute illness		
-CD4 cell count < 200="" cells="">		
-Monoclonal CD20 Antibody (Rituximab) in last 6 months		
-Intravenous Immunoglobulin infusion in last 6 months		
-None of the above		
-Other (Please enter details below)		

15. What is the lowest overall grade of chronic graft-versus-host disease (cGvHD) that would prompt a delay in administration of live attenuated and inactive vaccines given as part of RVP in your transplant programme?

Live attenuated vaccines include - MMR, intranasal influenza, varicella (Varivax) Inactive vaccines include - Pneumococcal conjugate, tetanus-

diphtheria-pertussis, Haemophilus influenza conjugate, meningococcal conjugate, inactivated polio, recombinant hepatitis B, inactivated influenza Please select one option for each of live attenuated and inactive vaccines *

	History of cGvHD	Grade 1 (mild)	Grade 2 (moderate)	Grade 3 (severe)	Would not delay for any grade
Live attenuated					
Inactive					

16. What is the lowest dose or combination of immunosuppressive agents that would prompt a delay in administration of live attenuated and inactive vaccines given as part of RVP in your transplant programme?

Live attenuated vaccines include - MMR, intranasal influenza, varicella (Varivax) Inactive vaccines include - pneumococcal conjugate, tetanus-diphtheria-pertussis, Haemophilus influenza conjugate, meningococcal conjugate, inactivated polio, recombinant hepatitis B, inactivated influenza Please select one option for each of live attenuated and inactive vaccines *

	Any single immunosuppressive agent including corticosteroid at	Prednisolone >0.5 mg/Kg body weight single agent	Prednisolone >0.5 mg/Kg body weight plus second immunosuppressive agent	Any three-agent combination immunosuppressive therapy	Would not delay for any dose or combination
Live attenuated					
Inactivate					

17. Which of the following vaccines would ordinarily be included in RVP in your transplant programme assuming no contraindications? Please select all that apply

-Pneumococcal Vaccine
-Tetanus-Diphtheria-Pertussis Vaccine
-Haemophilus Influenza B Conjugate Vaccine
-Meningococcal Vaccine
-Inactivated Poliovirus Vaccine
-Hepatitis B Vaccine
-Inactivated Influenza Vaccine
-Measles-Mumps-Rubella (Live) in HSCT recipient seronegative for measles at time of vaccination
-Human Papillomavirus Bivalent (HPV2) or Quadrivalent (HPV4) in female HSCT recipients age 11-26

-Human Papillomavirus Quadrivalent (HPV4) in male HSCT recipients age 11-26
-Varicella (Varivax) in HSCT recipients seronegative at time of vaccination
Other (please specify):

18. For which of the following vaccine-preventable infections does your transplant programme routinely monitor or recommend monitoring of antibody levels to assess response to vaccination? Please select all that apply

-Pneumococcus
-Haemophilus Influenza B
-Measles
-Tetanus
-Diphtheria
-Polio
-Hepatitis B
-No specific recommendation / at discretion of hospital or GP practice administering vaccine
-Other (please specify):

19. Are there any specific clinical situations in which your transplant programme would monitor or recommend monitoring of antibody levels to assess response to vaccination? Please select all that apply *

-Active GVHD
-Ongoing immunosuppressive therapy
-Illness caused by a vaccine preventable infection (e.g. pneumococcal infection)
-None
-Other (please specify):

Vaccine Specific Questions
The following questions ask about the specific vaccines used in RVP in your transplant programme It may help you to have your guidelines or SOP available for reference

20. Which pneumococcal vaccine does your transplant programme use or recommend first-line as part of RVP? Please select one answer *

-Pneumococcal Polysaccharide 23-valent (PPSV23 - Pneumovax)
-Pneumococcal Conjugate 7-Valent (PCV7 - Prevnar)
-Pneumococcal Conjugate 13-valent (PCV13 - Prevnar13)
-PCV7 or PCV13 (Prevnar) followed by PPSV23 (Pneumovax)
-No specific recommendation / at discretion of hospital or GP practice administering

-Not applicable (pneumococcal vaccine not recommended as part of RVP)
Other (please specify):

21. Which diphtheria-tetanus-pertussis vaccine does your transplant programme use or recommend first-line as part of RVP? Please select one answer *

-Full dose diphtheria-tetanus-pertussis (DTaP)
-Reduced dose diphtheria - reduced dose pertussis - full dose Tetanus (Tdap)
-Full dose (DTaP) **or** reduced dose (Tdap) depending on patient age
-No specific recommendation / at discretion of hospital or GP practice administering
-Not applicable (diphtheria-tetanus-pertussis vaccine not recommended as part of RVP)
-Other (please specify):

22. Which Meningococcal vaccine does your transplant programme use or recommend first-line as part of RVP? Please select one answer *

-Meningococcal C conjugate (NeisVac-C, Menjugate Kit, Meningitec)
-Combined Haemophilus influenzae B / meningococcal C Conjugate (Menitorix)
-Meningococcal ACWY Conjugate (Menveo, Nimenrix)
-Meningococcal B conjugate (Bexsero)
-No specific recommendation / at discretion of hospital or GP practice administering
-Not applicable (meningococcal vaccine not recommended as part of RVP)
Other (please specify):

23. At what time point post HSCT does your transplant programme recommend commencing routine vaccination with seasonal influenza vaccine assuming no contraindications? *

	3 months	6 months	9 months	12 months	18 months	>18 months
Sibling donor allogeneic or syngeneic HSCT						
Unrelated donor allogeneic HSCT						

24. Which seasonal influenza vaccine does your transplant programme recommend to family members and close contacts of HSCT recipients? Please

select all that apply to each of child and adult family members and close contacts of HSCT recipients

	Child family members and close contacts	Adult family members and close contacts
Live attenuated influenza vaccine (Intranasal)		
Inactive influenza vaccine (Intramuscular)		
No specific influenza vaccine recommendation / at discretion of hospital or GP practice administering		
Not applicable (influenza vaccination of family members and close contacts not recommended)		

25. Does your transplant programme recommend that family members and close contacts of HSCT recipients consider vaccination with varicella vaccine (Varivax)? *

Yes
No

Appendix 3 – Notice of Ethical Approval, Patient Information Sheet, and Consent Form for Chapter 3

Health Research Authority

NRES Committee Yorkshire & The Humber - South Yorkshire

Unit 001
Jarrow Business Centre
Rolling Mill Road
Jarrow
Tyne and Wear
NE32 3DT

Telephone: 0191

428 3561 12 August 2015

Dr Paul Miller

Anthony Nolan Research Institute

Royal Free Hospital

Pond Street

NW3 2QG

Dear Dr Miller

Study title:	**A pilot study comparing the microneutralization assay and haemagglutination inhibition assay as measures of the immunogenicity of seasonal inactive influenza vaccine in recipients of reduced intensity conditioning allogeneic haematopoietic stem cell transplants during the first-year post-transplant**
REC reference:	**15/YH/0394**
IRAS project ID:	**183540**

The Proportionate Review Sub-committee of the NRES Committee Yorkshire & The Humber - South Yorkshire reviewed the above application on 12 August 2015.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this favourable opinion letter. The expectation is that this

information will be published for all studies that receive an ethical opinion but should you wish to provide a substitute contact point, wish to make a request to defer, or require further information, please contact the REC Assistant Miss Kerry Dunbar, *nrescommittee.yorkandhumber-southyorks@nhs.net* Under very limited circumstances (e.g. for student research which has received an unfavourable opinion), it may be possible to grant an exemption to the publication of the study.

Ethical opinion

On behalf of the Committee, the sub-committee gave a favourable ethical opinion of the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below.

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission ("R&D approval") should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of approvals from host organisations.

Registration of Clinical Trials

All clinical trials (defined as the first four categories on the IRAS filter page) must be registered on a publicly accessible database. This should be before the first participant is recruited but no later than 6 weeks after recruitment of the first participant.

There is no requirement to separately notify the REC but you should do so at the earliest opportunity e.g. when submitting an amendment. We will audit the registration details as part of the annual progress reporting process.

To ensure transparency in research, we strongly recommend that all research is registered but for non-clinical trials this is not currently mandatory.

If a sponsor wishes to request a deferral for study registration within the required timeframe, they should contact hra.studyregistration@nhs.net. The expectation is that all clinical trials will be registered, however, in exceptional circumstances non-registration may be permissible with prior agreement from NRES. Guidance on where to register is provided on the HRA website.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Ethical review of research sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion").

Summary of discussion at the meeting

Social or scientific value; scientific design and conduct of the study

Members requested that the title of the application be written in lay language.

You replied that it was assumed that this referred to the short title. This had been changed on the IRAS form and added this to the participant Information Sheet and Consent Form which were both attached. The title was within the 70-character limit on the IRAS form.

The Sub Committee was satisfied with the response given to the issue raised.

Favourable risk benefit ratio; anticipated benefit/risks for research participants (present and future)

Members requested clarification on payment of transport costs if a second visit needed to be for research alone. If this was not to be paid, or was to be paid then it needed to be clearly stated in the participant information sheet.

You replied that the detail had been added to the participant information sheet

The Sub Committee was satisfied with the responses given to the issues raised.

Informed consent process and the adequacy and completeness of participant information

Members requested that the participant information sheet needed to state clearly that potential participant can say "No, if you do not wish to." under the heading "Do I have to take part".

You replied that the detail had been added to the participant information sheet

Members stated the participants may be expert patients but a lay title would be of great benefit and it should be used on all participant documents in addition to the full title, therefore members requested that all titles were amended as appropriate.

You agreed with this.

Members requested clarification on the paragraph "What will happen to the results of this study", the last sentence is confusing needs to be amended accordingly.

You agreed this was not clear and did not fit well under this heading, therefore the sentence had been removed.

The Sub Committee was satisfied with the responses given to the issues raised.

Suitability of research summary

Members requested that the "Summary of the Study" be rewritten in lay language.

You replied that this had been re-written under section A6-1 of the IRAS form.

The Sub Committee was satisfied with the response given to the issue raised.

Approved documents

The documents reviewed and approved were:

Document	*Version*	*Date*
Evidence of Sponsor insurance or indemnity (non NHS Sponsors only)		28 July 2015
GP/consultant information sheets or letters	1.3	28 July 2015
Instructions for use of medical device	V5403/07-14	07 April 2015
IRAS Checklist XML [Checklist_03082015]		03 August 2015
Letter from funder		22 July 2015
Other [Dr Karl Pegg's CV]		03 August 2015
Other [PHE HI Assay Protocol]	V5403/07-14	07 April 2015
Other [PHE MN Assay Protocol]	V5463/07-13	08 November 2013
Other [Full Dataset Trial Form]		09 August 2015
Participant consent form	Version 1.2	09 August 2015

Participant information sheet (PIS)	Version 1.9	09 August 2015
REC Application Form [REC_Form_03082015]		03 August 2015
Referee's report or other scientific critique report		28 July 2015
Research protocol or project proposal	2.0	28 July 2015
Summary CV for Chief Investigator (CI) [CV - Professor Alejandro Madrigal]		03 August 2015
Summary CV for student [Dr Paul Miller CV]		30 July 2015
Summary CV for supervisor (student research) [Professor John Snowden CV]		03 August 2015

Membership of the Proportionate Review Sub-Committee

The members of the Sub-Committee who took part in the review are listed on the attached sheet.

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After ethical review

Reporting requirements

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- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports

x Notifying the end of the study

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http://www.hra.nhs.uk/about-the-hra/governance/quality-assurance/

HRA Training

We are pleased to welcome researchers and R&D staff at our training days – see details at http://www.hra.nhs.uk/hra-training/

With the Committee's best wishes for the success of this project.

15/YH/0394 Please quote this number on all correspondence

Yours sincerely

pp

Dr Ian Woollands Chair

Email: nrescommittee.yorkandhumber-southyorks@nhs.net

Enclosures: *List of names and professions of members who took part in the review*

"After ethical review – guidance for researchers"

Copy to: *Mr Dave Wilson, University College London (UCL)*
Ms Julie Curtis, Research and Development Department
Professor Alejandro Madrigal, Anthony Nolan Research Institute

NRES Committee Yorkshire & The Humber - South Yorkshire

Attendance at PRS Sub-Committee of the REC meeting on 12 August 2015 via correspondence.

Committee Members:

Name	*Profession*	*Present*	*Notes*
Dr Ahmed H Abdelhafiz	Consultant Physician, Elderly Medicine	Yes	
Dr Rhona Bratt	Retired Multimedia Project Manager	Yes	
Dr Ian Woollands (Chair)	Retired Clinical Director, Occupational Health	Yes	

Also in attendance:

Name	*Position (or reason for attending)*
Miss Kerry Dunbar	REC Assistant

PARTICIPANT INFORMATION SHEET

Short Title
Immune response to the seasonal flu vaccine after stem cell transplant

Full Title
A pilot study comparing the microneutralization assay and haemagglutination inhibition assay as measures of the immunogenicity of seasonal inactive influenza vaccine in recipients of reduced intensity conditioning allogeneic haematopoietic stem cell transplants

Invitation to participate in the study

Before you decide whether to take part, it is important for you to understand why the research is being done and what it will involve.

Please take time to read the following information carefully. Discuss it with friends and relatives if you wish.

You are free to decide whether or not to take part in this study. If you choose not to take part, this will not affect the care you get from your doctors.

Ask us if there is anything that is not clear or if you would like more information

Study Summary

This research study will be conducted between September 2015 and March 2016. We hope to recruit approximately 50 patients to the study.

The aim of the seasonal flu vaccine is to protect people who are at risk of flu and its complications.

International guidelines recommend that patients who have had a stem cell transplant receive the seasonal flu vaccine each winter, usually if they are more than 6 months after transplantation. This is based on evidence that the seasonal flu vaccine is not effective for patients who have been treated with full-intensity (myeloablative) stem cell transplants until at least 6 months after their transplant. We do not know how soon after transplant the seasonal flu vaccine is effective for patients who have had reduced-intensity (non-myeloablative) stem cell transplants and earlier vaccination may be beneficial.

At The Royal Marsden Hospital we recommend that as long as it is safe for them, patients who have had a reduced-intensity stem cell transplant receive the seasonal flu vaccine each winter, regardless of how long ago they had their transplant. We consider this the best approach based on our current understanding,

We want to compare two laboratory techniques to see if one is better at detecting a response to the seasonal flu vaccine in patients who have received a stem cell transplant.

We want to use this study and build on it with future studies to find the best time to give the seasonal flu vaccine to patients who have had a reduced-intensity stem cell transplant.

Do I have to take part in the study?

No, you do not have to take part if you do not wish to

Why have I been asked to participate?

You have received a reduced intensity stem cell transplant.

Your transplant doctor has recommended that you receive the seasonal flu vaccine this winter.

Your transplant doctor thinks that it is safe for you to receive the seasonal flu vaccine this winter.

What would taking part in the study involve?

We will give you the seasonal flu vaccine here at The Royal Marsden Hospital.

We will give you the same flu vaccine that is being given to all eligible patients in the United Kingdom this winter.

We will take two extra blood samples so we can test how you respond to the seasonal flu vaccine. We will take one blood sample just before we give you the seasonal flu vaccine. We will take the second blood sample about 4 weeks after we give you the seasonal flu vaccine. Each blood sample will be approximately 5ml (equivalent to 1 teaspoon) in volume.

We will try to make sure that we take the second blood sample at a time you would be visiting the hospital anyway. If this isn't possible we may need to ask you to make a journey to the hospital specifically for this blood test. Unfortunately, we are not able offer any payment to cover travel costs.

We will give you a letter confirming you have received the seasonal influenza vaccine, and with your consent we will send a copy of the letter to your GP so they also know you have received the seasonal influenza vaccine.

What will happen if I don't take part?

You will continue to receive all your normal care at The Royal Marsden Hospital.

We will recommend that you make an appointment at your GP Surgery to receive the seasonal flu vaccine and you will not be asked to have any additional blood tests.

What are the possible benefits of taking part?

You may find it more convenient to be given the seasonal flu vaccine at your transplant center than at your GP surgery.

What are the possible disadvantages of taking part?

We will ask you for two additional blood samples. If possible we will take these samples at the same time as other blood samples we would be taking anyway.

We may need to ask you to make an extra visit to the hospital to have the second blood sample taken.

The flu vaccination doesn't usually cause side effects. However, some people may experience a sore arm at the site of injection, mild fever and slight muscle aches for a day or so, or in very rare cases an allergic reaction the flu vaccination. These are the same side effects you may experience to the seasonal flu vaccine whether or not you take part in the study.

What will happen if I don't want to carry on with the study?

You can withdraw from the research study at any time, and this decision will not affect your ongoing medical care. All data collected as part of the study will be destroyed, and blood samples collected as part of the study will be disposed of.

What information will be collected and will it be kept confidential?

In addition to taking blood samples we will use your medical records to find out other important information about you. This will include details such as your age,

the date of your stem cell transplant, the medication you are currently receiving and whether or not you have experienced graft versus host disease.

All information that is collected about you during the course of the research will be kept strictly confidential and will comply with data protection legislation. All information will be stored securely at The Royal Marsden Hospital and the Anthony Nolan Research Institute.

Any information about you will have your name and address removed so that you cannot be recognised. Your data will be stored under a code and not under your name. Only the study team will have access to the code key.

To monitor the implementation of the study it may be necessary to give access to regulatory authorities and the trust's sponsor representatives. Your research data will be stored for 20 years.

What will happen to the samples I give?

The blood samples will be stored under a code and not under your name. Only the study team will have access to the code key.

The samples provided will be processed in the laboratory at The Royal Marsden Hospital. Frozen samples will be transferred to the Public Health England laboratories where laboratory analysis be performed. The blood samples will be tested for response to the seasonal influenza vaccine. This will involve testing for antibody levels.

Your anonymised blood samples will be stored until the end of the period of laboratory analysis and then destroyed. All blood samples will be destroyed by December 2016.

Involvement of other health care professionals

Your transplant doctor will be informed and if you give us permission, we will also write to your GP about the study and let them know that you have received the seasonal flu vaccine as part of the study.

Who has reviewed the study?

All research in the NHS is looked at by an independent Research Ethics Committee to protect your interests. This study has been reviewed and given a favourable opinion by the National Research Ethics Service Committee.

What will happen to the results of the study?

The results of this study may be published or presented at scientific meetings and may be used for further research, but your data will be presented in an anonymous format. If you would like a summary of the results from the study please let the doctor in clinic know.

We will not be able to provide individual patients with results of their blood tests.

Who is organizing and funding the study?

The Chief Investigator for the study is Professor Alejandro Madrigal, Consultant Haematologist and Scientific Director of the Anthony Nolan Research Institute.

The study is organized by Dr Paul Miller who is undertaking a clinical research degree (MD Res) at the Anthony Nolan Research Institute and the University College London Cancer Research Institute. The Study is being funded by the Anthony Nolan Research Institute, and the study is sponsored by University College London.

What if something goes wrong?

If you wish to complain or have any concerns about any aspect of the way you have been approached or treated by members of staff you may have experienced due to your participation in the research, National Health Service or UCL complaints mechanisms are available to you. Please ask your research doctor if you would like more information on this.

In the unlikely event that you are harmed by taking part in this study, compensation may be available.

If you suspect that the harm is the result of the Sponsor's (University College London) or the hospital's negligence then you may be able to claim compensation. After discussing with your research doctor, please make the claim in writing to Professor Alejandro Madrigal who is the Chief Investigator for the research and is based at the Anthony Nolan Research Institute, Royal Free Hospital, Pond Street, NW3 2QG. The Chief Investigator will then pass the claim to the Sponsor's Insurers, via the Sponsor's office. You may have to bear the costs of the legal action initially, and you should consult a lawyer about this.

Further Information and Contact Details

For further information please contact the Chief Investigator, Lead Researcher or Principle Investigator at your study site

Chief Investigator
Professor Alejandro Madrigal
Anthony Nolan Research Institute
Pond Street
London
NW3 2QG

alejandro.madrigal@anthonynolan.org
0303 303 0303

Student Researcher
Dr Paul Miller
Anthony Nolan Research Institute
The Royal Free Hospital
Pond Street
London
NW3 2QG

0303 303 0303
Paul.miller@anthonynolan.org

Principal Investigator, Royal Marsden Hospital.
Dr Chloe Anthias
Royal Marsden Hospital
Downs Road
Sutton
SM2 5TP

020 8642 6011
chloe.anthias@rmh.nhs.uk

Principal Investigator, Royal Hallamshire Hospital
Dr Thushan de Silva
Royal Hallamshire Hospital.
Glossop Road
Sheffield
South Yorkshire
S10 2JF

0114 271 1900
thushan.desilva@sth.nhs.uk

Centre Number:

Study Number:

Patient Identification Number for this study:

CONSENT FORM

The impact of seasonal influenza infection and vaccination health beliefs on vaccination intent among adult recipients of autologous and allogeneic haematopoietic stem cell transplant

Chief Investigator: **Professor Alejandro Madrigal**

Please initial all boxes

1. I confirm that I have read and understand the information sheet dated **24 March 2016 version number 1.3** for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

2. I understand that my participation is voluntary, and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. ☐

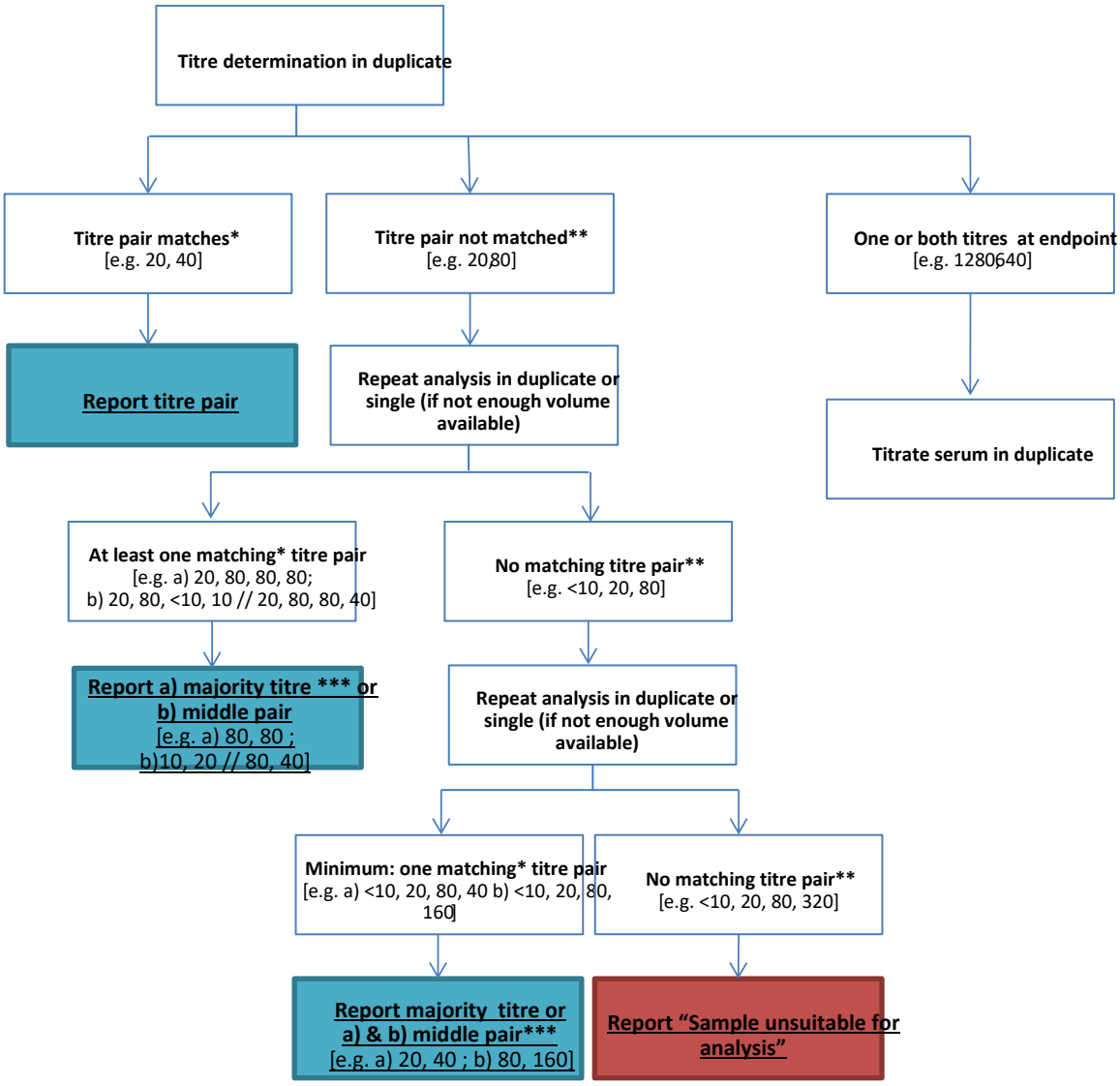
3. I understand that relevant sections of my medical notes and data collected during the study, may be looked at by individuals from the sponsor of the trial (University College London) and responsible persons authorized by the sponsor, from regulatory authorities or from the NHS Trust, where it is I relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐

4. I agree to take part in the above study. ☐

Name of Participant	Date	Signature
Name of Person taking consent.	Date	Signature

Appendix 4 – Public Health England Reporting Strategy for HAI and VMN Assays

Reporting of repeat strategy for HAI and MN

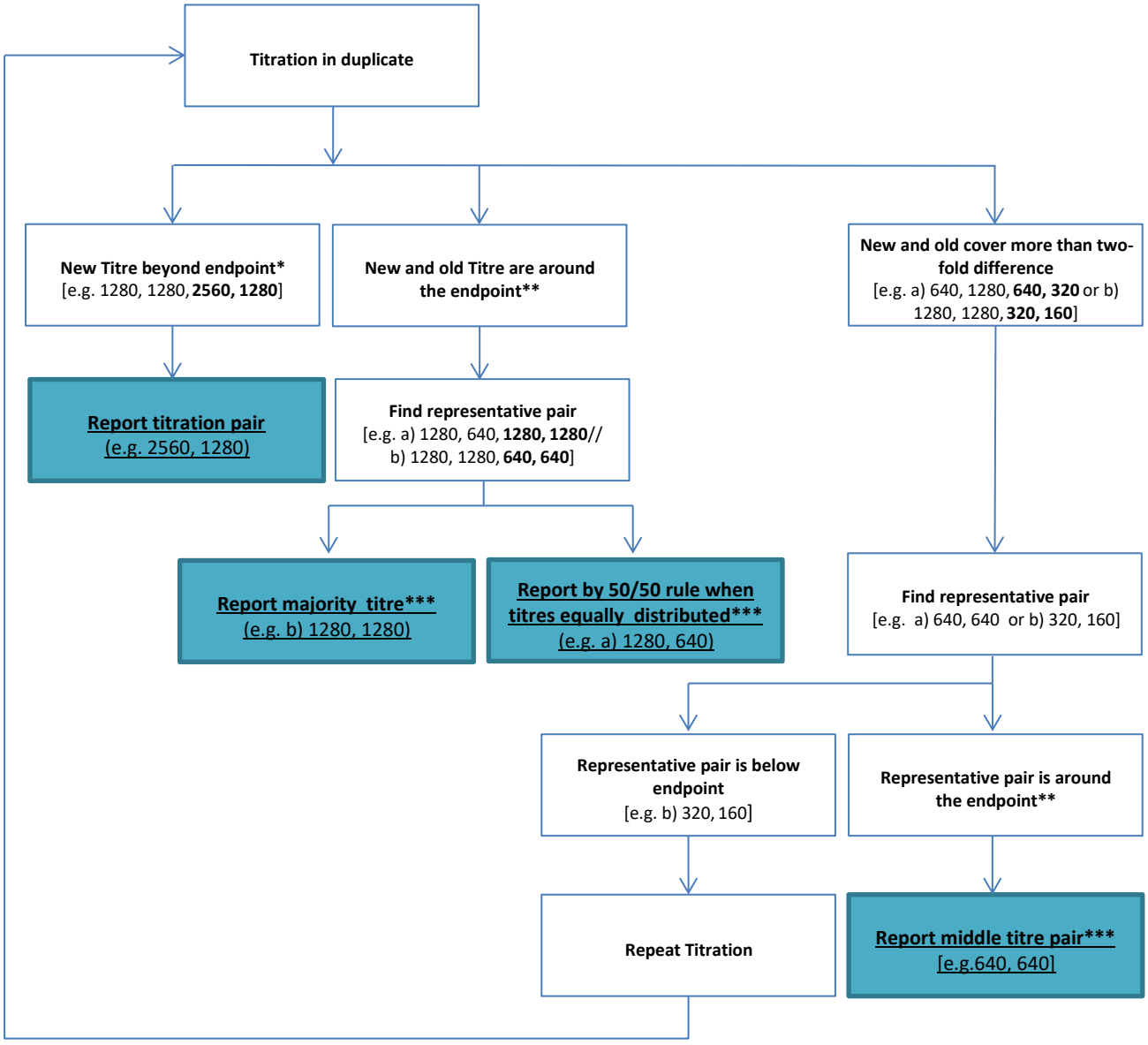


* ≤ 2-fold difference
** > 2-fold difference

*** if more than one rule applies, find the MOST representative titre, with the ordered preference as follows: majority titre (more than 50% of titres) > middle pair

Strategy for titrations

To be performed if required by sender of the samples or specific question relating to true titre (titrate pre- and post-sera if one of the two has titres around endpoint**)



* ≥ 1280

** equal to 640 or 1280

*** if more than one rule applies, find the MOST representative titre, with the ordered preference as follows: majority titre (more than 50% of titres are equal) > 50/50 rule > middle pair

Appendix 5 – Immunogenicity Cumulative Frequency Tables for HAI and VMN

A(H1N1)pdm09	HAI		VMN		VMN ELISA	
Titre	Pre	Post	Pre	Post	Pre	Post
<10 (negative)	18(64.3)	17(60.7)	15(53.6)	14(50.0)	11(39.3)	11(39.3)
≤40	22(78.6)	23(82.1)	22(78.6)	23(82.1)	17(60.7)	16(57.1)
≤80	26(92.9)	27(96.4)	23(82.1)	24(85.7)	18(64.3)	17(60.7)
≤160	27(96.4)	27(96.4)	25(89.3)	27(96.4)	22(78.6)	22(78.6)
≤320	27(96.4)	27(96.4)	26(92.9)	27(96.4)	25(85.7)	26(92.9)
≤640	28(100.0)	28(100.0)	27(96.4)	27(96.4)	26(92.9)	27(96.4)
≤1280	28(100.0)	28(100.0)	27(96.4)	27(96.4)	27(96.4)	27(96.4)
≤2560	28(100.0)	28(100.0)	28(100.0)	27(96.4)	27(96.4)	27(96.4)
≤5280	28(100.0)	28(100.0)	28(100.0)	28(100.0)	28(100.0)	28(100.0)

A(H3N2)	HAI		VMN	
Titre	Pre	Post	Pre	Post
<10 (negative)	15(53.6)	13(46.4)	3(10.7)	3(10.7)
≤40	25(89.3)	26(92.9)	8(28.6)	6(21.4)
≤80	27(96.4)	27(96.4)	11(39.3)	14(50.0)
≤160	27(96.4)	27(96.4)	17(60.7)	15(53.6)
≤320	27(96.4)	27(96.4)	20(71.4)	23(82.1)
≤640	28(100.0)	28(100.0)	25(89.3)	25(89.3)
≤1280	28(100.0)	28(100.0)	27(96.4)	27(96.4)
≤2560	28(100.0)	28(100.0)	28(100.0)	28(100.0)

B(Phuket)	HAI		VMN	
Titre	Pre	Post	Pre	Post
<10 (negative)	14(50.0)	19(67.9)	23(82.1)	24(82.8)
≤40	24(85.7)	24(85.7)	27(96.4)	28(96.6)
≤80	25(89.3)	25(89.3)	27(96.4)	29(100.0)
≤160	26(92.9)	27(96.4)	28(100.0)	29(100.0)
≤320	27(96.4)	27(96.4)	28(100.0)	29(100.0)
≤640	28(100.0)	28(100.0)	28(100.0)	29(100.0)

Appendix 6 – Notice of Ethical Approval, Patient Information Sheet, and Consent Form for Chapter 4

Health Research Authority

West Midlands - South Birmingham Research Ethics Committee

Royal Standard Place
Nottingham
NG1 6FS

15 March 2016

Dr Paul Miller

Anthony Nolan Research Institute

Royal Free Hospital

Pond Street

NW3 2QG

Dear Dr Miller

Study title:	**The impact of seasonal influenza infection and vaccination health beliefs on vaccination intent amongst adult recipients of autologous and allogeneic haematopoietic stem cell transplant**
REC reference:	**16/WM/0144**
Protocol number:	**15/0875**
IRAS project ID:	**193912**

The Proportionate Review Sub-committee of the West Midlands - South Birmingham Research Ethics Committee reviewed the above application on 15 March 2016.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this favourable opinion letter. The expectation is that this information will be published for all studies that receive an ethical opinion but should you wish to provide a substitute contact point,

wish to make a request to defer, or require further information, please contact the REC Assistant, Tadeusz Jones, nrescommittee.westmidlands-southbirmingham@nhs.net. Under very limited circumstances (e.g. for student research which has received an unfavourable opinion), it may be possible to grant an exemption to the publication of the study.

Ethical opinion

On behalf of the Committee, the sub-committee gave a favourable ethical opinion of the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below.

Conditions of the favourable opinion

The REC favourable opinion is subject to the following conditions being met prior to the start of the study.

1. Amend the participant information sheet to add a sentence stating that PALS can be contacted if participants needed to speak with an independent person about participating.

You should notify the REC once all conditions have been met (except for site approvals from host organisations) and provide copies of any revised documentation with updated version numbers. Revised documents should be submitted to the REC electronically from IRAS. The REC will acknowledge receipt and provide a final list of the approved documentation for the study, which you can make available to host organisations to facilitate their permission for the study. Failure to provide the final versions to the REC may cause delay in obtaining permissions.

Management permission must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements. Each NHS organisation must confirm through the signing of agreements and/or other documents that it has given permission for the research to proceed (except where explicitly specified otherwise).

Guidance on applying for HRA Approval (England)/ NHS permission for research is available in the Integrated Research Application System, www.hra.nhs.uk or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of management permissions from host organisations.

Registration of Clinical Trials

All clinical trials (defined as the first four categories on the IRAS filter page) must be registered on a publically accessible database. This should be before the first participant is recruited but no later than 6 weeks after recruitment of the first participant.

There is no requirement to separately notify the REC but you should do so at the earliest opportunity e.g. when submitting an amendment. We will audit the registration details as part of the annual progress reporting process.

To ensure transparency in research, we strongly recommend that all research is registered but for non-clinical trials this is not currently mandatory.

If a sponsor wishes to request a deferral for study registration within the required timeframe, they should contact hra.studyregistration@nhs.net. The expectation is that all clinical trials will be registered, however, in exceptional circumstances non-registration may be permissible with prior agreement from the HRA. Guidance on where to register is provided on the HRA website.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Ethical review of research sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion").

Summary of discussion at the meeting

- **Social or scientific value; scientific design and conduct of the study**

The Sub-committee discussed whether a consent form was needed as returning the questionnaire could imply consent.

- **Informed consent process and the adequacy and completeness of participant information**

The Sub-committee agreed the participant information sheet needed a sentence adding stating that PALS can be contacted if participants needed to speak with an independent person about participating.

Approved documents

The documents reviewed and approved were:

Document	*Version*	*Date*
Evidence of Sponsor insurance or indemnity (non-NHS Sponsors only) [Insurance Confirmation]		12 January 2016
IRAS Checklist XML [Checklist_03032016]		03 March 2016
Letter from funder [Funding Confirmation]		14 January 2016

Non-validated questionnaire [HB Questionnaire]	6.2	08 January 2016
Other [Supervisor CV - KP]		01 August 2015
Other [Insurance Certificate]		12 January 2016
Other [Data Protection Registration]		12 January 2016
Participant consent form [Consent Form]	1.2	24 January 2016
Participant information sheet (PIS) [PIS]	1.2	24 January 2016
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Referee's report or other scientific critique report [Peer Review]		08 January 2016
Research protocol or project proposal [Research Protocol]	2.2	24 January 2016
Summary CV for Chief Investigator (CI) [CI CV - AM]		31 July 2015
Summary CV for student [PM CV]		30 July 2015
Summary CV for supervisor (student research) [JS CV]		03 August 2015

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http://www.hra.nhs.uk/about-the-hra/governance/quality-assurance/

HRA Training

We are pleased to welcome researchers and R&D staff at our training days – see details at http://www.hra.nhs.uk/hra-training/

With the Committee's best wishes for the success of this project.

16/WM/0144	**Please quote this number on all correspondence**

Yours sincerely

Professor Simon Bowman

Chair

Email: nrescommittee.westmidlands-southbirmingham@nhs.net

Enclosures:	*List of names and professions of members who took part in the review*
	"After ethical review – guidance for researchers" [SL-AR2]
Copy to:	*Ms Smaragda Agathou*
	Ms Julie Curtis, Research and Development Department
	Professor
	Alejandro Madrigal

West Midlands - South Birmingham Research Ethics Committee

Attendance at PRS Sub-Committee of the REC meeting on 15 March **2016**

Committee Members:

Name	*Profession*	*Present*	*Notes*
Professor Simon Bowman	Consultant Rheumatologist	Yes	Chair
Rev'd Dr Barry Clark	Retired Hospital Chaplain	Yes	
Dr John David Cochrane	Retired GP	Yes	

Also in attendance:

Name	*Position (or reason for attending)*
Mr Tad Jones	REC Assistant

Partner Organisations:

Health Research Authority, England NIHR Clinical Research Network, England

NHS Research Scotland NISCHR Permissions Co-ordinating Unit, Wales

HSC Research & Development, Public Health Agency, Northern Ireland

Notification of Non-Substantial/Minor Amendments(s) for NHS Studies

This template must only be used to notify NHS/HSC R&D office(s) of amendments, which are NOT categorised as Substantial Amendments. If you need to notify a Substantial Amendment to your study then you MUST use the appropriate Substantial Amendment form in IRAS.

Instructions for using this template

' For guidance on amendments refer to htto://www.hra.nhs.uk/research-community/during-your-researchproiect/amendments/

● This template should be completed by the Cl and optionally authorised by Sponsor, if required by sponsor guidelines.

● This form should be submitted according to the instructions provided for NHS/HSC R&D at http://www.hra.nhs.uk/research-community/during-your-research-proiect/amendments/which-reviewbodies-need-to-approve-or-be-notified-of-which-types-of-amendments/ . If you do not submit your notification in accordance with these instructions then processing of your submission may be significantly delayed.

1. Study Information

Full title of study:	The impact of seasonal influenza infection and vaccination health beliefs on vaccination intent amongst adult recipients of autologous and allogeneic haematopoietic stem cell transplant
IRAS Project ID:	193912
Sponsor Amendment Notification number:	
Sponsor Amendment Notification date:	
Details of Chief Investigator:	
Name [first name and surname]	Professor Alejandro Madrigal

Address	Anthony Nolan Research Institute Royal Free Hospital Pond Street
Postcode	NW3 2QG
Contact telephone number:	0303 303 0303
Email address	alejandro.madrigal@anthonynolan.org
Details of Lead Sponsor:	

Notification of non-substantial / minor amendments; version 1.0; November 2014

Partner Organisations:

Health Research Authority, England NIHR Clinical Research Network, England

NHS Research Scotland NISCHR Permissions Co-ordinating Unit, Wales

HSC Research & Development, Public Health A enc Northern Ireland

Name:	Tabitha Kavoi University College London
Contact email address:	randd@uclh.nhs.uk
Details of Lead Nation:	
Name of lead nation delete as appropriate	England / Northern Ireland / Scotland / Wales
If England led is the study going through CSP? delete as appropriate	Yes / No
Name of lead R&D office:	RandD Department Royal Marsden Hospital SM2 5PT

Partner Organisations:
Health Research Authority, England
NHS Research Scotland
HSC Research & Development, Public Health Agency, Northern Ireland
NIHR Clinical Research Network, England
NISCHR Permissions Co-ordinating Unit, Wales

2. Summary of amendment(s)

This template **must only** be used to notify NHS/HSC R&D office(s) of amendments, which are **NOT** categorised as Substantial Amendments.
If you need to notify a Substantial Amendment to your study then you MUST use the appropriate Substantial Amendment form in IRAS.

No.	Brief description of amendment *(please enter each separate amendment in a new row)*	Amendment applies to *(delete/ list as appropriate)*		List relevant supporting document(s), including version numbers *(please ensure all referenced supporting documents are submitted with this form)*		R&D category of amendment *(category A, B, C) For office use only*
		Nation	**Sites**	**Document**	**Version**	
1	Addition of study site under existing protocol:_ St George's NHS Foundation Trust Blackshaw Road Tooting London SW17 0QT PI – Dr Matthias Klammer Consultant Haematologist 0208672 1255	England	All sites or list affected sites	N/A		
		Northern Ireland	All sites or list affected sites			
		Scotland	All sites or list affected sites			
		Wales	All sites or list affected sites			
2						
3						
4						
5						

[Add further rows as required]

Partner Organisations:

Health Research Authority, England NIHR Clinical Research Network, England

NHS Research Scotland NISCHR Permissions Co-ordinating Unit, Wales

HSC Research & Development, Public Health Agency, Northern Ireland

3. Declaration(s)

Declaration by Chief Investigator

I confirm that the information in this form is accurate to the best of my knowledge and I take full responsibility for it.

I consider that it would be reasonable for the proposed amendment(s) to be implemented.

Signature of Chief Investigator:

Print name:

Date:

Optional Declaration by the Sponsor's Representative (as per Sponsor Guidelines)

The sponsor of an approved study is responsible for all amendments made during its conduct.

The person authorising the declaration should be authorised to do so. There is no requirement for a particular level of seniority; the sponsors rules on delegated authority should be adhered to.

I confirm the sponsor's support for the amendment(s) in this notification.

Signature of sponsors representative: ……

Print name:……

Post: ……

Organisation:……

Date:……

PARTICIPANT INFORMATION SHEET

The impact of seasonal influenza infection and vaccination health beliefs on vaccination intent among adult recipients of autologous and allogeneic haematopoietic stem cell transplant

Invitation to participate in the Study

Before you decide whether to take part, it is important for you to understand why the study is being done and what it will involve.

Please take time to read the following information carefully. Discuss it with friends and relatives if you wish.

If you would like to speak with an independent person the PALS (Patient Advice and Liaison Service) Team at your hospital can be contacted to discuss participation in the study with you.

You are free to decide whether or not to take part in this study. If you choose not to take part, this will not affect the care you get from your doctors and transplant team.

Ask us if there is anything that is not clear or if you would like more information

Study Summary

This research study will be conducted between March and September 2016. We hope to recruit 114 patients to the study.

The aim of the seasonal flu vaccine is to protect people who are at risk of flu and its complications.

International guidelines recommend that patients who have had a stem cell transplant receive the seasonal flu vaccine each winter. However, recent studies indicate that only around 60 to 70% of stem cell transplant recipients will actually receive the vaccine. There are a number of possible reasons for this. Studies of other patient groups have shown that their thoughts and feelings (known as "health beliefs") about seasonal flu and the flu vaccine impact on their intention to receive

the vaccine. The aim of this study is to find out how stem cell transplant patients' health beliefs impact on their intention to receive the flu vaccine.

Do I have to take part in the study?

No, you do not have to take part in the study if you do not wish to

Why have I been asked to participate?

You have received a stem cell transplant and will be eligible to receive the seasonal flu vaccine next winter.

You may have received the seasonal flu vaccine in the past, but you have not received the seasonal flu vaccine since your stem cell transplant.

What would taking part in the study involve?

You will be asked to complete a questionnaire that asks about your transplant, your beliefs about seasonal flu and the seasonal flu vaccine, and finally a few questions about yourself.

There are 41 questions in total. The questionnaire should take about 15minutes to complete.

All questions have tick boxes for you to respond. At the end you can write down anything else you think is important that has not been covered by the questionnaire.

When you have completed the questionnaire, you will be asked to seal it in the envelope provided.

You can complete the questionnaire today or take it home to complete and return it at your next appointment.

What will happen if I don't take part?

You will continue to receive all your normal care at Newcastle Freeman Hospital.

What are the possible advantages of taking part?

Taking part may help you to think about the seasonal flu and seasonal flu vaccine before you are offered the vaccine in Winter 2016. Taking part may help you to think of questions you have for your transplant team.

What are the possible disadvantages of taking part?

The questionnaire will take up some of your time – approximately 15 minutes.

It is possible that completing the questionnaire will raise concerns for you about your transplant, the seasonal flu or the seasonal flu vaccine. If you have any questions or concerns after you have completed the questionnaire, your transplant team will be available to discuss these with you.

What will happen if I don't want to answer all the questions?

If there are any questions you don't want to answer you can leave these blank. You can stop completing the questionnaire at any point.

What will happen if I start or complete the questionnaire and then decide I don't want to take part?

You can decide you don't want to take part in the study at any time, and this decision will not affect your ongoing medical care. The questionnaire you have completed will be destroyed and your responses will not be included in the study data analysis.

What information will be collected and will it be kept confidential?

All the information needed will be in the questionnaire. We will not gather any further information about you from your transplant team or medical records.

The questionnaire is anonymous. Your transplant team will not see your responses to the questionnaire. Only the study investigators will see your answers. Your answers will be collated with those of other participants and reported anonymously.

The questionnaire will be transferred securely from your transplant hospital to the Anthony Nolan Research Institute for analysis. Although anonymous, the completed questionnaires will be held strictly confidential at all times and will be stored in accordance with data protection legislation.

Involvement of other health care professionals

We will not be contacting any other health care professionals, including your GP, to notify them of your participation.

Who has reviewed the study?

All research in the NHS is looked at by an independent Research Ethics Committee to protect your interests. This study has been reviewed and given a favourable opinion by the National Research Ethics Service Committee.

What will happen to the results of the study?

The results of the study may be published or presented at scientific meetings and may be used for further research, but your data will be presented in an anonymous format. If you would like a summary of the results from the study please let the clinic doctor know.

Who is organizing and funding the study?

The Chief Investigator for the study is Professor Alejandro Madrigal, Consultant Haematologist and Scientific Director of the Anthony Nolan Research Institute.

The study is organized by Dr Paul Miller who is undertaking a clinical research degree (MD Res) at the Anthony Nolan Research Institute and the University College London Cancer Research Institute. The Study is being funded by the Anthony Nolan Research Institute, and the study is sponsored by University College London.

What if something goes wrong?

If you wish to complain or have any concerns about any aspect of the way you have been approached or treated by members of staff you may have experienced due to your participation in the research, National Health Service or UCL complaints mechanisms are available to you. Please ask your research doctor if you would like more information on this.

In the unlikely event that you are harmed by taking part in this study, compensation may be available.

If you suspect that the harm is the result of the Sponsor's (University College London) or the hospital's negligence then you may be able to claim compensation. After discussing with your research doctor, please make the claim in writing to Professor Alejandro Madrigal who is the Chief Investigator for the research and is based at the Anthony Nolan Research Institute, Royal Free Hospital, Pond Street, NW3 2QG. The Chief Investigator will then pass the claim to the Sponsor's Insurers, via the Sponsor's office. You may have to bear the costs of the legal action initially, and you should consult a lawyer about this.

Thank you for taking the time to read this patient information sheet.

Further Information and Contact Details

For further information please contact the Chief Investigator, Student Researcher or Principal Investigator at your study site

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Study Number:

Patient Identification Number for this study:

CONSENT FORM

The impact of seasonal influenza infection and vaccination health beliefs on vaccination intent among adult recipients of autologous and allogeneic haematopoietic stem cell transplant

Chief Investigator: **Professor Alejandro Madrigal**

Please initial all boxes

5. I confirm that I have read and understand the information sheet dated **24 March 2016 version number 1.3** for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

6. I understand that my participation is voluntary, and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. ☐

7. I understand that relevant sections of my medical notes and data collected during the study, may be looked at by individuals from the sponsor of the trial (University College London) and responsible persons authorised by the sponsor, from regulatory authorities or from the NHS Trust, where it is I relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐

8. I agree to take part in the above study. ☐

Name of Participant	Date	Signature
Name of Person taking consent.	Date	Signature

Appendix 7 – Seasonal Inactivated Influenza Health Belief Study Questionnaire

Seasonal flu and seasonal flu vaccine health beliefs in recipients of allogeneic and autologous stem cell transplant

Most people who have received a stem cell transplant are advised to receive the seasonal flu vaccine each winter.

The aim of this questionnaire is to understand how your beliefs may affect your intention to receive the seasonal flu vaccine next winter.

Below are a series of statements about your intentions and beliefs regarding the seasonal flu and the seasonal flu vaccine. Please read each statement and put a tick or cross in the box that is closest to your belief.

Your responses will be collated with those of other study participants and reported anonymously. Your transplant team will not find out your individual responses to these statements. Your responses will not affect the healthcare you receive.

First, please could you tell us a few details about your transplant, and whether you had the seasonal flu vaccine before your transplant?

1. What type of transplant have you received?

Autologous (Your own stem cells given back to you)	Allogeneic (Another person's stem cell given to you)
☐	☐

2. What condition was your stem cell transplant treating?

☐ AML (Acute Myeloid Leukaemia)
☐ ALL (Acute Lymphoid Leukaemia)
☐ CML (Chronic Myeloid leukaemia)
☐ CLL (Chronic Lymphocytic Leukaemia)
☐ MDS (Myelodysplastic Syndrome)
☐ Aplastic Anaemia
☐ Multiple Myeloma
☐ Non-Hodgkin Lymphoma
☐ Hodgkin Lymphoma
☐ Autoimmune disease
☐ Other (please specify)
_____

3. How long ago was your stem cell transplant?				
0-3 months ☐	4-6 months ☐	7-9 months ☐	10-12 months ☐	More than 12 months ☐

4. Did you receive the seasonal flu vaccine at **any point in time before** your stem cell transplant?		
Yes ☐	No ☐	Not Sure ☐

5. Have you received **any vaccines since** your stem cell transplant?		
Yes ☐	No ☐	Not Sure ☐

6. Have your transplant team given you any information about the seasonal flu vaccine before today?		
Yes ☐	No ☐	Not Sure ☐

Please now read each of the following statements. Please put a tick or cross in the box next to the <u>best single option</u> to indicate whether you STRONGLY DISAGREE, DISAGREE, NEITHER AGREE NOR DISAGREE, AGREE or STRONGLY AGREE with each statement.

7. I **intend to receive** the seasonal flu vaccine next winter				
Strongly disagree ☐	Disagree ☐	Neither agree Nor disagree ☐	Agree ☐	Strongly Agree ☐

8. Now I have had a stem cell transplant I can catch the seasonal flu more easily than other people my age				
Strongly disagree ☐	Disagree ☐	Neither agree Nor disagree ☐	Agree ☐	Strongly Agree ☐

9. Now I have had a stem cell transplant I can catch the seasonal flu more easily than before my transplant

Strongly disagree	Disagree	Neither agree Nor disagree	Agree	Strongly Agree
☐	☐	☐	☐	☐

10. I will **choose not to receive** the seasonal flu vaccine next winter

Strongly disagree	Disagree	Neither agree Nor disagree	Agree	Strongly Agree
☐	☐	☐	☐	☐

11. My chances of catching seasonal flu next winter will be high if I **do not receive** the seasonal flu vaccine

Strongly disagree	Disagree	Neither agree Nor disagree	Agree	Strongly Agree
☐	☐	☐	☐	☐

12. I am more likely than other people my age to catch seasonal flu next winter if I **do not receive** the seasonal flu vaccine

Strongly disagree	Disagree	Neither agree Nor disagree	Agree	Strongly Agree
☐	☐	☐	☐	☐

13. Now I have had a stem cell transplant it is more likely that I will catch seasonal flu next winter if **I do not receive** the seasonal flu vaccine

Strongly disagree	Disagree	Neither agree Nor disagree	Agree	Strongly Agree
☐	☐	☐	☐	☐

14. If I **do not receive** the seasonal flu vaccine and caught the seasonal flu next winter this would be a serious illness for me				
Strongly disagree ☐	Disagree ☐	Neither agree Nor disagree ☐	Agree ☐	Strongly Agree ☐

15. If I **do not receive** the seasonal flu vaccine and caught the seasonal flu next winter this would have a negative impact on my recovery from my stem cell transplant				
Strongly disagree ☐	Disagree ☐	Neither agree Nor disagree ☐	Agree ☐	Strongly Agree ☐

16. If I **do not receive** the seasonal flu vaccine and caught the seasonal flu next winter I would become more unwell than other people my age				
Strongly disagree ☐	Disagree ☐	Neither agree Nor disagree ☐	Agree ☐	Strongly Agree ☐

17. I am worried about side effects of the seasonal flu vaccine				
Strongly disagree ☐	Disagree ☐	Neither agree Nor disagree ☐	Agree ☐	Strongly Agree ☐

18. If I receive the seasonal flu vaccine next winter it may make me feel unwell with the flu or a flu-like illness				
Strongly disagree ☐	Disagree ☐	Neither agree Nor disagree ☐	Agree ☐	Strongly Agree ☐

19. If I receive the seasonal flu vaccine next winter I am more likely to experience side effects than other people my age

Strongly disagree	Disagree	Neither agree Nor disagree	Agree	Strongly Agree
☐	☐	☐	☐	☐

20. If I receive the seasonal flu vaccine next winter it may have a negative impact on my recovery from my stem cell transplant

Strongly disagree	Disagree	Neither agree Nor disagree	Agree	Strongly Agree
☐	☐	☐	☐	☐

21. Now I have had a stem cell transplant the seasonal flu vaccine may not work as well for me as it does for other people my age

Strongly disagree	Disagree	Neither agree Nor disagree	Agree	Strongly Agree
☐	☐	☐	☐	☐

22. I would prefer to have the seasonal influenza vaccine next winter at my transplant centre instead of my GP surgery

Strongly disagree	Disagree	Neither agree Nor disagree	Agree	Strongly Agree
☐	☐	☐	☐	☐

23. If I receive the seasonal flu vaccine next winter it may help to prevent me from catching the seasonal flu

Strongly disagree	Disagree	Neither agree Nor disagree	Agree	Strongly Agree
☐	☐	☐	☐	☐

24. If I receive the seasonal flu vaccine next winter it may help to prevent me from passing the seasonal flu to other people around me

Strongly disagree	Disagree	Neither agree Nor disagree	Agree	Strongly Agree
☐	☐	☐	☐	☐

25. If I receive the seasonal flu vaccine next winter, but still catch the flu, it may help to prevent me from becoming seriously unwell

Strongly disagree	Disagree	Neither agree Nor disagree	Agree	Strongly Agree
☐	☐	☐	☐	☐

26. If I receive the seasonal flu vaccine next winter I will worry less about catching the seasonal flu

Strongly disagree	Disagree	Neither agree Nor disagree	Agree	Strongly Agree
☐	☐	☐	☐	☐

27. The thought of catching seasonal flu next winter worries me

Strongly disagree	Disagree	Neither agree Nor disagree	Agree	Strongly Agree
☐	☐	☐	☐	☐

28. If my transplant team advised me to receive the seasonal flu vaccine next winter I would definitely have it

Strongly disagree	Disagree	Neither agree Nor disagree	Agree	Strongly Agree
☐	☐	☐	☐	☐

29. If my GP advised me to receive the seasonal flu vaccine next winter I would definitely have it

Strongly disagree	Disagree	Neither agree Nor disagree	Agree	Strongly Agree
☐	☐	☐	☐	☐

30. My GP understands my condition enough to know if the seasonal flu vaccine is right for me

Strongly disagree	Disagree	Neither agree Nor disagree	Agree	Strongly Agree
☐	☐	☐	☐	☐

31. My transplant team understand my condition enough to know if the seasonal flu vaccine is right for me

Strongly disagree	Disagree	Neither agree Nor disagree	Agree	Strongly Agree
☐	☐	☐	☐	☐

32. I have enough information and am able to decide whether the seasonal flu vaccine is right for me?

Strongly disagree	Disagree	Neither agree Nor disagree	Agree	Strongly Agree
☐	☐	☐	☐	☐

33. I would find it easy to attend my GP surgery next winter to receive the seasonal flu vaccine

Strongly disagree	Disagree	Neither agree Nor disagree	Agree	Strongly Agree
☐	☐	☐	☐	☐

34. I would regret it if I decided **not to receive** the seasonal flu vaccine next winter and became unwell with seasonal flu				
Strongly disagree ☐	Disagree ☐	Neither agree Nor disagree ☐	Agree ☐	Strongly Agree ☐

35. I would regret it if I **decided to receive** the seasonal flu vaccine next winter and became unwell with side effects				
Strongly disagree ☐	Disagree ☐	Neither agree Nor disagree ☐	Agree ☐	Strongly Agree ☐

36. If you have any other thoughts or comments about the seasonal flu or receiving the flu vaccine after your transplant please let us know about them below

Finally, please could you tell us a few details about yourself?

37. What is your age group?					
16-24 ☐	25-34 ☐	35-44 ☐	45-54 ☐	55-64 ☐	65+ ☐

38. What is your gender?	
Male ☐	Female ☐

39. What is your marital status?					
Single ☐	Married ☐	Cohabiting/living with partner ☐	Divorced/ Separated ☐	Widowed ☐	Prefer not to answer ☐

40. Please tick which best describes your living arrangements				
Rent from local authority/ housing association ☐	Rent from private landlord/agency ☐	Home owner ☐	Other ☐	Prefer not to answer ☐

41. What is the highest level of education or professional qualification you have obtained?		
☐ GCSE / O-level / GCE ☐ ONC / BTEC ☐ A-levels or equivalent	☐ Degree or equivalent ☐ Post graduate qualification ☐ No formal qualification	☐Other ☐ Prefer not to answer

42. What is your ethnic background?				
White	**Mixed / multiple ethnic groups**	**Asian / Asian British**	**Black / African / Caribbean / Black British**	**Other ethnic group**
☐ English / Welsh / Scottish/Nother n Irish / British ☐ Irish ☐ Other white background ☐Any other white background. Please specify: ……	☐ White and black Caribbean ☐white and black African ☐white and Asian ☐Any other mixed/multiple ethnic background please specify: …… …	☐ Indian ☐Pakistani ☐Bangladeshi ☐Chinese ☐Other Asian background please specify …… …	☐ African ☐Caribbean ☐Any other black/African/ Caribbean background please specify …… …	☐Arab ☐Any other ethnic background please specify: …… …

Thank you very much for taking the time to complete this questionnaire. Please place the questionnaire in the envelope provided, seal the envelope and return it to your transplant nurse.

If completing this questionnaire has prompted any questions or concerns about seasonal flu or the seasonal flu vaccine please speak with your transplant nurse who will be happy to discuss this with you.

Appendix 8 – Minimum Essential Data C form: Autoimmune cytopenias (AIC) following allogeneic HSCT for acquired aplastic anaemia

Restricted MED-C

Auto-immune Cytopenias after SCT in SAA

TEAM

EBMT Centre Identification Code (CIC) ……

Hospital …… Unit ……

Contact person……

Telephone…… Tax ……

e-mail……

Date of this report .... - .... - ....

yyyy *mm* *dd*

PATIENT

Unique Identification Code (UIC) ……

Hospital Unique Patient Number or Code ……

Initials …… ……

Date of birth .... - .... - ....

yyyy *mm* *dd*

Sex: ☐ Male ☐ Female

DATE AA DIAGNOSIS, HSCT AND AIC DIAGNOSIS

Date of diagnosis aplastic anaemia .... - .... - ....

yyyy *mm* *dd*

Date of HSCT: .... - .... - .... HSCT number:…… (first HSCT only)

yyyy *mm* dd

Date diagnosis auto-immune cytopenia .... - .... -..... .....

yyyy *mm* *dd*

To be included patients need to be diagnosed with AA, have a first transplant between 2002 and 2012 and a diagnosis of AIC post first allograft.

AUTO-IMMUNE CYTOPENIA POST HSCT

DIAGNOSTIC CRITERIA *(please tick the diagnosed type(s) of AIC and complete the corresponding questions)*

☐ **Immune thrombocytopenia (ITP)**

New or worsening thrombocytopenia with Plt <100 ☐ Yes ☐ No ☐ Unknown

Bone marrow aspiration - *select <u>one</u> option*

☐ Not performed

☐ Performed showing normo- or hyper regenerative megakaryopoiesis

☐ Performed other morphological/histological findings

Exclusion of other causes of thrombocytopenia - *tick yes if excluded!*

Disease relapse	☐ Yes	☐ No	☐ Unknown
New haematological malignancy	☐ Yes	☐ No	☐ Unknown
Drug induced	☐ Yes	☐ No	☐ Unknown
Active infection	☐ Yes	☐ No	☐ Unknown
Thrombocytopenic microangiopathy	☐ Yes	☐ No	☐ Unknown
Allo-immune thrombocytopenia	☐ Yes	☐ No	☐ Unknown

☐ **Autoimmune haemolytic anaemia (AIHA)**

Direct agglutinin test (DAT) - *select one option*

☐ Negative ☐ IgG Positive ☐ Not done

☐ C3d Positive ☐ IgG and C3d positive

New or worsening anaemia (Hb drop >/=20g/L) ☐ Yes ☐ No ☐ Unknown

Features of haemolysis

Rise in lactate dehydrogenase (LDH) level	☐ Yes	☐ No	☐ Unknown
Unconjugated hyper bilirubinaemia	☐ Yes	☐ No	☐ Unknown

Reduced Haptoglobin ☐ Yes ☐ No ☐ Unknown

Reticulocytosis ☐ Yes ☐ No ☐ Unknown

Blood film showing spherocytes ☐ Yes ☐ No ☐ Unknown

Exclusion of other causes of haemolytic anaemia - *tick yes if excluded!*

Disease relapse ☐ Yes ☐ No ☐ Unknown

New haematological malignancy ☐ Yes ☐ No ☐ Unknown

Drug induced ☐ Yes ☐ No ☐ Unknown

Active infection ☐ Yes ☐ No ☐ Unknown

Microangiopathic haemolytic anaemia ☐ Yes ☐ No ☐ Unknown

Allo-immune haemolytic anaemia ☐ Yes ☐ No ☐ Unknown

☐ **Evans Syndrome (Autoimmune haemolytic anaemia and immune thrombocytopenia) – please complete diagnostic criteria under ITP and AIHA**

☐ **Autoimmune neutropenia (AIN)**

New or worsening neutropenia ☐ Yes ☐ No ☐ Unknown

Positive serum anti-neutrophil antibodies by either

direct / indirect method ☐ Yes ☐ No ☐ Unknown

Exclusion of other causes of neutropenia - *tick yes if excluded!*

Disease relapse ☐ Yes ☐ No ☐ Unknown

New haematological malignancy ☐ Yes ☐ No ☐ Unknown

Active viral / bacterial infection ☐ Yes ☐ No ☐ Unknown

Drug induced ☐ Yes ☐ No ☐ Unknown

TREATMENT AUTO-IMMUNE CYTOPENIA

Multiple treatments for AIC ☐ No

☐ Yes: Number of treatments: .....

FOR MULTIPLE TREATMENTS, COPY THIS AND THE NEXT PAGE

AS MANY TIMES AS NECESSARY

Date treatment started .... - .... - ....

yyyy *mm* *dd*

SEQUENTIAL NUMBER OF THIS TREATMENT EPISODE: .....

Treatment in this episode:

☐ Corticosteroid dose_____ mg/kg

☐ Intravenous Immunoglobulin dose _____g/kg

☐ Anti CD20 Monoclonal Antibody (Rituximab) dose _____mg/m2

☐ Cyclosporin

☐ Mycophenolate Mofetil

☐ Anti CD25 Monoclonal Antibody (Alemtuzumab)

☐ Cyclophosphamide dose _____mg/kg

☐ Plasma Exchange

☐ Splenectomy

☐ Other (please specify):

_____

RESPONSE TO THIS AIC TREATMENT EPISODE

please tick one response classification and complete the corresponding questions

☐ Complete response

Normalisation or return to baseline of:-

- Haemoglobin, platelet or neutrophil count ☐ Yes ☐ No ☐ Unknown

- Biochemical markers of haemolysis (For AIHA and Evan's Syndrome) ☐ Yes ☐ No ☐ Unknown

Ongoing maintenance therapy required? ☐ Yes ☐ No ☐ Unknown

Other (Please give details)_____

☐ Partial response

Improvement but **NOT** normalisation or return to baseline of

- Haemoglobin, platelet or neutrophil count ☐ Yes ☐ No ☐ Unknown

- Biochemical markers of haemolysis (For AIHA and Evan's Syndrome) ☐ Yes ☐ No ☐ Unknown

Ongoing maintenance therapy required? ☐ Yes ☐ No ☐ Unknown

Other (Please give details)_____

☐ No response

No improvement or further decline of

- Haemoglobin, platelet or neutrophil count ☐ Yes ☐ No ☐ Unknown

- Biochemical markers of haemolysis (For AIHA and Evan's Syndrome) ☐ Yes ☐ No ☐ Unknown

Other (Please give details)_____

Date response achieved .... - .... -..... .....

yyyy *mm* *dd*

Ongoing Response ☐ Yes

☐ No. Date of loss of CR/PR .... - .... -..... .....

yyyy *mm* *dd*

ADDITIONAL NOTES IF APPLICABLE

COMMENTS

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Your reference number	
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Number of figure/table/extracts	1
Description of figure/table/extracts	Fig 1 Symptoms and complications of influenza. Complicated influenza is defined as an infection that requires hospital admission
Will you be translating?	No
Circulation/distribution	5
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