INSTITUTE OF CLINICAL TRIALS AND METHODOLOGY

Repurposing medicines for the adjuvant treatment of cancer: An evaluation of aspirin and metformin

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Declaration

I, Christopher Coyle, confirm that the work presented in this thesis is my own. I have stated the contribution from other parties within the acknowledgement section. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

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Dr Christopher Coyle

Abstract

Introduction: Evidence from pre-clinical studies and observational data suggest that metformin and aspirin are good candidates for adjuvant therapies, though definitive phase III trials have not been completed. Prior to the initiation of this work, the Add-Aspirin trial had been conceived and funded with several potential challenges related to the implementation and design identified. Evidence to support the evaluation of metformin in a phase III adjuvant basket trial had not been systematically evaluated.

Methods: I examined the implementation and conduct of the Add-Aspirin trial during its first year at individual UK research centres. Baseline clinical characteristics, and the feasibility and effect of the run-in period, in the first 500 participants was also examined. Additionally, I conducted a systematic review and meta-analysis to investigate the effect of metformin use on survival outcomes for individual tumour types in the adjuvant setting.

Results: Centres recognised the efficiencies offered from a basket trial design particularly in terms of gaining approvals, staffing and data entry, though some unanticipated set-up and recruitment challenges have been identified. The baseline clinical characteristics were largely as expected. Overall, 88% of participants were randomised. The run-in period was effective in identifying, and preventing randomisation of participants who had less than 80% adherence (5.0%), and participants who developed significant aspirin related toxicities (1.2%). Other non-randomisations were mostly due to minor toxicity and/or personal choice. A systematic review and meta-analysis found that metformin use was associated with significant benefits in recurrence-free survival, overall survival and cancer-specific survival in early-stage colorectal and prostate cancer.

Conclusion: Opening a large multi-tumour type basket trial with an active run-in period was found to be feasible, but minor conduct modifications have been recommended and protocol amendments implemented. Metformin could be a useful adjuvant agent, and randomised-control trials in colorectal and prostate cancer are advocated.

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Abbreviations

Abbreviation	Expansion
AARP	Association of American Retired Persons Diet and Health Study
ADMET	Absorption, distribution, metabolism, excretion and toxicity
AMP	Adenosine monophosphate
AMPK	Adenosine monophosphate activated protein kinase
ATP	Adenosine triphosphate
BCG	Bacillus Calmette-Guerin
BMI	Body mass index
CALGB	Cancer and leukemia group B
cAMP	Cyclic adenosine monophosphate
CaPP1	Cancer Prevention Project 1
CaPP2	Cancer Prevention Project 2
CaPSURE	Cancer of the Prostate Strategic Urologic Research Endeavour
CI	95% confidence interval
COX	Cyclooxygenase
CRF	Case report forms
CRT	Chemoradiotherapy
CSS	Cancer specific survival
CT	Chemotherapy
CTCAE	Common terminology criteria for adverse events
DFS	Disease free survival
DM	Type II diabetes mellitus
DNA	Deoxyribonucleic acid
ECR	Eindhoven Cancer Registry
eGFR	Estimated glomerular filtration rate
EMBASE	Excerpta Medica Database
EME	European Medicines Agency

ER	Oestrogen receptor
FAP	Familial Adenomatous Polyposis
FDA	US Food and Drug Administration
g	Grams
GBP	British pound
GCP	Good Clinical Practice
GLP-1	Glucagon-like peptide-1
GLUT4	Glucose transporter type 4
GP	General Practitioner
H2	Histamine receptor 2
HbA1c	Glycosylated haemoglobin A1c
HDI	Human Development Index
HEAT trial	Helicobacter Eradication Aspirin Trial
HER-2	Human Epidermal Growth Factor 2
HLA	Human Leukocyte Antigen
HPFS	Health Professionals follow-up study
HR	Hazard ratio
HRA	Health Research Authority
IL-6	Interleukin 6
IQR	Interquartile range
ISRCTN	International Standard Randomised Controlled Trials Number
LKB1	Liver kidney B1
LMIC	Low and middle income countries
MCS	Moffitt Cancer Centre
MDT	Multidisciplinary Team Meeting
MEDLINE	Medical Literature Analysis and Retrieval System Online
Met	Metformin
mg	Milligrams
ml/min/1.73m^2	Millilitre per minute per 1.73 metres squared
MHRA	Medicines and Healthcare products Regulatory Agency

mmol/L	Millimole per litre
mTOR	Mechanistic target of rapamycin
mTORC1	mTOR complex 1
n	Number
N/A	Not applicable
NF-kappa B	Nuclear factor kappa B
NHS	National Health Service
NICE	National Institute for Health and Care Excellence
NIHR	National Institute for Health Research
NMI	Non-muscle invasive
NOS	Newcastle-Ottawa quality assessment scale
NSAID	Non-steroidal anti-inflammatory drug
OCT-1	Organic cation transporter-1
OR	Odds-ratio
OS	Overall survival
PET	Positron emission tomography
PI	Principal Investigator
PI3K/mTOR	Phosphatidylinositol-3-kinase/mammalian target of rapamycin
PIK3CA	Phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit alpha
PKA	Protein kinase A
PR	Progesterone receptor
PRISMA	Preferred reporting items for systematic reviews and meta-analyses
PTGS	Prostaglandin endoperoxide synthetase
RFS	Recurrence-free survival
Rheb	RAS homologue enriched in brain
RMH	Royal Melbourne Hospital
RT	Radiotherapy
SD	Standard deviation
SNP	Single nucleotide polymorphism
TBX2	11-dehydro-thromboxane B_2

TDM-1	Ado-trastuzumab emtansine
TGF-β	Transforming growth factor beta
TMG	Trial Management Group
TNF-α	Tumour necrosis factor alpha
TNM	Tumour, nodes, metastasis
TSC	Trial Steering Committee
TSC2	Tuberous sclerosis complex 2,
TURBT	Transurethral resection of bladder tumour
UK	United Kingdom
US	United States
WHS	Women's Health Study
WNT	Wingless-related integration site
χ^2	Chi-squared test

Chapter 1. Introduction

1.1 The global burden of cancer

In 2014, there were around 357,000 new cancer diagnoses and 163,000 cancer deaths in the United Kingdom (UK) (1). Earlier cancer detection, combined with developments in cancer treatment have meant that survival after a cancer diagnosis has doubled in the last 40 years, however despite this about half of those diagnosed with cancer in the UK still die from their disease within 10 years (1).

Cancer is not just a disease of well-resourced countries, in 2012 there were an estimated 14.1 million new cancer diagnoses and 8.2 million cancer deaths globally (2). Cancer is an increasing problem in low and middle income countries (LMIC), which accounts for 57% of cancer diagnoses, and 65% of cancer deaths worldwide (2). To examine trends in the global distribution of cancer, the Human Development Index (HDI) (based on life expectancy, education and gross domestic product per capita), has been used to group countries into four categories of socioeconomic development, ranging from low HDI (resource poor countries) to very high HDI (resource rich countries). Between 2008 and 2030 the absolute incidence of cancer is predicted to increase by 93%, 78%, 60% and 39% in low, medium, high and very high HDI countries respectively (3). The shift in the global burden of cancer towards lower HDI countries is thought to result from rapid increases in population growth, life expectancy, and exposure to risk factors including alcohol, infectious diseases, diet, industrial exposures, and smoking (4). Cancer survival rates are also poorer in low HDI countries, which has been attributed to a later stage at diagnosis (due to inadequate access to healthcare, health education and screening programmes) and limited access to affordable cancer treatments (5).

1.2 The treatment of cancer

When feasible, the cornerstone of treatment for many cancer types is surgical resection. Beyond surgery, there are a number of other modalities of cancer treatment which can be

broadly divided into cytotoxic therapy (e.g. chemotherapy and radiation therapy), treatment that interferes with tumour growth and survival pathways (e.g. hormone therapy and molecularly targeted therapy), and treatment that modifies the host environment (e.g. immunotherapy).

Cytotoxic cancer therapies work by causing damage to cancer cells to a greater extent than normal tissue. Radiation therapy does this through the administration of ionising radiation to damage the deoxyribonucleic acid (DNA) of cancer cells leading to cell death. To spare normal tissue, radiation is applied either using a shaped beam or directly through the use of radioactive implants (brachytherapy). Radiation therapy is also administered in temporally separated doses (fractions) to utilise the greater ability of normal tissue to recover between administrations. Chemotherapy uses chemical substances as cellular poisons, mainly through oral or intravenous administration and very occasionally through intrathecal or intraperitoneal routes. Like radiation therapy, chemotherapy also damages normal tissue, but due to biological differences between cancer cells and normal tissue (for example cancer cells undergo rapid cellular division), cancer cells are subjected to greater and more irreversible damage. Chemotherapy is also administered in temporally separated doses (cycles) to allow normal tissue to recover between administrations. Traditional chemotherapy acts either by causing damage to DNA (for example alkylating agents or topoisomerase inhibitors), or by preventing cell division/DNA replication (anti-metabolites or anti-mitotic agents). Different chemotherapeutic agents are often used in combination to minimise the chance of resistance developing and to allow lower doses to be given whilst maintaining/maximising therapeutic benefit. Combining chemotherapy and radiation therapy can lead to therapeutic synergy.

Cancer can also be treated by interfering with the molecular pathways involved in tumour growth and survival. Hormone therapy involves the disruption of endocrine signalling through the administration of exogenous hormones, or medications which inhibit the production or actions of hormones, thereby inhibiting cancer growth for some tumour types (examples include prostate cancer and hormone receptor positive breast cancer). Targeted therapy uses

small molecules or monoclonal antibodies, designed to target and interfere with specific proteins or enzymes involved in the molecular pathways controlling tumour growth and survival. Examples include the small molecule pazopanib, which inhibits the enzyme tyrosine kinase, reducing growth signalling in renal cell carcinoma (6), and the monoclonal antibody bevacizumab, which inhibits vascular endothelial growth factor, reducing blood vessel formation in a number of tumour types (7). Targeted therapy can also be combined with chemotherapy, as a single molecule, to allow targeted cancer cell cytotoxicity, for example the antibody-cytotoxic conjugate ado-trastuzumab emtansine (T-DM1) used in the treatment of human epidermal growth factor 2 (HER-2) receptor positive metastatic breast cancer (8).

Most recently, developments in the treatment of cancer have focused on modifying the host environment to exert an anti-cancer effect. Immunotherapy is a treatment strategy that stimulates a patient's immune system to target cancer cells. This can either be achieved actively, by directing the immune system to target specific proteins and other macromolecules on the surface of cancer cells, or passively, by enhancing an existing immune response to cancer cells. Recent successes in the field of cancer immunotherapy for the treatment of melanoma (9) highlight the potential efficacy of new therapies that modify the host and tumour microenvironment rather than working through direct tumour cytotoxicity.

1.2.1 The adjuvant treatment of cancer

Those diagnosed with cancer at an early stage often have primary treatment administered with curative intent (surgery or another radical treatment), however a risk of cancer returning (recurrence) remains for many cancer types. Adjuvant therapy, (therapy given in addition to primary treatment) can reduce the risk of recurrence, thus avoiding the burden of subsequent treatment and associated morbidity and mortality.

A number of distinct modalities of adjuvant treatment have been shown to be effective in decreasing cancer recurrence and improving survival outcomes. Combinations of chemotherapeutic agents are often given for a number of tumour types including colorectal, breast, soft tissue sarcoma, endometrial, pancreatic, testicular and non-small cell lung cancer

(10). Other treatment modalities include hormone therapy (for example in prostate cancer), radiotherapy (for example in endometrial cancer) or molecularly targeted therapy (for example trastuzumab for the treatment of HER-2 receptor positive breast cancer). Improvements in adjuvant cancer outcomes have been made by combining modalities, either concurrently or sequentially, for example, a combination of chemotherapy and radiation therapy is used in the adjuvant treatment of small cell lung cancer, glioblastoma multiforme and head and neck cancer (11).

The incremental benefits of combining treatment modalities can be illustrated by examining estimated survival gains in the adjuvant treatment of breast cancer using an on-line prognostication tool (12). After surgery alone for a patient with T4N2M0 luminal-B breast cancer, and without any adjuvant therapy, 77 out of 100 women are estimated to have died at five years. The use of adjuvant combination chemotherapy results in an extra 18 women alive at five years, and the addition of adjuvant radiotherapy can also improve local control rates further. Moreover, by adding a year of molecularly targeted therapy (trastuzumab), and five years of hormone therapy (tamoxifen), a further 10 and 13 extra women are alive at five years respectively. Further gains are also possible by extending adjuvant hormone therapy to 10 years (13). Despite the benefits of combining adjuvant therapies in this scenario, 27 women out of 100 will still not be alive at five years. Likewise, adjuvant therapy only partially reduces the risk of recurrence for most tumour types (10), added to which, some cancers, including renal cell carcinoma and hepatocellular carcinoma, currently have no proven adjuvant therapies available. As a consequence, new adjuvant cancer treatments that can be given in isolation or combined with existing therapies, are urgently needed.

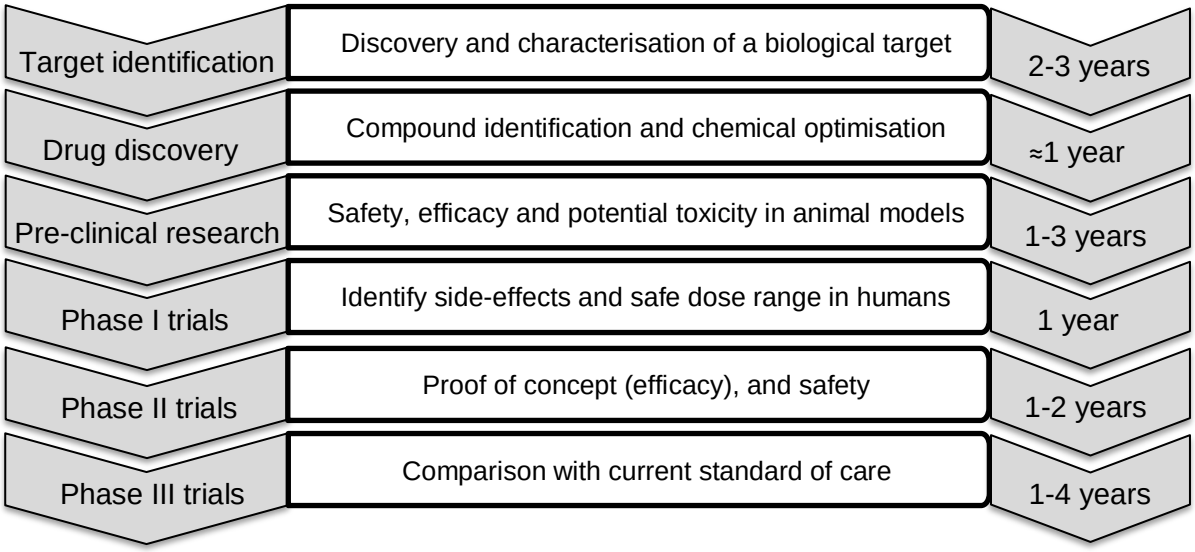
1.3 Drug discovery and development

In ancient times, medicines are thought to have been discovered through trial and error experimentation using natural products (often herbs, plants, roots or fungi) and observation of their biological effects (14). In the late 19th century, developments in medicinal chemistry

resulted in the discovery of new drugs through the identification of the active chemical ingredients that are responsible for the biological activity of traditional remedies, for example acetylsalicylic acid (aspirin). In the mid-20th century, the advent of classical pharmacology allowed drugs to be discovered through the use of chemical libraries administered to cell cultures or organisms, to look for a desired biological effect, which led to the development of traditional chemotherapy, for example paclitaxel. Later in the twentieth century, advances in the understanding of the molecular pathways underlying cancer, led to the era of modern drug discovery and the development of biological cancer therapies (15).

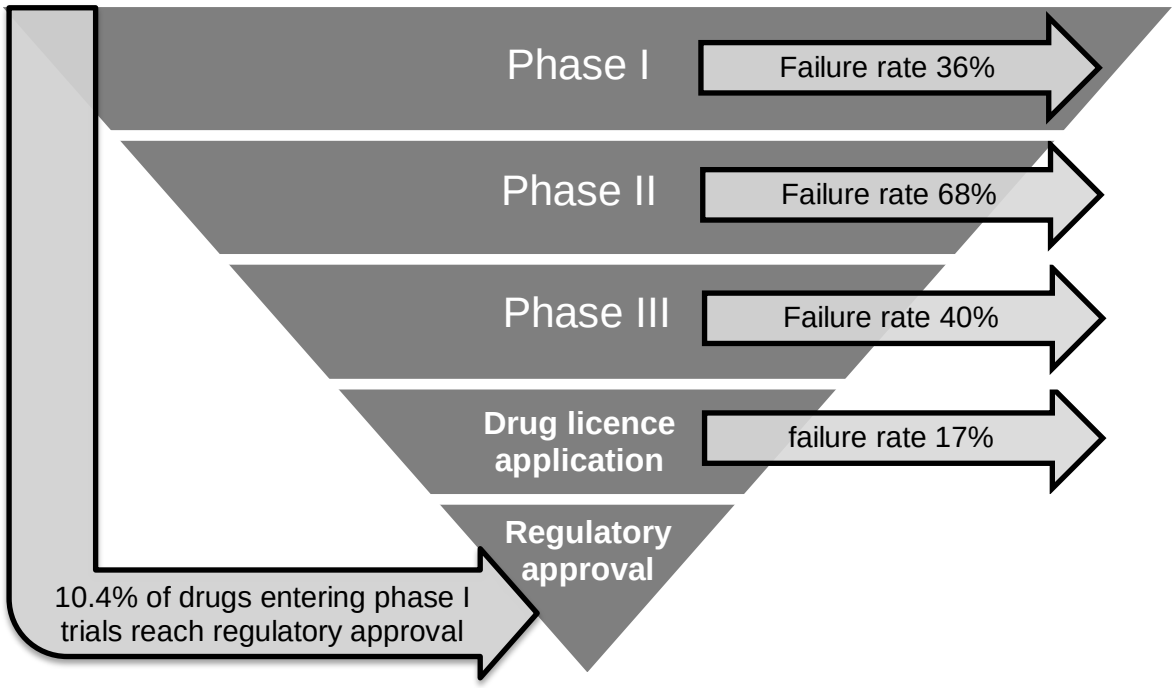
Modern cancer drug discovery usually starts with laboratory based research to find and characterise a biological target that has been implicated in the growth and development of malignancy. A molecular screening library is then searched for a compound that has the greatest affinity for that target. Once identified, the compound then undergoes a process of chemical optimisation using pre-clinical studies designed to investigate and potentially improve absorption, distribution, metabolism, excretion and toxicity (ADMET) (16). When a potential candidate therapy is selected, pre-clinical studies (*in-vivo* and *in-vitro)* then evaluate its safety, efficacy and potential toxicity before beginning clinical testing. Phase I trials test the drug in people for the first time to identify side-effects and determine a safe dosage range. This is normally done by escalating the dose given to successive people to establish the highest dose that does not cause unacceptable side effects (called the maximum tolerated dose). If no concerning toxicities develop, the drug will enter phase II trials to examine efficacy and investigate side-effects further. Agents shown to be sufficiently effective and tolerated in phase II trials then enter phase III testing. Phase III trials are designed to compare a new treatment to the current standard of care in terms of efficacy, toxicity, and often resource requirements and quality of life. If shown to be beneficial, an application is then made for a licence for routine use (regulatory approval). The path from the discovery of a drug to clinical use is a lengthy journey, and is estimated to take between 10 and 17 years (17) as illustrated in figure 1.1.

Figure 1.1 Modern drug development pathway timelines (17)



In order to move through the drug development process, a new therapy needs to be successful at each of the steps outlined above. It is estimated that about 10% of new cancer treatments entering phase I trials pass through all the steps required to gain regulatory approval (18). Figure 1.2 illustrates the attrition rate for new drugs as they pass through the clinical trials process.

Figure 1.2 Clinical trial phases and attrition rates (18)

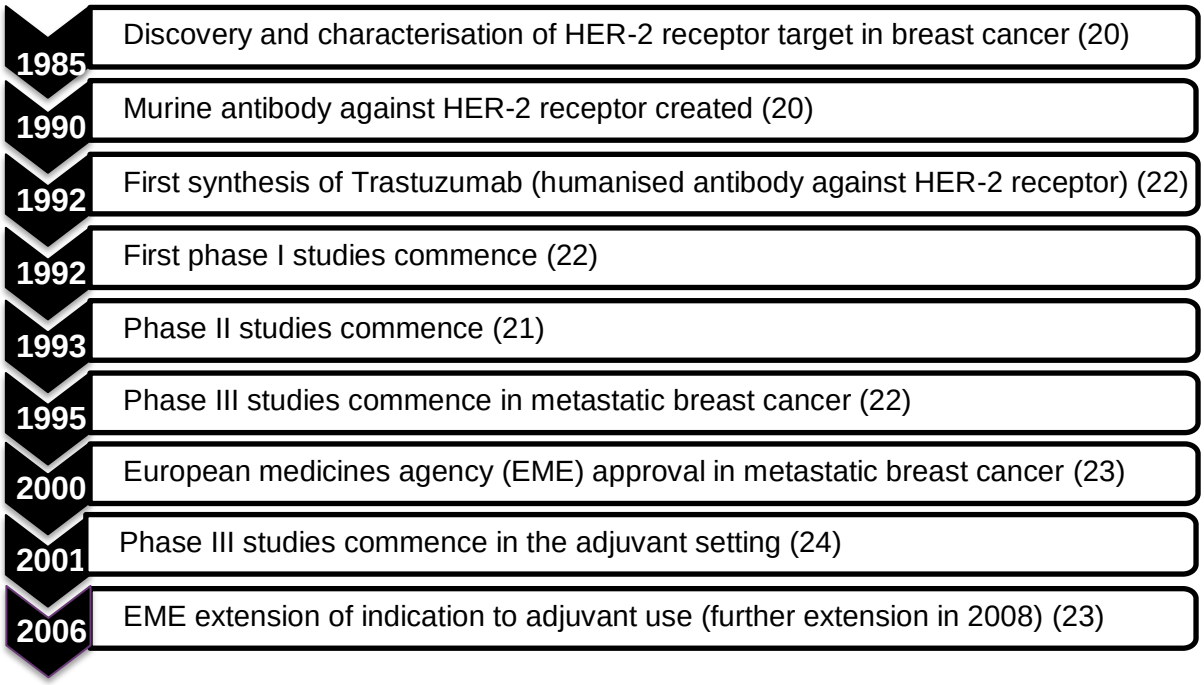


Developing new medicines is not only time consuming, but is also expensive. A recent analysis estimates that it costs 350 million US (United States) dollars for the pharmaceutical industry to discover a new medicine and make it available to patients (19). The high attrition rate means that if you include the costs of all the medicines that fail during this process it is estimated to cost five billion US dollars per new medicine (19).

1.3.1 The drug development process for adjuvant cancer therapies

Phase III trials of adjuvant cancer therapies often investigate clinical outcomes which take a number of years to occur (for example cancer recurrence). This can extend the timelines for drug development, beyond those described in figure 1.1. The time and cost limitations of the modern drug development process can be illustrated by the pathway taken by trastuzumab from discovery to use in the adjuvant setting. Trastuzumab is now part of standard treatment for the adjuvant treatment of high risk, early stage, HER-2 receptor positive breast cancer. In the UK, for a patient to receive standard adjuvant treatment with trastuzumab (administered for 1 year) it currently costs £21,184 GBP (British pounds) for the drug alone (20). It took 14 years from its discovery to gain regulatory approval in the adjuvant setting (21 years from the identification of its target), as illustrated in figure 1.3.

Figure 1.3 Development timeline of trastuzumab (21-25)



To have any significant impact on the global cancer burden in the near future, new cancer treatments not only need to be efficacious but also need to emerge quickly and be universally affordable. To achieve this, therapeutic candidates for phase III trials need to successfully emerge from the drug discovery and development process more rapidly and cost-effectively.

1.4 Repurposing of established medicines as anti-cancer therapies

In the quest for new cancer treatments, a number of established and widely used medications taken for non-oncological indications have been considered for repurposing as anti-cancer therapies on account of evidence to suggest anti-cancer activity. Repurposing is the process of finding new uses beyond the original indication for an existing drug, and has also been referred to as repositioning, reprofiling, redeployment and redirecting. Established medicines are more likely to be overlooked by modern drug discovery, firstly due to lack of commercial interest because of generic availability (as a result of patent expiry), and possibly because the modern drug development process is designed to detect short-term dramatic anti-cancer effects (for example tumour response rates in phase II trials), rather than more gradual long-term anti-cancer effects (possibly through modification of the host environment), which may be associated with established medicines.

Repurposing established medicines as cancer treatments is attractive for a number of reasons:

i. Data on anti-cancer efficacy may already be available from data collected on existing users in other indications.
ii. Safety, toxicity and interaction profiles are already well established through longstanding widespread use.
iii. Existing clinical data (as described by i and ii above) has the potential to fulfil many of the roles of early phase trials making the drug development process quicker and more economical.

iv. Established medicines are normally available in a generic formulation and are therefore inexpensive compared to novel therapies.

v. Most generic medicines are easily available world-wide, particularly in LMIC countries, widening their potential impact on the global burden of cancer.

Outside the field of oncology, there is a tradition of repurposing as a method of drug discovery, with new potential indications often emerging from unanticipated side-effects. Examples include sildenafil, originally used as a treatment for heart disease, now commonly used for erectile dysfunction, and minoxidil, developed as an anti-hypertensive, now used as a treatment for hair loss (26).

Prior to the molecular and genomic era of cancer research, a number of new cancer treatments emerged from medicines in non-cancer indications. Thalidomide was first developed as a sedative and anti-emetic for use during pregnancy, but was found to cause phocomelia (congenital limb abnormality) and withdrawn from the market for that indication as a consequence. After the discovery that thalidomide had anti-angiogenic properties (27), in-vitro studies investigated its potential as an anti-cancer therapy (28), eventually leading to its use as a treatment for multiple myeloma (29).

In the adjuvant cancer setting there have also been some drugs that have been successfully repurposed. Tamoxifen, which was originally discovered as part of a contraceptive research programme (30), and is now commonly used in the treatment of breast cancer, and Bacillus Calmette-Guerin (BCG), first developed as a vaccine against tuberculosis, now also used as an adjuvant treatment for early stage bladder cancer (by direct administration into the bladder) (31). A number of candidate drugs have recently been identified for evaluation in the adjuvant cancer setting, examples include aspirin (32), vitamin D (33), statins (34), cimetidine (35), ketorolac (36), low molecular weight heparin (37), oestrogen patches (38) and metformin (39). A further example is the bisphosphonate, zoledronic acid, used in the prevention and treatment of osteoporosis. A number of phase III trials examining the adjuvant role of zoledronic acid, in breast cancer had conflicting results (40-42), however a recently published

meta-analysis (October 2015) showed significant benefits in breast cancer survival in post-menopausal women, hazard ratio (HR) 0.82, 95% confidence interval (CI) 0.73-0.93) (43). The use of zoledronic acid in this setting is beginning to be acknowledged in clinical guidelines (44), emphasising the value of meta-analysis in repurposing. Bisphosphonates are thought to prevent metastases by modifying the bone microenvironment (45), and provide another example of an adjuvant therapy that works by causing biological changes in the host rather than directly in cancer cells.

1.4.1 Established medicines and the drug development process

The aim of a phase I trial is to investigate safety and tolerability of a drug. For an established medicine, this information is often already available, either through historical clinical trials, or data on existing users, in non-cancer indications. In the cancer setting, phase I cancer trials usually aim to establish a maximum tolerated dose to optimise short-term anti-cancer effect, in-order to treat established malignancy. However, in the adjuvant setting, many therapies are also given long-term as maintenance against micrometastases (46), and in the primary prevention setting the priority is the long-term suppression of neoplastic processes. Consequently, long-term tolerability could be more of a priority in the adjuvant and primary prevention setting. Phase II cancer trials frequently aim to demonstrate efficacy by showing tumour shrinkage usually measured as response rates. In the adjuvant and primary prevention setting, short-term tumour shrinkage may not reflect efficacy (46), and therefore data on cancer incidence and outcomes in those who take an established medicine, long-term, in a non-cancer indication, could provide valuable insights.

Aspirin is an example of an established medicine where there is now a considerable body of evidence underpinning its anti-cancer effect, which is considered sufficient to warrant a number of phase III trials. The evidence providing justification for phase III cancer trials did not emerge from the traditional steps of the drug development process, as illustrated in figure 1.1, but emerged as described in the following section.

1.5 Evidence supporting aspirin as an intervention in phase III trials

Aspirin has been taken by patients for almost 120 years (47), originally prescribed for its analgesic and anti-inflammatory properties, it is now mostly used for the secondary prevention of cardiovascular disease. Aspirin exhibits many of the attributes that make established medicines attractive as potential cancer therapies, including affordability, (it can be bought over the counter at three pence per tablet in the UK (48)), and widespread previous use, with a known toxicity profile.

1.5.1 Aspirin - mechanistic hypothesis and pre-clinical evidence

For a medication to be considered as a cancer therapy, a plausible mechanism of action in the intended treatment setting is required. The mode of action for novel cancer therapies is often known because they are designed to meet a particular mechanistic specification, however for established medicines, efficacy is often discovered before the underlying anti-cancer mechanism, which then needs to be identified through pre-clinical research.

Extensive *in-vitro* and *in-vivo* research into the biological effects of aspirin has already been undertaken as a result of interest in the mechanisms underlying its cardiovascular effects. Aspirin is known to inactivate both isoforms of the enzyme cyclooxygenase (COX) (49), also known as prostaglandin endoperoxide synthetase (PTGS). Many of the downstream mediators of the COX pathways are thought to be involved in the development and spread of malignancy (50), however, a divided daily dose of greater than 2000mg of aspirin would be required to achieve consistent inhibition of COX in tissues (51) because aspirin has a short half-life (approximately 20 minutes) and nucleated cells can resynthesize COX enzymes within a few hours. Aspirin has mostly been used in doses of 75-300 milligrams (mg) once daily over the last 40 years, therefore an indirect mechanism, possibly through modification of the host environment, rather than a direct cytotoxic effect may be more plausible.

Aspirin is known to reduce platelet aggregation at commonly used doses (75-300mg), through inhibition of COX-1 (which is not re-synthesised by the anucleate platelet), which represents another potential mechanism for its anti-cancer effects (51). The first evidence suggesting

aspirin has anti-cancer activity emerged in 1972 from mouse models to suggest that aspirin prevented metastases, possibly through an anti-platelet mechanism (52, 53). This is consistent with more recent evidence showing that platelets are key mediators in the metastatic process (54). Platelets are thought to promote the adhesion of cancer cells to leukocytes and endothelium leading to transmigration (55), and may also act as a barrier between circulating cancer cells and natural killer cells to prevent immune mediated clearance (56). It is also thought that platelets may play a more active role in promoting metastatic spread by active signalling to cancer cells through the TGF-β (transforming growth factor beta) and NF-kappa B (nuclear factor kappa B) pathways resulting in a pro-metastatic phenotype that facilitates tumour cell extravasation and metastasis formation (57).

As well as COX inhibition, a number of additional COX-independent anti-cancer mechanisms have been proposed for aspirin (58). Aspirin is known to directly inhibit activation of NF-kappa B (59), which is thought to play a key role in tumour growth and invasion (60), as well as promoting apoptosis (61), and inhibiting angiogenesis (62). There is also *in-vitro* evidence to suggest that aspirin can directly influence other molecules and pathways involved in the development and growth of cancer, including Tumour Necrosis Factor, B-catenin, polyamine metabolism and the DNA mismatch repair and WNT (wingless-related integration site) signalling (63-65).

1.5.2 Aspirin - observational evidence

Being one of the most commonly used medications in the world, data on cancer incidence and outcomes in users of aspirin for existing indications has emerged. Case-control and cohort studies have been conducted using patient data from sources including hospital electronic health records, cancer registries, prescription databases, prospective surveys and concomitant medication data from clinical trials of other interventions. This has provided some evidence of an anti-cancer effect, and its relation to dose and duration, thus fulfilling many of the requirements of early phase trials. As the mechanisms underlying the anti-cancer effect of

aspirin could differ according to the therapeutic setting, evidence in the primary prevention setting and treatment setting are described separately in the following section.

1.5.2.1 Aspirin - observational evidence in the primary prevention setting

The first observational study to show that regular aspirin use was associated with a decreased risk of developing cancer was published in 1988 (66). Since then there have been well over 100 case-control and cohort studies investigating the use of aspirin and cancer risk (67).

A systematic review and meta-analysis of case-control and cohort studies in 2012 (67) examined the evidence in 12 individual tumour types and found evidence showing that aspirin was associated with a significantly lower risk of developing colorectal, oesophageal, gastric, breast and prostate cancer. There was no benefit seen in lung, pancreatic, endometrial, ovarian, bladder and kidney cancer, however this could be due to insufficient numbers to detect a treatment effect in these tumour types. Table 1.1 provides further details of these results. Findings also suggest that at least five years of aspirin use was required to convey these effects.

Table 1.1 Meta-analysis of observational data on aspirin in the primary prevention setting (67).

Tumour type	Observational studies *(n)*	Diagnoses *(n)*	Relative risk (95% Confidence interval)
Breast cancer	*32*	*52,926*	0.90 (0.85-0.95)
Colorectal cancer	*30*	*37,519*	0.73 (0.67-0.79)
Prostate cancer	*24*	*37,452*	0.90 (0.85-0.98)
Gastric cancer	*13*	*4,519*	0.67 (0.54-0.83)
Oesophageal and gastric cardia adenocarcinoma	*11*	*3,723*	0.64 (0.52-0.78)
Oesophageal squamous cell carcinoma	*11*	*2,193*	0.61 (0.50-0.76)

Since this meta-analysis, a number of large observational studies have provided further supporting data. The Association of American Retired Persons Diet and Health study (AARP) included 301,240 adults aged between 50 and 71 years. An estimated 14% reduction in colorectal cancer incidence was observed with daily aspirin use (HR 0.86, CI 0.79-0.94) during 10 years of follow-up (68). A Danish case-control study of 10,280 colorectal cancer cases and 102,800 controls showed a reduction in the risk of colorectal cancer (odds-ratio (OR) 0.73, CI 0.54-0.99) for those continuously taking aspirin for at least 5 years (69). In a US population based cross-sectional questionnaire based study, (*n=11,657*) regular aspirin use was associated with a significantly lower prevalence of prostate cancer (OR 0.60, CI 0.38-0.94) (70).

1.5.2.2 Aspirin - observational evidence in the treatment setting

Observational studies have also shown improvements in cancer outcomes with aspirin use after a diagnosis of cancer, suggesting efficacy in the adjuvant setting. Table 1.2 summarises the observational studies assessing the effects of aspirin after a cancer diagnosis.

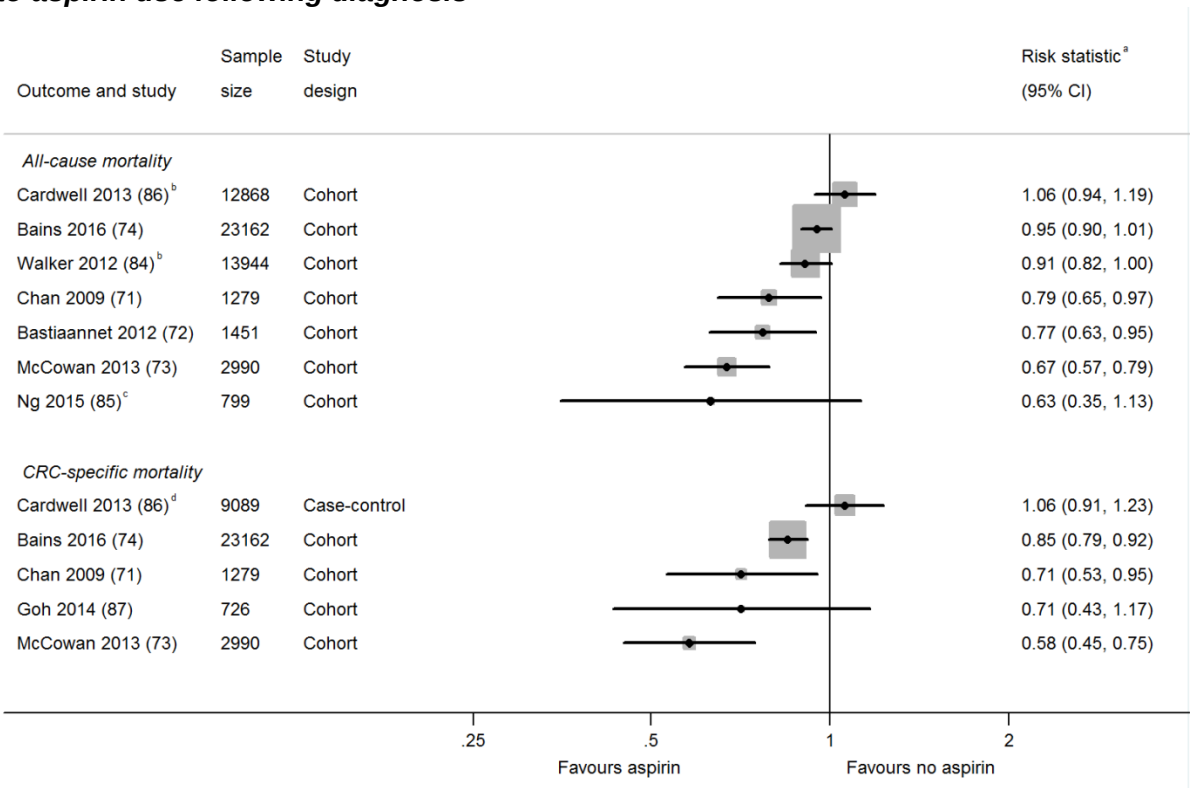
Table 1.2 Summary of key observational data assessing the effects of aspirin after a cancer diagnosis by tumour type

Cancer type	Study (sample size)	Treatment effect with aspirin (except where indicated)
Colorectal cancer	Nurses' Health and HPFS (71) *(n=1,279)*	Colorectal CSS- HR 0.71, CI 0.53-0.95 OS- HR 0.79, CI 0.65-0.97
	Eindhoven Cancer Registry, Holland (72) *(n=4,481)*	OS- RR 0.65 (0.50-0.84)
	Tayside, Scotland (73) *(n=2990)*	Colorectal CSS- HR 0.58, CI 0.45-0.75 OS- HR 0.67, CI 0.57-0.79
	Cancer registry of Norway (74) *(n=23,162)*	Colorectal CSS- HR 0.85, CI 0.79-0.92 OS- HR= 0.95, CI 0.90-1.01
Breast cancer	Nurses' Health Study (75) *(n=4164)*	Breast CSS- RR 0.36, CI 0.24-0.65 OS RR- 0.54, CI 0.41-0.70 (daily users)
	Tayside, Scotland (76) *(n=4,627)*	breast CSS- HR 0.42 (0.31-0.55) OS HR 0.53 (0.45-0.63)
Prostate cancer	Philadelphia, US (77) (post-radiotherapy) *(n=2,051)*	Interval to biochemical failure in aspirin non-users vs users- OR 2.05, CI 1.33-3.17
	CaPSURE study (78) (post-radical therapy)*(n=5,995)*	Prostate CSS- HR 0.43, CI 0.21-0.87
	Cancer prevention study-II (79) *(n=7,118)*	Prostate CSS- HR 0.60, CI 0.37-0.97 (high risk non-metastatic)
Gastro-oesophageal cancer	Henbei University, China (80) *(n=1,600)*	5-year OS aspirin 51.2%, placebo 41%, no tablet 42.3%. No HR/RR presented.
	Eindhoven Cancer Registry, Holland (81) *(n=560)*	OS- RR 0.42, CI 0.30-0.57
Non-small cell lung cancer	Liverpool thoracic surgical database (82) *(n=1,765)*	OS- HR 0.84, *p=0.05*
Head and neck cancer	Primary care electronic records, Scotland (83) *(n=1,195)*	OS- HR 0.56, CI 0.44-0.71
CSS=cancer specific survival, OS=overall survival, RR=relative risk		

The largest body of observational evidence in the treatment setting pertains to colorectal cancer. In the Nurses' Health Study, and Health Professionals Follow-up Study (HPFS), two large, prospective studies, aspirin use after a diagnosis of colorectal cancer was associated with a significant reduction in colorectal cancer deaths (HR 0.71, CI 0.53-0.95), as well as overall mortality, with larger effects observed for daily users (71). Similar improvements in colorectal cancer outcomes from aspirin use after diagnosis have been seen in population databases including the Eindhoven Cancer Registry, Holland (OS RR 0.65, CI 0.50-0.84) (72), Tayside and Fife, Scotland (OS HR 0.67, CI 0.57-0.79) (73), Cancer Registry of Norway (colorectal cancer specific survival (CSS) HR 0.85, CI 0.79-0.92) (74), and the UK General Practice Research Database (All-cause mortality HR 0.83, CI 0.75-0.92 at 5 years) (84). Data

is also available in clinical trials designed to examine other interventions. The CALGB 89803 trial (which compared two different adjuvant chemotherapy regimens in patients with stage III colon cancer), collected concomitant medication data on aspirin use. A post-hoc analysis of the trial found a trend towards improved OS (HR 0.63, CI 0.35-1.12) and disease-free survival (DFS) (HR 0.68, CI 0.42-1.11) in those patients using aspirin both during and after chemotherapy (85). Other population databases have not shown improvements, including the UK clinical practice research data link (all-cause mortality OR 1.06, CI 0.94-1.19, CSS OR 1.06, CI 0.91-1.23) (86) and the Singapore Hospitals dataset (CSS HR 0.71, CI 0.43-1.17) (87) Figure 1.4 summarises the results of the observational studies investigating the effect of aspirin use after a colorectal cancer diagnosis.

Figure 1.4 Observational studies investigating colorectal cancer outcomes according to aspirin use following diagnosis



Footnotes: No summary statistic is presented given the high heterogeneity of studies. Multivariate (adjusted) statistics are presented in all cases. a: All risk statistics are hazard ratios except the study by Cardwell 2013 which is an odds-ratios, and Bastiaannet 2012 which is a rate-ratio. b: Studies have an overlapping population, both using UK General Practice Data. c: Cohort taken from randomised chemotherapy trial and is limited to colon cancer. d: Cohort only matched for CRC-specific mortality analysis.

In breast cancer, results from the Nurses' Health Study indicated that 6-7 days of aspirin use per week following a diagnosis of breast cancer may improve breast CSS (RR 0.36, CI 0.24-0.54), and the risk of distant recurrence (RR 0.57, CI 0.39-0.82) (75). Significant improvements have also been seen with post-diagnostic aspirin use in a population cohort study based in Tayside, Scotland (OS HR 0.53, CI 0.45-0.63, and Breast CSS HR 0.42, CI 0.31-0.55) (76).

There is also observational evidence to support aspirin use after a diagnosis of prostate cancer. A large longitudinal observational study of men with prostate cancer (CaPSURE study) found that in men who had radical surgery or radiotherapy (*n=5,955*), aspirin was associated with a significant reduction in prostate CSS (HR 0.43, CI 0.21-0.87) (78). Additionally, an analysis of hospital data from Philadelphia showed that aspirin non-use after radiation therapy for prostate cancer is associated with early biochemical failure (OR 2.05, CI 1.33-3.17) (77). Most recently, an analysis of a large prospective cohort from the Cancer Prevention Study *(n=7,118)* found that post-diagnostic aspirin use in men with high risk non-metastatic prostate cancer resulted in a reduction in prostate CSS (HR 0.60, CI 0.37-0.97) (79).

There is also evidence of improvement in cancer outcomes for post-diagnostic aspirin use in other tumour types including gastro-oesophageal cancer (80, 81), non-small cell lung cancer (88), and upper aerodigestive tract cancers (89).

1.5.3 Aspirin - randomised evidence

Whilst observational data can be adjusted for known confounding factors, the influence of unknown confounding factors cannot be eliminated. Randomised data (on an established medicine in other indications) reduces the chance of confounding and provides the most robust evaluation outside a phase III trial specifically designed to answer a question. Such evidence in the primary prevention and treatment setting are described separately.

1.5.3.1 Aspirin - randomised evidence supporting use in the primary prevention setting

Meta-analyses of individual participant data on cancer incidence in randomised trials designed to investigate the effect of aspirin on vascular disease provide the strongest evidence for aspirin in the primary prevention setting. In a meta-analysis of four large vascular randomised controlled trials with aspirin as an intervention (*n=14,033*) a 24% relative reduction in the risk of developing colorectal cancer was identified (HR 0.76, CI 0.63–0.94), improving to 32% if taken for greater than five years (HR 0.68, CI 0.54–0.87) (90). A meta-analysis of six vascular randomised controlled trials with aspirin as an intervention (*n=35,535*) showed aspirin reduced the overall incidence of cancer from three years onwards (OR 0.76, CI 0.66-0.88) however numbers were insufficient for analysis according to individual cancer types (91). Since these meta-analyses were conducted, long-term follow-up from the Women's Health Study (WHS), a randomised placebo-controlled trial designed to assess the effects of aspirin (100mg on alternate days) in the primary prevention of cardiovascular disease, has also showed that allocation to aspirin reduced the incidence of colorectal cancer by 20% (HR 0.80, CI 0.67–0.97) after 18 years of follow-up, with survival curves suggesting the benefit emerged after 10 years, suggesting a delayed effect (92).

Phase III trials investigating the role of aspirin for cancer prevention in the general population are likely to be challenging due to the low event rate and the associated length of follow-up and number of participants required, however randomised trials are more feasible in groups that are at higher risk of developing a particular type of cancer. For example, in Lynch syndrome (Hereditary Non-Polyposis Colorectal Cancer), the most common inherited colorectal cancer syndrome, the CaPP2 (Cancer Prevention Project 2) trial randomly allocated patients with Lynch Syndrome to 600mg aspirin daily or placebo and found a reduction in colorectal cancer incidence in those that remained on aspirin for more than two years (HR 0.41, CI 0.19-0.86), again suggesting a delayed effect (93). Another familial colorectal cancer syndrome, Familial Adenomatous Polyposis (FAP), has also been the subject of a randomised controlled trial. The CaPP1 trial randomised patients with FAP (prior to preventive surgery), in

a 2x2 factorial design, to 600mg daily aspirin, resistant starch, or placebo. They found a trend towards reduced polyp load in aspirin users, however this did not reach statistical significance (relative risk 0.77, CI 0.54-1.10) (94). The median duration of aspirin use was only 17 months and it is plausible that a treatment effect may have emerged with longer exposure.

1.5.3.2 Aspirin - randomised evidence supporting use in the treatment setting

Meta-analyses of data on cancer outcomes in randomised trials designed to investigate the effect of aspirin on vascular disease have also provided potential evidence in the cancer treatment setting.

A meta-analysis of individual participant data from seven trials (*n=23,535*) showed a reduction in deaths from all cancers after five years of follow up (HR 0.66, CI 0.5-0.87) and at 20 years of follow-up (HR 0.80, CI 0.72-0.88). The effect was largest for adenocarcinomas (HR 0.53, CI 0.35-0.81) and for gastro-intestinal cancers (HR 0.65, CI 0.54-0.78). For those aged over 65 years and over, absolute reduction in 20 year risk of cancer death was 7% (95).

A further meta-analysis of individual participant data from five trials (*n=17,285*) revealed a reduction in the risk of having metastases when an adenocarcinoma is diagnosed (HR 0.69, CI 0.50-0.95) and of subsequently developing them during follow-up when not present at diagnosis (HR 0.45, CI 0.28-0.72) (96), which is supportive of efficacy as a treatment for cancer, particularly in the adjuvant setting. Analyses for individual cancers showed some evidence of improvements in mortality in colorectal (HR 0.27, CI 0.11-0.66), breast (HR 0.16, CI 0.02-1.19) and prostate cancer (HR 0.34, CI 0.12-0.99) (96) however these results are based on very small numbers and therefore need to be interpreted with caution.

1.5.4 Aspirin - existing safety data

A major advantage of an established medicine is the pre-existence of an abundance of safety data which can be used to fulfil the safety role of early phase trials, and often provide more detailed and accurate information, because an established medicine has been widely used for a long period of time allowing sufficient efficacy and safety data to accumulate.

Regulatory agencies including the Medicines and Healthcare products Regulatory Agency (MHRA), EMA and US Food and Drug Administration (FDA) already provide extensive safety information on aspirin from existing indications and list the main contraindications to aspirin use as a history of active or recurrent peptic ulceration, active gastrointestinal bleeding, previous intracranial haemorrhage, a haemorrhagic diathesis or a coagulation disorder (97). Co-administration of other NSAIDs (Non-steroidal anti-inflammatory drug), anti-coagulants or corticosteroids is known to increase the risk of adverse effects. The most common concern in relation to aspirin use is the risk of bleeding. Due to the large number of clinical trials in other indications, extensive safety data has accumulated from clinical trial adverse event reporting allowing a number of meta-analyses of existing safety data. A meta-analysis of data from six cardiovascular primary prevention randomised controlled trials, (*n=95,000*) found that aspirin increased the risk of serious bleeding (excluding intracranial haemorrhage) by 0.04 % per year (from 6.6 events per year in 10,000 individuals to 10.2 events) and intracranial haemorrhage by less than 0.01 % per year (from 2.7 events in 10,000 individuals treated for a year in the control groups to 3.5 events in the aspirin groups), HR 1.39, CI 1.08–1.78) (98). The meta-analysis also revealed that elevated mean blood pressure is associated with an increased risk of intracranial haemorrhage (rate ratio 2.18, CI 1.65–2.87 for every 20mmHg elevation) (99). Systematic review has been used to examine risk factors for bleeding and age has been shown to be a key predictor with the finding that the risk of major bleeding increases between three- and four-fold between the ages of 50–54 and 70–74 years (98). Long-term and widespread use of aspirin has allowed enough data to accumulate to rarer side-effects of aspirin including an increased risk of macular degeneration (100) and tinnitus (97), which are unlikely to have been identified by phase I and II trials due to the association with long-term exposure. This safety information can be used to design eligibility criteria for phase III trials to protect those at the highest risk of aspirin related toxicity.

1.5.5 Aspirin - risk benefit considerations

Existing data from aspirin use in other indications might also be used to identify sub-populations who stand to benefit most, and those who do not benefit, influencing subsequent

clinical trial design, for example, observational data has suggested that tumour PIK3CA (Phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit alpha) mutation status, expression of COX-2 (71) and Human Leukocyte Antigen (HLA) class-I (101), along with certain germline polymorphisms, might all help to identify individuals with colorectal cancer who stand to gain most from aspirin use after diagnosis. PIK3CA mutation status (affecting approximately 15% of those with colorectal cancer (101-103)) has been implicated as a biomarker of benefit in both a large cohort study (102) and in a small ad hoc analysis of the VICTOR trial, where rofecoxib (a COX-2 inhibitor) was being evaluated after colorectal cancer resection but the trial was closed early when rofecoxib was withdrawn from the market due to concerns about cardiovascular toxicity (103). Data on PIK3CA mutation status as a potential predictive biomarker is encouraging, but comes from small numbers of participants and findings are inconsistent, which are summarised in table 1.3.

Table 1.3 Studies examining PIK3CA mutation status, aspirin use and colorectal cancer outcomes

Study	**PIK3CA mutation (%)**	**PIK3CA Mutant**					**PIK3CA Wild-Type**				
		No aspirin	Aspirin	Outcome	HR	95% CI p value	No aspirin	Aspirin	Outcome	HR	95% CI p value
NHS & HPFS (102)	16.7%	95	66	OS	0.54	0.31-0.94 p=0.01	466	337	OS	0.94	0.75-1.17 p=0.96
				CSS	0.18	0.06-0.61 P<0.001			CSS	0.96	0.69-1.32 p=0.76
VICTOR Trial (103)	11.6%	90	14	OS	0.29	0.04-2.33 P=0.19	681	111	OS	0.95	0.56-1.61 p=0.26
				CSS	0.11	0.001-0.83 p=0.027			CSS	0.94	0.59-1.49 p=0.79
MCS & RMH (104)	12.4%	136	49	OS	0.96	0.58-1.57 p=0.86	Study of PIK3CA mutated tumours only				
				CSS	0.60	0.34-1.16 p=0.14					
ECR (101)**	15.8%	73	27	OS	0.73*	0.33-1.63 p=0.4	348	147	OS	0.55	0.40-0.75 P<0.001

Multivariate (adjusted) statistics are presented in all cases. *=rate ratio, **colon cancer only, OS=overall survival, CSS=colorectal cancer-specific survival, RFS=recurrence-free survival, NHS= Nurses' Health Study, HPFS=Health Professionals Follow-up Study, MCS=Moffitt Cancer Centre, RMH=Royal Melbourne Hospital, ECR=Eindhoven Cancer Registry.

Another potential predictive biomarker is HLA class-I antigen expression. It has been hypothesised that aspirin, through its anti-platelet effects, could expose circulating tumour cells to immune-mediated destruction by natural killer cells (105) and that this effect would be

restricted to tumours with low or absent HLA class I expression. Analyses of a randomly selection of colon tumour samples (*n=999*) from the Eindhoven Cancer Registry found that the benefit in OS from aspirin therapy was largely restricted to tumours expressing HLA class-I antigens (OS risk ratio 0.53, CI 0.38-0.74), and benefit was not seen in those who had lost expression (OS risk ratio 1.03; CI 0.66-1.61) (101). This interesting observation, contrary to the original study hypothesis, requires validation. HLA class-I expression is seen in about a third of colorectal tumours and so could identify a sizeable group who might benefit from aspirin after a colorectal cancer diagnosis.

Observational data can also be used to identify individuals who might be at increased risk of aspirin related toxicity. A history of gastro-oesophageal reflux disease or dyspepsia prior to starting aspirin has been shown to be strongly predictive of upper gastrointestinal symptoms on aspirin (OR 17.6, CI 11.52-26.88) (106) which is useful to guide eligibility criteria in phase III trials. Helicobacter pylori infection has been proposed to be a marker of increased risk of developing dyspepsia and a bleeding gastrointestinal ulcer with aspirin (98), however most data supporting this association relates to non-aspirin NSAID (non-steroidal anti-inflammatory drug) use, therefore further data is needed to confirm a relationship with aspirin (107). The HEAT trial (Helicobacter Eradication Aspirin Trial) (ISRCTN10134725), examining helicobacter pylori eradication to prevent ulcer-related bleeding and dyspepsia in aspirin users is ongoing.

Genome-wide association studies can identify genetic variants that are associated with developing side-effects. These studies have identified certain genetic polymorphisms, proposed as potential biomarkers for NSAID induced gastrointestinal ulceration and bleeding (including aspirin). A study in a Japanese population (*n=480*) found that a functional single nucleotide polymorphism (SNP) of the COX-1 gene (rs1330344) were significantly associated with gastric ulceration (OR 5.80, CI 1.59–21.1) (108). Additionally, two polymorphisms of Cytochrome P450 2C9 (an enzyme responsible for the metabolism of aspirin) have been found to be significantly associated with bleeding risk in NSAID users (108, 109).

1.5.6 Dose considerations

The mechanism of action underlying the anti-cancer effects of aspirin is unproven and it is plausible that there may be different mechanisms that act to prevent metastases and the development of the primary tumour which could be dose dependent. Meta-analyses of cancer outcomes in cardiovascular trials where aspirin is an intervention have evaluated low dose aspirin (75-100mg daily) and shown beneficial effects on cancer outcomes (91, 95). However, the Nurses' Health Study and Health Professionals Follow-Up Study suggest that higher doses of aspirin (greater than 6 x 325mg tablets per week) have a greater effect than lower doses (0.5-5 x 325mg tablets per week) on both colorectal cancer and overall mortality (75). The dose of aspirin needs to be balanced against the risks of aspirin toxicity which is thought to increase with both age and aspirin dose (98). Another key consideration is the acceptability of prescribing different doses of aspirin. A recent survey of General Practitioner (GP) attitudes towards prescribing aspirin for carriers of Lynch syndrome found that 91.3%, 81.8% and 62.3% of GPs were comfortable prescribing 100mg, 300mg and 600mg of daily aspirin respectively (110). Further research is needed to investigate the relationship between dose and the potential anti-cancer effects of aspirin.

The evidence supporting a phase III trial with aspirin as an adjuvant therapy for cancer is significant, and includes data to suggest how the risk/benefit profile might be optimised, however a phase III trial in this setting presents a number of challenges.

1.6 Challenges to evaluating aspirin in an adjuvant phase III cancer trial

There are a number of methodological and practical challenges presented by an adjuvant phase III cancer trial with aspirin as an intervention, many of which may apply to other trials of generic medicines. The main challenges are summarised below:

1. Lack of commercial interest in generic interventions from pharmaceutical companies mean that all financial support for the trial is required from governmental, academic and charity sources, and significant cost-efficiencies need to be incorporated into trial design and conduct.
2. Operational procedures including drug packaging, labelling, blinding, distribution, supply management and unblinding provision need to be provided without industry support.
3. Maintained long-term adherence is likely to be required for the anti-cancer effects of aspirin to emerge (90, 92, 93), posing challenges including length of follow-up, maintained adherence, and extended exposure increasing the risk of developing toxicity.
4. Aspirin is available for purchase over the counter without the need for a prescription, and as such there is the potential for control arm contamination.
5. Large numbers of participants are required to achieve adequate power to detect a long-term treatment effect in individual tumour types.

1.7 Overview of the Add-Aspirin trial

Add-Aspirin is a randomised phase III trial which aims to assess whether regular aspirin use after standard potentially curative primary therapy can prevent recurrence and prolong survival in individuals with common early stage solid tumours. The trial employs a number of methodological approaches to meet the challenges described in the previous section. Traditionally, clinical trials have been designed to evaluate a single intervention in a homogenous group of patients. A basket trial design investigates a single intervention in

different types of patients, whereas a platform trial design uses a single master protocol in which multiple interventions (or doses) are evaluated simultaneously (111). The design for the Add-Aspirin trial incorporates a single protocol across four tumour types, and investigates two doses of aspirin (compared to placebo) both across and within each tumour type, and as such, incorporates elements of both platform and basket trial design, but the term “basket design” will be used from this point onwards. The tumour types selected for the trial were based on the following factors:

(i) The strength of the evidence relating to potential benefit of aspirin (section 1.5).

(ii) The size of the potential impact (numbers of cases diagnosed at an early stage).

(iii) Feasibility of recruitment.

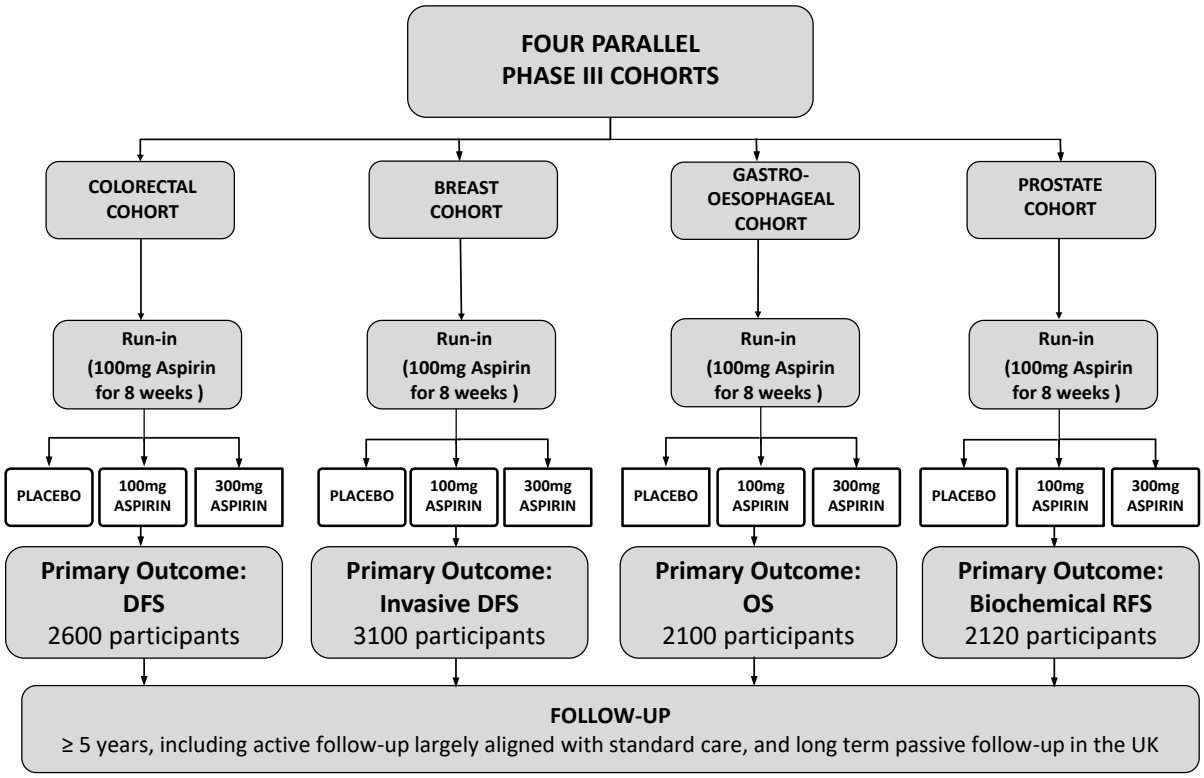
Four tumour types were selected, (breast, colorectal, gastro-oesophageal and prostate tumours) which, together, accounts for approximately one third of all cancer cases and cancer deaths (1).

There is uncertainty surrounding the optimal aspirin dose required to achieve anti-cancer effects as described in section 1.5.6. Given this, the length of time needed to for sufficient events to occur, evaluating only one dose would not allow dose effect to be investigated, potentially wasting valuable research time and resources. To answer this question, two doses of aspirin were selected (100mg daily and 300mg daily, compared with matched placebo) to be investigated both within, and across cohorts. The dose of aspirin in its lowest formulation varies between countries, with 75mg, 81mg and 100mg commonly used in the UK, US and Europe respectively. A 100mg dose was selected over 75mg and 81mg because it could be provided by Bayer AG. These small differences in dose are not anticipated to be clinically significant.

Uncommonly for a cancer trial, the design includes a run-in period where all potential participants are asked to take aspirin 100mg daily for 8 weeks to assess adherence and

tolerance, prior to being selected for the randomised phase of the trial. Figure 1.5 shows a summary schema of the trial design.

Figure 1.5 Add-Aspirin trial schema



DFS = disease free survival, OS = overall survival, RFS = recurrence free survival.

Funding for the trial has been secured from charity and governmental sources. All doses of the blinded active intervention and matched placebo have been donated by industry (Bayer AG), but not the packaging, labelling, blinding or distribution, which therefore represented a major operational and funding challenge. These challenges were met by outsourcing some of these processes and development of an in-house drug supply management system to track stock levels at sites and automatically trigger re-orders based on projected demand.

To ensure recruitment is sufficient to detect trial outcomes in individual tumour types, it is necessary for the trial to open in most centres treating cancer across the UK as well as additional centres in India, where patients will be recruited to the breast and gastro-oesophageal cohorts. The design for the Add-Aspirin trial also has the capacity to add

additional interventions alongside aspirin, presenting the opportunity to evaluate other established medicines where sufficient supporting evidence exists.

1.8 Metformin as an adjuvant anti-cancer therapy

There is a growing body of evidence to suggest that metformin, commonly used to treat type II diabetes mellitus (DM), has anti-cancer activity. Like aspirin, metformin also has many of the qualities that make established medicines in non-cancer indications, attractive as anti-cancer treatments. Metformin is available generically worldwide, and is generally well tolerated with long term use. It is also low in cost, in the UK it is available to the National Health Service (NHS) at two pence per tablet (500mg) (112).

There have been a number of calls for systematic reviews and meta-analyses to be conducted as part of the scientific justification, and to inform the design, of new clinical trials (113, 114), and these are likely to provide the most objective evaluation of a drug candidate, and represent the corner stone of the evidence that supports aspirin as an intervention in phase III trials. The utility of meta-analysis to detect more subtle, but important treatment effects of a repurposed cancer agent has also been demonstrated with bisphosphonates as described in section 1.4 (16). The ability to perform a meaningful systematic review and meta-analysis relies on the availability of sufficient homogenous data. A robust evaluation of observational data needs to be conducted in a population and disease setting that most closely reflects that which is proposed for repurposing, therefore data is best analysed separately for the primary prevention, adjuvant and advanced setting. Many anti-cancer effects are also tumour specific, and therefore it is important to examine observational data in the context of individual tumour types rather than across them. To-date, evidence to support the use of metformin as an adjuvant therapy in individual cancer types has not been presented.

1.9 Aims and objectives of thesis

I joined the Add-Aspirin trial team in September 2013, at which point the funding for the trial was in the process of being secured, and the protocol was in development. I led the writing for the clinical aspects of the protocol (appendix A) and the design for the trial procedures investigated by this thesis including; the method for assessing adherence and tolerance in the trial run-in period; the timing of trial registration across tumour types; and the management of toxicity and guidance around concomitant medication. I also designed the trial case report forms (CRF) (available in appendix D) and coordinated applications for regulatory approval, which was granted by Research Ethics Committee on the 4th June 2014 and the MHRA on 25th November 2014. I led clinical site training and managed the clinical aspects of the trial on a day to day basis, with support from the Chief Investigator. I was the first point of contact for clinical and eligibility queries and clinical review of adverse events between the trial opening and September 2016.

During my research fellowship (September 2013—September 2016), I formulated four research questions to improve the conduct of this and similar trials. Each of the following research questions is addressed in the subsequent chapters of this thesis.

1) What are the advantages and challenges of delivering the Add-Aspirin trial design at site, and how is the trial design perceived?
2) How accurate are the anticipated recruitment rates provided by sites for each tumour type, and how might this be improved?
3) Are patients entering the trial as expected and are any adjustments to the run-in period design necessary?
4) Is there sufficient evidence to undertake a phase III trial of metformin in the adjuvant setting, or add metformin as an intervention to the Add-Aspirin trial?

Chapter 2. An evaluation of the set-up processes and delivery of the Add-Aspirin trial at sites, and the acceptability of the design

2.1 Introduction

Some aspects of the Add-Aspirin trial design may present both advantages and challenges to the conduct of the trial at NHS research sites. An evaluation was undertaken to investigate the benefits, obstacles, and perceptions of the trial design in the first year of the trial.

The basket design of Add-Aspirin aims to address multiple questions in a single study, including dose, toxicity, cancer and non-cancer benefits, both within and across tumour types, and is the first major UK phase III trial in cancer using this design. Keeping all four cohorts within a single protocol aims to keep the management of each cohort as comparable as possible (with the exception of some tumour type specific procedures) and save many years of research time. This also allows a combined analysis across cohorts, providing a much larger sample size to study small effects, which are of interest since aspirin is low cost and easy to administer. These include cross cohort secondary analyses of toxicity, cardiovascular, cognitive, and other health benefits, thus increasing the overall potential impact of the trial. A combined analysis also allows a co-primary outcome measure of overall survival to be investigated across tumour types. To deliver these benefits, the trial needed to be successfully set-up and recruit to multiple tumour cohorts at the majority of UK NHS research sites that treat cancer. A trial conduct survey was designed to examine the experience at site of trial set-up and recruitment, both overall, and to individual tumour cohorts, with the intention of improving the conduct of this and other similar trials.

The basket design also has the potential to provide economies of scale both centrally and at site, including site set-up, regulatory approval, central staffing, coordination, oversight and data management. The resulting potential cost efficiencies improve the financial viability of the trial given the lack of industry support, and provide value for money for charitable and

governmental funders. The second objective of the trial conduct survey is to examine whether there were efficiencies in opening and running the trial at site through the use of a basket design.

The trial design also includes an active run-in period where all participants take 100mg aspirin (one tablet per day) in an open-label manner for a period of approximately eight weeks prior to randomisation. This approach aims to identify those individuals who are unlikely to be able to tolerate aspirin, as well as those who are unlikely to be able to adhere to the protocol treatment schedule. The background and methodology for the run-in period are described in detail in chapter four. The third objective of the trial conduct survey is to establish the views of site staff on the effectiveness of the run-in period in selecting a population with enhanced adherence and tolerance to trial treatment.

Allowing participants who are already taking part in other trials to co-enrol in the Add-Aspirin trial provides a greater pool of potential participants and maximises the opportunities for patients to participate in trials. Trial co-enrolment also allows an assessment of the efficacy of aspirin in participants who have received both current and potentially future standard of care treatment, helping to ensure the future relevance of both trials. Whilst co-enrolment has a number of potential benefits, it is not routinely adopted by the majority of trials. The fourth objective of the trial conduct survey is to determine the attitudes of NHS research site staff towards trial co-enrolment. The objectives of this chapter are summarised below.

2.1.1 Objectives

1. Examine the challenges experienced at site in setting up and recruiting to the Add-Aspirin trial overall, and to individual tumour cohorts.
2. Establish whether there are efficiencies in the conduct of the Add-Aspirin trial at site through the use of a basket design.
3. Establish the views of site staff on the effectiveness of the run-in period in selecting a population with enhanced adherence and tolerance to trial treatment.
4. Determine the attitudes of NHS research sites towards trial co-enrolment.

2.2 Methods

2.2.1 Setting up the Add-Aspirin trial

After gaining full regulatory approval, the first Add-Aspirin trial site training was conducted on the 5th May 2015. To allow sites to open promptly, weekly online training sessions were conducted via a video link, and a training video was provided on the trial website (training slides and the training video is available at www.addaspirintrial.org). To open, sites had to have a tumour-specific lead investigator for each recruiting cohort, and an overall Principal Investigator (PI), selected from the tumour specific lead investigators, who has responsibility for trial oversight and regulatory approval. This approach was designed and implemented to provide the individual tumour type expertise required to manage and provide oversite for each cohort effectively, whilst maintaining efficiency in gaining regulatory approval with one overall PI.

2.2.2 Evaluation of site opening

Data on the opening of individual cohorts at UK NHS research sites was collected between the 8th October 2015 and the 1st November 2016, and includes the first 12 full months of the trial.

2.2.3 Trial conduct survey

A survey was designed to meet the aims and objectives described in section 2.1.1 An email containing a web link to an online survey was sent to the main points of contact of 184 UK NHS research sites (237 individuals), where the trial was open, or where an interest in opening had been previously expressed. Completion of the survey was voluntary and instructions were given to seek input from other colleagues at their site as necessary. The survey was sent to sites on the 26th August 2016, with responses accepted until the 1st November 2016.

The survey was created using an on-line survey platform (www.surveymonkey.co.uk), and consisted of 30 questions overall, with some questions automatically skipped depending on previous responses to ensure applicability. Question types included single answer multiple choice (n=5), multi-answer multiple choice (n=10), rating scale (n=9), open ended free-text

(n=5), and ranking of options (n=1) style questions. Multi-answer multiple choice questions presented a list of pre-set options, created based on common themes that emerged from communication with sites during the set-up of the trial, and also included an option to provide other answers using free-text which were not available as existing options. Free-text responses were analysed using a qualitative approach and coded according to common themes. Where free-text responses matched the theme of a pre-set option that was already selected, it was excluded from the analysis to avoid duplication, however if a matching pre-set option had not already been selected, then it was counted with that pre-set option.

Rating scale questions used a five-point Likert scale which allows respondents to specify their level of agreement or disagreement on a symmetric agree-disagree scale for a series of statements, with a scale from one for strongly agree, through to five for strongly disagree. The results were interpreted by calculating a median response for each question to capture the intensity of opinions for a given question, rather than the mean, because the data is categorical and therefore unlikely to be normally distributed (115).

Ranking questions were used to allow respondents to compare options by placing them in an order of preference. A mean ranking was calculated for each option to determine the preferred choice by weighting each response according to the ranking attributed (with weighting of one for first ranked response through to a weighting of five for fifth ranked response) and calculating the mean. Where “not given as a reason” was selected, these we not factored into mean rankings. This approach was used so that the options could be compared in a way which reflects all the ranking given by respondents for each option, rather than just comparing the most frequent response (mode), which could be misrepresentative.

Trial conduct survey responses were linked to individual NHS research sites through a site code to determine the number of separate sites that had responded and their participation status, but all other analyses were blinded to site, and no other identifiers were collected for individual respondents. Statistical analysis for numerical data was mostly descriptive with

percentages being reported. A copy of the trial conduct survey is available in appendix B. All data were analysed using Microsoft Excel.

2.3 Results

2.3.1 Site opening at one year

The trial recruited its first participant on the 8th October 2015 and, in the first year of opening (by 1st November 2016), 155 sites had opened to recruitment, meaning that 66.7% of NHS trusts and boards in the UK had at least one recruiting site (NHS trusts in England, Health Boards in Scotland and Wales, and Social Care Trusts in Northern Ireland). 81.3% of these sites were able to open in at least three of the four tumour cohorts. For trusts in England, the National Institute for Health Research (NIHR) Clinical Trials Network has set a 30 day target from NHS permission to first subject recruited as a higher level objective (116). The median time from a site opening to recruiting its first participant (censoring sites yet to recruit) was 43 days (IQR 27-71 days), exceeding the NIHR higher level objective. Table 2.1 shows the number of sites open to the trial, broken down by each devolved nation. Table 2.2 shows the number of individual cohorts open at sites. Table 2.3 summarises site opening at one year by cohort.

Table 2.1 NHS trusts and boards* open to the Add-Aspirin trial at one year

	NHS sites open (n)	NHS Trusts and boards* with an open site (n)	Total number of NHS trusts or boards* in the UK	**Percentage of all NHS trusts and boards* open**
England	133	106	154	**68.9%**
Scotland	10	7	14	**50%**
Wales	12	7	7	**100%**
Ireland	0	0	5	**0%**
UK total	155	120	180	**66.7%**

**NHS trusts and boards (NHS trusts in England, Health Boards in Scotland and Wales, and Social Care Trusts in Northern Ireland).*

Table 2.2 Add-Aspirin trial cohort opening after one year, by cohort

Cohort	Sites with cohort open (%)	Open sites with at least one participant (%)
Breast	144 (92.9%)	114 (79.2%)
Colorectal	143 (92.3%)	101 (70.65%)
Gastro-oesophageal	114 (73.5%)	31 (27.2%)
Prostate	128 (82.6%)	68 (53.1%)
Total	**155**	**127**

Table 2.3 Add-Aspirin cohorts open at individual sites after one year of being open

Number of open cohorts	Number of sites (Total 155)	Percentage of sites
Four cohorts	97	62.6%
Three cohorts	29	18.7%
Two cohorts	17	11.0%
One cohort	12	7.7%

2.3.2 Data return from the trial conduct survey

Between the 26th August 2016, and the 1st November 2016, 54.0% (128/237) of the individuals approached completed the trial conduct survey. Responses were available from 58.0% (90/155) of open sites, and 39% (16/41) of sites yet to open (which had expressed interest). The data therefore provides a broad reflection of UK site experience of the Add-Aspirin trial. Surveys were mostly completed by clinical trial nurses or sisters (60.9%), or by clinical trial practitioners or coordinators (28.1%), suggesting that respondents were directly involved with delivering the trial at site. A breakdown of the job title of those completing the trial conduct survey is provided in table 2.4. No data is available on the job title of non-responders.

Table 2.4 Job title of trial conduct survey respondents

Job title	Respondents (%)	Job title	Respondents (%)
Clinical trials nurse	53 (41.4%)	Clinical trials manager	2 (1.6%)
Clinical trials sister	25 (19.5%)	Clinical trials administrator	2 (1.6%)
Clinical trials coordinator	19 (14.8%)	Research and development lead	2 (1.6%)
Clinical trials practitioner	17 (13.3%)	Clinical trials assistant	2 (1.6%)
Clinical trials officer	3 (2.3%)	Data manager	1 (0.8%)
Clinical trials pharmacist	2 (1.6%)	***Total***	***128***

2.3.3 Barriers to opening the Add-Aspirin trial at site

The vast majority of sites expressing an interest in the trial had completed set-up and were open by the end of the first year of recruitment (84.7%). Respondents identifying their site as yet to open (*n=16,* 57.1% of all sites yet to open overall), were directed to a question to establish the reasons for this. Respondents were asked to select all the reasons that applied from a list, and were given the option to specify additional reasons as free-text. Table 2.5 shows the reasons given to account for not opening the trial.

Table 2.5 Reasons given for not opening the Add-Aspirin trial at sites expressing an interest

Reasons given for not opening *(n=16, able to select multiple options)*	***n (%)***	**Reasons offered but not selected**	***n (%)***
Delay in sponsor opening site	4 (25.0%)	Lack of interest in study	0 (0%)
Insufficient staff	4 (25.0%)	Competing trials	0 (0%)
Concerns about excess trial costs	2 (12.5%)	Lack of familiarity with a basket design	0 (0%)
Unable to identify PI(s)	1 (6.3%)	Lack of familiarity with a run-in period design	0 (0%)
Other	7 (43.8%)	Concerns about variations in follow-up schedules	0 (0%)

Delays at the coordinating trial unit were attributed to be the reason for failing to open by four respondents (25%). Initially, site set-up was coordinated centrally by one trial manager and four data managers, which increased to two trial managers and four data managers by the end of the first year. This provided efficiencies in central staffing compared to that normally required for setting-up sites for four separate trials, but may have been the rate-limiting step in opening for some sites. Whilst central staffing didn't prevent recruitment targets being met

in the breast, colorectal and prostate cohorts, opening sites earlier to the gastro-oesophageal cohort may have helped meet its recruitment target. Sites looking to open this cohort may have benefited from being prioritised.

Insufficient staffing at site was a reason given by four respondents (25%) for failure to open the trial. Sites often have a fixed number of facilitators who are responsible for setting up all trials, and time allocated by the NIHR does not account for variation in the complexity of phase III trials (117). The use of a complexity score to allocate funding for study set-up facilitators could be considered by the NIHR to allow more sites to open.

Only one site reported that difficulties finding a tumour specific cohort lead investigator resulted in a delay in opening, demonstrating that the model of having an overall PI with with a tumour-specific lead investigator for each cohort was not a common barrier.

In a qualitative analysis of "other reasons" provided in free-text (*n=7*), four responses followed a theme that the trial had not opened due to delays in regulatory approval (either Health Research Authority (HRA) or local research and development approval). It is possible that the trial took longer to achieve local research and development approvals because of lack of familiarity with a basket design. In March 2016 the requirement for research ethics committee, MHRA and local NHS research and development approvals was replaced by a single HRA approval, which simplified the regulatory approvals process in England, and may prevent this being a cause for delays in the future for similar trials. The other three reasons given in free-text were; "having four arms," "difficult to find suitable patients", and "study team to update Good Clinical Practice (GCP) training first".

2.3.4 Barriers to opening individual tumour cohorts at site

Respondents indicating their site had opened to the trial (n=97, 62.6% of sites open overall), were asked which cohorts were yet to open. Opening all cohorts was optional, however sites were encouraged to open as many cohorts as possible. The most frequented unopened cohort was the gastro-oesophageal cohort (*n=20*), followed by the prostate (*n=12*), colorectal (*n=3*)

and breast (n=2), cohort(s). Respondents reporting that a particular cohort had not opened at their site were asked to select all the reasons for this from a list, and were given the option to specify additional reasons as free-text. Table 2.6 shows the reasons given to account for not opening each cohort.

Table 2.6 Reasons for not opening individual Add-Aspirin cohorts at site

Reasons given for not opening cohort	Gastro-oesophageal *(n=20)*	Prostate *(n=12)*	Colorectal *(n=3)*	Breast *(n=2)*
Tumour cohort not treated at site	7 (35%)	5 (42%)	0 (0%)	0 (0%)
Unable to identify PI	5 (25%)	1 (8%)	0 (0%)	0 (0%)
Insufficient staffing	3 (15%)	2 (17%)	1 (33%)	0 (0%)
Competing trials	1 (5%)	1 (8%)	0 (0%)	0 (0%)
Differences in follow-up schedules	1 (5%)	0 (0%)	0 (0%)	0 (0%)
Other	1 (5%)	0 (0%)	2 (67%)	0 (0%)
Intention to open soon	6 (30%)	5 (42%)	2 (67%)	2 (100%)

The most commonly cited reason for not opening the gastro-oesophageal and prostate cohorts was that these tumour types were not treated at their site (35% and 42% of respondents respectively), which was not a reason given for the breast and colorectal cohorts. It is more common for the primary treatment of gastro-esophageal and prostate cancer to be centralised at specialist centres, however where patients return to their local site for follow-up and/or adjuvant therapy, patients could be registered for the trial at both sites. Encouraging those sites that only manage part of the treatment pathway to open may enhance recruitment.

In the gastro-eosophageal cohort, the second most common reason was the inability to identify a cohort specific lead investigator (25% of respondents), however this may reflect that not all sites are involved in the treatment pathway for gastro-oesophageal cancer.

Insufficient staffing was identified as a barrier to opening most frequently for the gastro-oesophageal cohort (three respondents), followed by the prostate (two respondents) and colorectal cohorts (one respondent), and was not reported in the breast cohort. This suggests that staffing might be prioritised to open particular cohorts over others. As previously proposed, the allocation of funding for study set-up facilitators according to the complexity of a trial could be considered, and may allow more cohorts to open.

Only one site cited a competing trial as the reason for not opening a cohort. That trial (Neo-AEGIS), has subsequently agreed to co-enrolment, suggesting that competing trials are not a barrier to site opening. In "other" reasons given as free-text, a respondent reported that the colorectal cohort had not opened because a "5 year scan required by the study not being standard of care," and another that "oncologist does not have sufficient time and capacity in his follow-up clinics". A respondent suggested the gastro-oesophageal cohort had not opened because there was "Insufficient capacity in oncology service".

2.3.5 Recruitment in the gastro-oesophageal cohort

At the time of designing the trial conduct survey, the gastro-oesophageal cohort had not met its monthly recruitment targets (chapter 3 table 3.1). Respondents reporting that the gastro-oesophageal cohort had opened at their site (*n=64*), were directed to a question to establish the reasons for poor recruitment. Respondents were asked to select all the reasons that applied from a list, and were given the option to specify additional reasons as free-text. Table 2.7 shows the reasons given to account for poor recruitment in the gastro-oesophageal cohort.

Table 2.7 Reasons given for poor recruitment in the gastro-oesophageal cohort

Reasons *(n=64, able to select multiple options)*	**Respondents**	**Percentage**
Few patients meet eligibility criteria	51	79.7%
Easier to recruit patients in other tumour types (cohorts)	22	34.4%
Concerns about toxicity	17	26.6%
Gastro-oesophageal multidisciplinary team meeting is at a different site	8	12.5%
Trial registration timelines	4	6.3%
Workload from other Add-Aspirin tumour types	3	4.7%
Other	5	7.8%

The most common reason given for poor recruitment in the gastro-oesophageal cohort was that fewer patients meet the eligibility criteria (79.7% of respondents). The incidence of patients with early stage gastro-oesophageal cancer is expected be lower than the other three cohorts, and this was considered when calculating anticipated recruitment rates for the trial. To explore the possibility of expanding the pool of patients considered for the gastro-

oesophageal cohort TMG discussions were held. Approximately 20% of patients with early stage gastro-oesophageal cancer have an R1 resection (118), but still have the potential for cure, and it was decided to adjust the eligibility criteria for the gastro-oesophageal cohort to patients with a positive surgical margins (R1 resection), and a protocol amendment has now been made.

The second most common reason given for poor recruitment in the gastro-oesophageal cohort (34.4% of respondents) was that it was easier to recruit to other cohorts. It was also reported that the workload from other cohorts impacted on recruitment (4.7% of respondents). It may be that sites are prioritising staffing for cohorts that are easier to recruit to, and are not providing staff time to screen for patients in the gastro-oesophgaeal cohort. Developing incentives to ring-fence staffing levels for each cohort with sites, or encouraging sites to reallocate staff time from over-recruiting cohorts, might result in more even recruitment.

26.6% of respondents reported concerns about toxicity as a cause of poor recruitment. Patients with gastro-oesophageal cancer commonly have tumour related ulceration or bleeding at first presentation, however once the tumour is treated, the risk of further ulceration and/or bleeding is minimised. Communication of interim safety data to sites in the early stages of the trial could act to reassure site staff about the risks of toxicity in this cohort, (see chapter 4) and this could be emphasised in the trial protocol and training slides.

12.5% of respondents reported that the gastro-oesophageal multidisciplinary team meeting (MDT) was located at a different site. Cross-site communication about potentially eligible patients, possibly in a regional MDT could aid recruitment.

Trial registration timelines were reported to inhibit recruitment by 6.3% of respondents. After further discussion with investigators, outside the trial conduct survey, it became apparent that patients receiving chemoradiotherapy often require a three month surveillance endoscopy, and recruitment could be improved by extending the timing of registration to be allowed up to

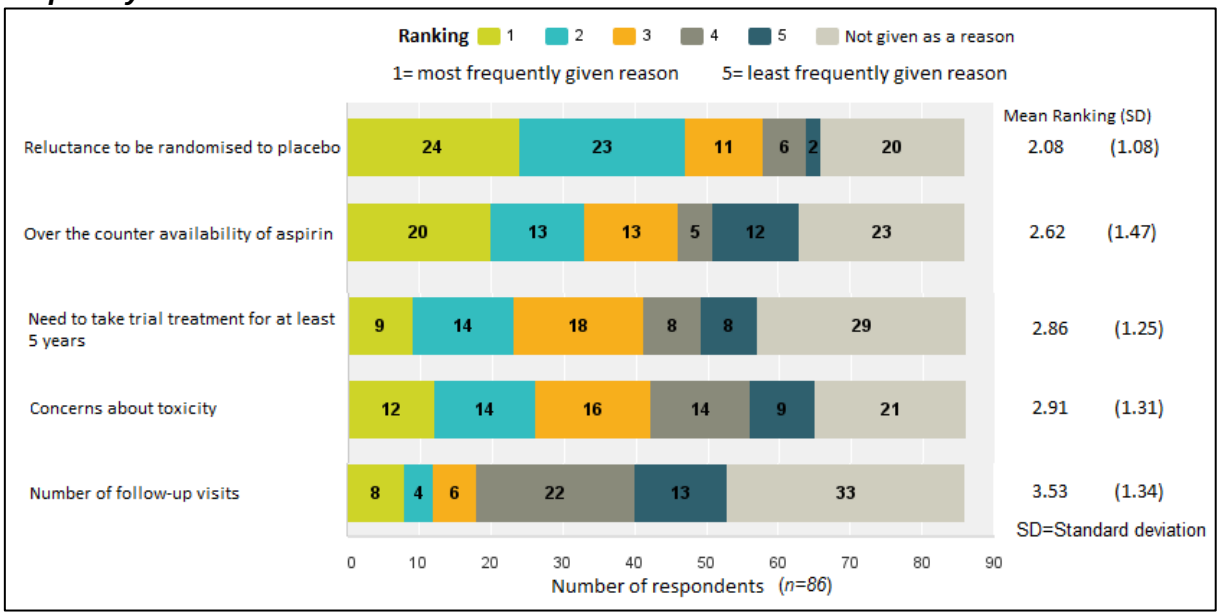
14 weeks after completing treatment (currently patients must register within twelve weeks of completion of chemoradiotherapy).

In a qualitative analysis of "other" reasons provided as free-text (*n=5*), three responses followed the theme that patients undergo part or all of their treatment for gastro-oesophageal cancer at other research sites. Other free-text reasons include; "no available research nurse to support this arm", and "competing non-compatible trial". Three respondents expanded on the reason that it was easier to recruit to other tumour types, with the following responses; "(*patients*) decline more so than in other cohorts", "are so exhausted after such intensive treatment and want a break", "some have had *pulmonary emboli* and are ineligible", emphasising the greater frequency of co-morbidities in this cohort.

2.3.6 Reasons given by patients for not registering for Add-Aspirin

A number of potential barriers to recruitment emerged from discussions with patient representatives and clinical staff during the development of the Add-Aspirin trial. These include; reluctance to be randomised to placebo, over the counter availability of aspirin, concern about toxicity, the need to take trial treatment for at least five years, and the number of follow-up visits. Establishing how frequently these reasons were expressed by patients can guide whether measures are necessary to address them. Respondents were asked to rank the five reasons above, in the order of how frequently they were given by patients for declining enrolment in the Add-Aspirin trial *(n=86)*. The most frequent reason was allocated a rank of one, through to the least frequent reason being allocated a rank of five. Respondents could also select "not reported" if a particular reason had not been expressed by patients. Figure 2.1 shows the ranking of how frequently reasons given by patients for not registering for the trial.

Figure 2.1 Ranking of reasons given by patients for not registering for Add-Aspirin by frequency

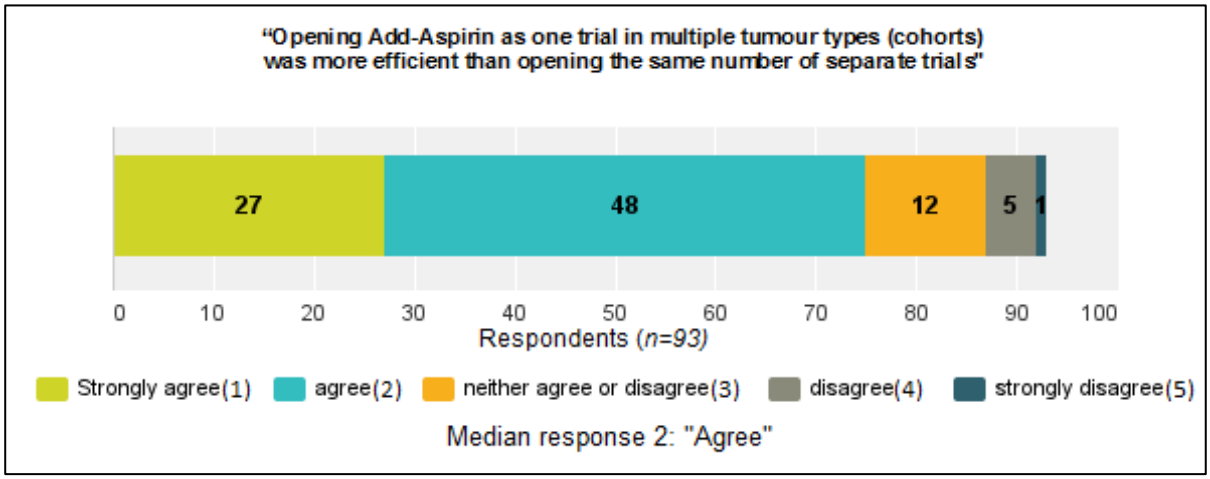


Respondents identified the most frequent reason given (mean ranking 2.08) as a reluctance to be randomised to placebo. This may reflect the large amount of media coverage on the anti-cancer effects of aspirin, and highlights the importance of that the role of aspirin in the adjuvant setting is unproven. This is relevant to any placebo controlled trial, but is of particular prominence to the Add-Aspirin trial because patients are often aware they can obtain aspirin over-the-counter without a prescription (second most frequent reason given, mean ranking 2.62). The requirement to take trial treatment for at least five years was ranked as the third most frequent reason given, (mean ranking 2.86). Concerns about toxicity (mean ranking 2.91), and the number of follow-up visits, (mean ranking 3.53) were ranked fourth and fifth and were not as highly ranked as anticipated. Training documents could enable site staff to address the most common patient concerns, particularly to emphasise that patients are twice as likely to be allocated aspirin as placebo (that randomisation to aspirin or placebo on a 2:1 basis), and the importance of taking an unproven investigational treatment within a clinical trial.

2.3.7 Efficiencies in trial set-up resulting from a basket design

Respondents indicating that two or more tumour cohorts have open at their site (n=93), were asked to indicate how strongly they agreed or disagreed with the statement: "Opening Add-Aspirin as one trial in multiple tumour types (cohorts) was more efficient than opening the same number of separate trials," using a scale of 1-5 where 1=strongly agree and 5=strongly disagree. There were 93 respondents, with 80.6% either agreed or strongly agreed with the statement. The median response was 2; "agree". Figure 2.2 shows the responses given.

Figure 2.2 Views on whether the basket design led to efficiencies in opening the trial



Respondents reporting that it was more efficient to open the Add-Aspirin trial as one trial with multiple tumour cohorts, were asked to identify the efficiencies they had witnessed from a list, with the option to provide additional free-text answers. The efficiencies identified are shown in table 2.8.

Table 2.8 Efficiencies in opening the Add-Aspirin trial resulting from a basket design

Efficiency *(n=64, able to select multiple options)*	**Respondents**	**Percentage**
Pharmacy set-up	54	84.4%
Regulatory approval	49	76.6%
Training	48	75.0%
Staffing	34	53.1%
Pathology departmental approval	30	46.9%
Radiology departmental approval	26	40.6%
Other	1	1.6%

When opening the trial, the majority of respondents witnessed efficiencies in pharmacy set-up, regulatory approval, training and staffing, however less than half witnessed efficiencies in gaining radiology and pathology departmental approvals. The imaging schedule for the trial varies by cohort, and approvals may be required from tumour-type specific radiologists and pathologists, rather than single individuals which limits efficiency. “Less repetition of paperwork” was given as a free-text reason by one respondent.

2.3.8 Inefficiencies in trial set-up resulting from a basket design

All respondents were asked to identify any inefficiencies they had witnessed in opening the Add-Aspirin trial as one trial rather than as multiple trials using free-text responses, or alternatively, indicating if no inefficiencies were identified. Table 2.9 shows the inefficiencies identified, categorised according to common themes.

Table 2.9 Inefficiencies in opening the Add-Aspirin trial resulting from a basket design

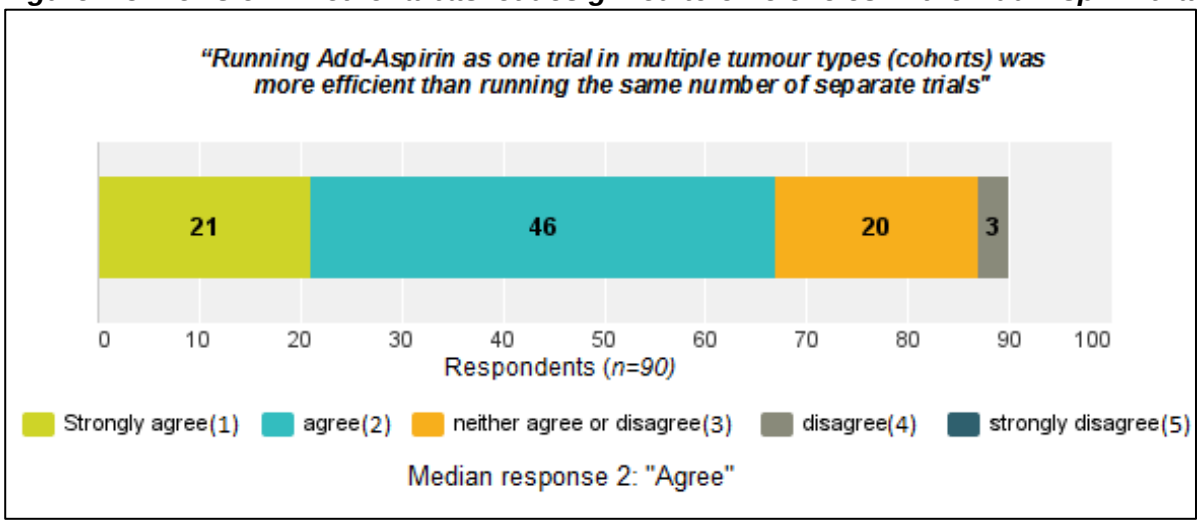
Inefficiency *(n=92)*	**Respondents**	**Percentage**
No inefficiencies identified	76	82.6%
More difficult to coordinate with different research teams	5	5.4%
Difficulties setting up one cohort delayed opening others	2	2.2%
Additional documentation required	2	2.2%
Burden of additional communications (email) not relevant to everyone	1	1.1%
Less available staff	1	1.1%
No difference	1	1.1%
Less efficient, but no reason given	1	1.1%
Unable to comment	3	3.3%

The most common inefficiency reported related to the challenge of coordinating site set-up between different research teams, highlighting that individual research staff members often work on trials in specific tumour types. A potential benefit from opening a trial with a basket design spanning tumour types could be to encourage a collaborative approach between trial staff to work across tumour types and share workload and experience.

2.3.9 Efficiencies in running the trial resulting from a basket design

Respondents indicating that two or more cohorts open at their site (*n=93*), were asked to indicate how strongly they agreed or disagreed with the statement; "Running Add-Aspirin as one trial in multiple tumour types (cohorts) was more efficient than running the same number of separate trials," using a scale of 1-5 where 1=strongly agree and 5=strongly disagree. There were 90 respondents, with 74.4% either agreeing or strongly agreeing with the statement. The median response was 2; “agree”. Figure 2.3 shows the responses given.

Figure 2.3 Views on whether a basket design led to efficiencies in the Add-Aspirin trial



Respondents reporting that it was more efficient to run the Add-Aspirin trial as one trial with multiple tumour cohorts were asked to identify the efficiencies they had witnessed from a list, with the option to provide additional free-text answers. Efficiencies identified are shown in table 2.10.

Table 2.10 Efficiencies in running the Add-Aspirin trial, resulting from a basket design

Efficiency *(n=64, able to select multiple options)*	**Respondents**	**Percentage**
Familiarity with common trial processes	60	93.8%
One overarching protocol	56	87.5%
Drug dispensing	42	65.6%
Staffing	39	60.9%
Data entry	32	50.0%
Other	2	3.1%

When running the trial, the majority of respondents witnessed efficiencies resulting from; a familiarity with trial processes common to all cohorts, the use of an overarching protocol, use of the same drug dispensing pathways, and shared staffing, however data entry was only thought to be an efficiency by half of respondents. A basket design would not be expected to reduce the amount of data required, but efficiencies might be possible for the collection and entry of data fields that are common to all tumour cohorts. Two free-text responses were provided, and followed the theme that amendments were easier to process as one rather than four separate protocols.

2.3.10 Inefficiencies in running the trial resulting from a basked design

All respondents were asked to identify inefficiencies they had witnessed in running the Add-Aspirin trial as one trial rather than as multiple trials using free-text responses, or alternatively, indicating that no inefficiencies were identified. Table 2.11 shows the inefficiencies identified, categorised according to common themes.

Table 2.11 Inefficiencies in running the Add-Aspirin, resulting from a basket design

Inefficiency *(n=90)*	**Number of respondents**	**Percentage**
No inefficiencies identified	73	81.1%
Insufficient staffing to run multiple cohorts	7	7.8%
Inefficiencies resulting from differences between cohorts	10	11.1%

Insufficient staffing was the most common inefficiency identified, again emphasising the need to balance staffing levels at site to support all cohorts.

11.1% of respondents reported that differences between cohorts led to inefficiencies in running the trial. Whilst trial processes and schedules were aligned as much as possible for all cohorts, some differences were necessary. Explaining the rationale for the differences between the cohorts in the protocol could prevent misunderstanding at site and minimise protocol violations.

2.3.11 Perceived effectiveness of a run-in period design

Sites were asked about their views on how effective they anticipate the run-in period to be with respect to predicting and improving adherence and tolerance in the main trial, based on their experiences of its use. Respondents were asked about their level of agreement or disagreement to a series of statements using a scale of 1-5 where 1=strongly agree and 5=strongly disagree (89 respondents). Responses are shown in figure 2.4.

Figure 2.4 Views on the effectiveness of the run-in period design

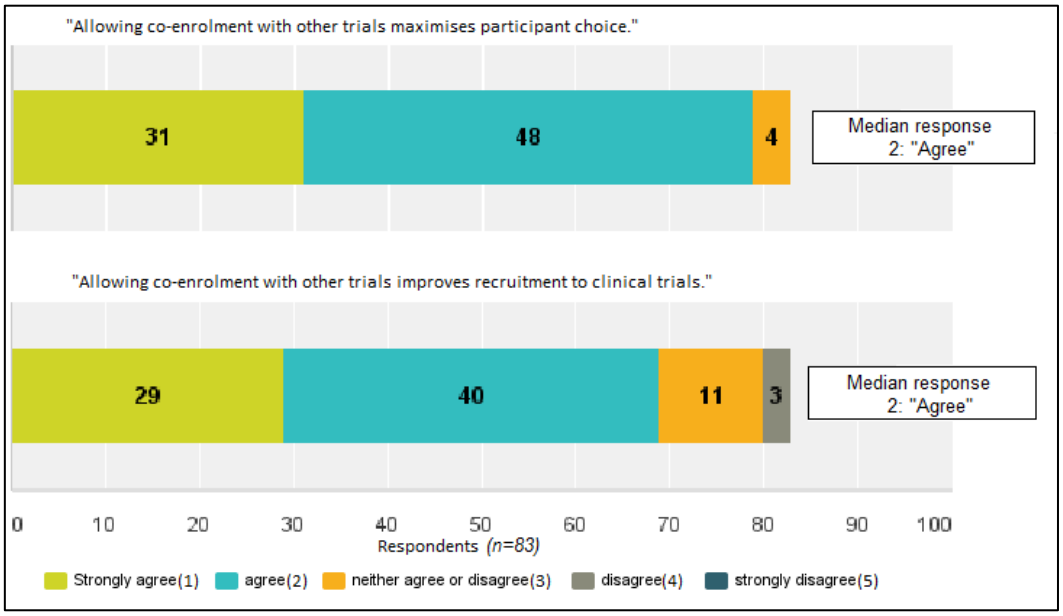
Respondents agreed (median response of 2) that the run-in period was likely to be a good predictor of adherence and tolerance, and improve both of these in the main trial. This is consistent with the aim of the run-in period and evidence of its effectiveness in other aspirin trials. Respondents neither agreed nor disagreed (median response of 3) that the trial run-in

period would reduce the number of participants who miss scheduled follow-up visits in the main trial.

2.3.12 Attitudes towards co-enrolment in other trials

Opportunities to offer patients already taking part in other trials the option to also register for the Add-Aspirin trial were sought from the outset of the trial. Co-enrolment is considered on a trial-by-trial basis, with an assessment of any conflicts in eligibility criteria, scheduling, and the potential impact on safety and the results of either trial, and a discussion with the relevant trial team. In the first year of the trial, co-enrolment has been agreed with 20 breast, 9 colorectal, 5 gastro-oesophageal, and 6 prostate cancer trials. This variation between cohorts could be due to differences in the amount of trial activity between tumour types, or even variation in the acceptability of co-enrolment to researchers and patients in different tumour groups. Sites were asked about their views on co-enrolment with other trials using a scale of 1 to 5 where 1=strongly agree and 5=strongly disagree (83 respondents). 95.2% either agreed or strongly agreed that “allowing co-enrolment with other trials maximises participant choice” (median response 2; “agree”). 83.1% of respondents either agreed or strongly agreed that; “Allowing co-enrolment with other trials improves recruitment to clinical trials” (median response 2; “agree”). Responses are shown in figure 2.5.

Figure 2.5 Attitudes towards co-enrolment in other trials



In an optional question, sites were asked to describe any concerns they had about co-enrolment in free-text and there were ten responses. Three respondents expressed concerns that there was additional burden for patients enrolling in multiple trials. Two respondents reported reservations about the challenge of aligning follow-up visits and schedules for multiple trials. Two respondents described perceptions about the risk of additional toxicity. Two respondents reported lack of clarity around which trials can be co-enrolled with, particularly with new trials. One respondent described apprehensions about the interpretation of trial results with the presence of additional confounding variables.

2.4 Discussion

One year after recruiting its first participant, the Add-Aspirin trial had successfully opened at least one site in the vast majority of NHS trusts and boards with cancer services, with most sites opening at least three of the four tumour cohorts. This proves that it is feasible to open a large multi-tumour type basket trial in the UK, and suggests that this type of design could be utilised for other phase III trials of other interventions with potential activity across tumour types. It also shows that appointing an overall site PI with tumour-specific cohort leads is a feasible strategy that could be applied effectively in similar trials.

The trial conduct survey also provided insights into the views of site staff on the trial methodology. The majority of sites were supportive of the basket trial design and felt it was more efficient than opening and running the same number of separate trials, however there were concerns around allocation of staffing between cohorts. Finding a balance between potential staffing efficiencies at site, and ensuring adequate recruitment to all cohorts is essential to the success of a trial with a design of this type.

On the whole, respondents were supportive of the use of a run-in period design and anticipated that the run-in period will select a population that is able to tolerate and more adherent with taking trial treatment. However the survey found that respondents didn't necessarily anticipate that it would result in a population that is more adherent to a trial

schedule for the duration of the trial. An analysis of the correlation between treatment adherence in the run-in period and trial visit attendance later in the trial may provide further insight.

Site staff were generally supportive of the principles of co-enrolment. There were a number of concerns about co-enrolment expressed by sites staff, which could be alleviated by providing specific information on trials approved for co-enrolment. This could include details of any additional burden in terms of follow-up visits and investigations, any extra risk of toxicity, and a summary of the perceived impact on the interpretation of the results of each trial.

I have used the findings of the trial conduct survey to provide a list of recommendations:

1) Developing incentives to ring-fence staffing time for each cohort with sites, to allow more cohorts to open and recruit more evenly.
2) The use of a complexity score to allocate funding for study set-up facilitators could be considered by the NIHR to allow more complex trials to open sites promptly.
3) Agreeing site staffing allocation with the Clinical Research Networks, according to the number of cohorts open, to encourage additional sites to open.
4) The treatment pathway for some tumour types is often divided between sites. Trial conduct could be improved by:
    a. Encouraging sites that only manage part of the treatment pathway to open.
    b. Promoting cross-site communication about potentially eligible patients at regional MDT meetings.
    c. Implementing processes centrally to ensure a smooth transfer of participants between sites.
    d. A national system of sharing credit for research activity and support costs more equitably when a patient's care involves multiple sites to encourage more sites to open.
5) Training documents to enable site staff to address common patient concerns, including:

a. Emphasising that there is twice the chance of being allocated aspirin as placebo in the trial.
b. Highlighting the importance of taking an unproven investigational treatment within a clinical trial, rather than purchasing over the counter.
c. Communication of existing safety data on the risks of toxicity, particularly in the context of aspirin and its high media profile.

6) Where differences exist in the trial protocol between tumour cohorts, (for example the timing of entry criteria), the rationale could be explained to avoid accidental protocol violations.
7) Concerns from patients and site staff about trial co-enrolment could be alleviated by providing specific information for each agreed trial on any additional burden in terms of follow-up visits and investigations, any extra risk of toxicity, and a summary of the perceived impact on the interpretation of the results.

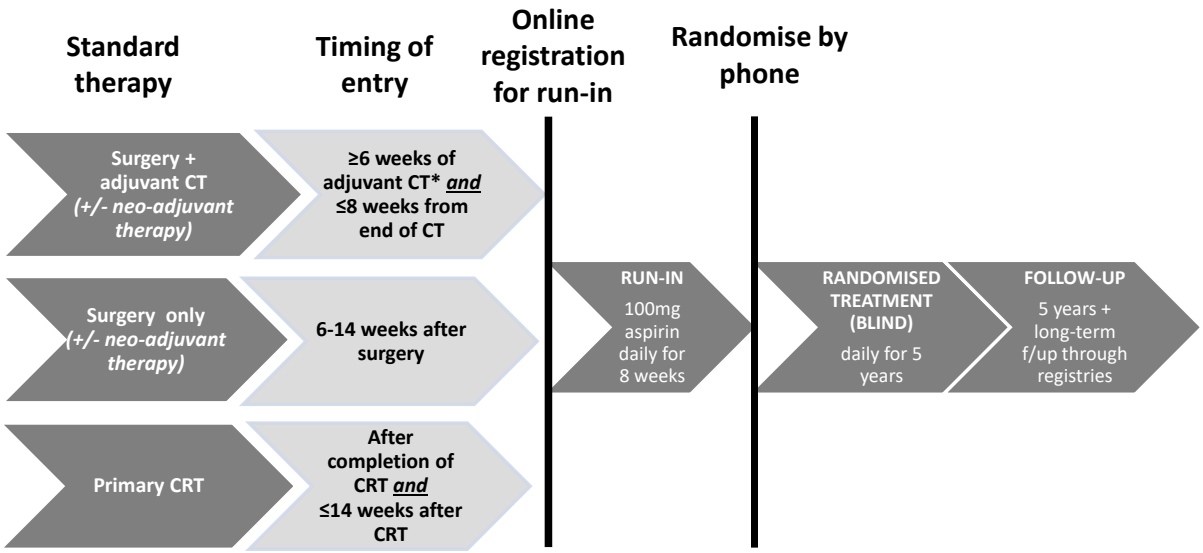
During the first year, recruitment exceeded monthly targets every month in the breast, colorectal, and prostate cohorts, but fell short for each month for the gastro-oesophageal cohort (chapter 3, figure 3.1), and only 27.2% (31/114) of sites which opened the gastro-oesophageal cohort had recruited a participant.

Using the results of the trial conduct survey I have provided a list of recommendations to improve recruitment in the gastro-oesophageal cohort:

1) Sites proposing to open the gastro-oesophageal cohort could be prioritised for opening.
2) Create incentives not to divert staffing away from the gastro-oesophageal cohort toward other cohorts where recruitment is easier, and to consider reallocating staff time from over-recruiting cohorts.
3) Promote cross-site communication about potentially eligible patients who receive primary treatment and follow-up for gastro-oesophageal cancer at different sites.

4) Training about the eligibility of patients with a history of tumour related ulceration or bleeding, who have already had cancer treatment.
5) Expansion of eligibility criteria so to not unnecessarily exclude patients:
    a. Inclusion of participants with an R1 resection (now implemented as a protocol amendment).
    b. Increase the end of the timing of entry (registration) window from 12 to 14 weeks after completing radical chemoradiotherapy for gastro-oesophageal cancer, (now implemented as protocol amendment, see figure 2.6).

Figure 2.6 Updated gastro-oesophageal cohort timing of entry flow diagram.



* If registration takes place whilst chemotherapy is ongoing, platelet count should be >100 x 109/L on day 1 of each preceding cycle. **CT= Chemotherapy, RT= Radiotherapy, CRT= Chemoradiotherapy**

Chapter 3. An evaluation of recruitment to the Add-Aspirin trial over the first year

3.1 Introduction

The success of the Add-Aspirin trial hinges on opening and recruiting to multiple tumour cohorts at the majority of UK NHS research sites that treat cancer. This represents a substantial undertaking for both research sites and the coordinating trial unit team.

Anticipating recruitment is a key consideration for the planning and funding of any trial. It is common practice for sites to provide predicted recruitment rates as part of a site evaluation process. Accurate recruitment predictions at sites allow more reliable overall trial recruitment projections, however these are often over optimistic, with less than a third of publically funded trials meeting their original recruitment targets (119). A number of different models for predicting recruitment have been proposed. Examples include the conditional model, where variation in monthly recruitment, according to the number of sites open and the time to reach maximum recruitment are considered (120), a Poisson model, which simulates the number of patients recruited in a given month according to the Poisson distribution (121), and a Bayesian model which uses ongoing accrual data in calculations to refine anticipated recruitment rates as the trial proceeds (122). To date, none of the models proposed have proven consistently effective (123).

Anticipated recruitment rates for each tumour cohort in the Add-Aspirin trial are largely based on recruitment rates in similar previous trials, incorporating a period for sites to reach peak recruitment and become familiar with recruiting to the trial. These estimates were made prior to site recruitment prediction becoming available for all sites. A trial with one protocol spanning four different tumour types represents an opportunity to understand how predicted recruitment rates relate to actual recruitment rates in different tumour types. The objectives for this chapter are as follows:

3.1.1 Objectives

1) Evaluate recruitment to the Add-Aspirin trial across cohorts over the first year.
2) Determine the accuracy of site predicted recruitment rates by comparing them with actual recruitment rates, across tumour types.
3) Suggest how the accuracy of site recruitment predictions and anticipated recruitment projections might be improved.

3.2 Methods

3.2.1 Site opening and recruitment

From the 1st November 2014, a site initiation form was sent, by email, to sites expressing an interest in opening the Add-Aspirin trial, and accepted if returned before the 1st of November 2016. Sites were asked to report their predicted average monthly recruitment rates for each tumour cohort. If respondents quoted an average annual recruitment estimate, it was divided by 12 to give an anticipated monthly recruitment figure, and where a range was given, the lower figure was taken to give the most conservative estimate. A copy of the site initiation form is available in appendix C.

The trial opened to recruitment on the 8th October 2015, and recruitment data were extracted from the trial registration server on the 1st of November 2016, providing data on the first 12 full months of trial recruitment. The mean and maximum monthly recruitment was calculated for individual tumour cohorts at each site, from the time of the first participant recruited for each cohort, until the 1st of November 2016. NHS trusts and boards (NHS trusts in England, Health Boards in Scotland and Wales, and Social Care Trusts in Northern Ireland), often comprise a number of separate research sites. Where one site initiation form contained combined predicted recruitment for a number of sites within a single NHS trust or board, actual recruitment data for those sites was combined to allow comparison. Data were analysed for sites that provided predicted recruitment and recruited at least one participant to a cohort. Monthly predicted recruitment was compared to the mean actual monthly recruitment as a

ratio. Monthly predicted recruitment was also compared to maximum actual monthly recruitment as a ratio. The data was summarised by calculating the median and interquartile range (IQR), rather than the mean and standard deviation, to minimise the influence of outliers.

Data on recruitment to individual cohorts at UK NHS research sites was collected between the 8th October 2015 and the 1st November 2016, and includes the first 12 full months of the trial. All data were analysed using Microsoft Excel and STATA version 14.

3.3 Results

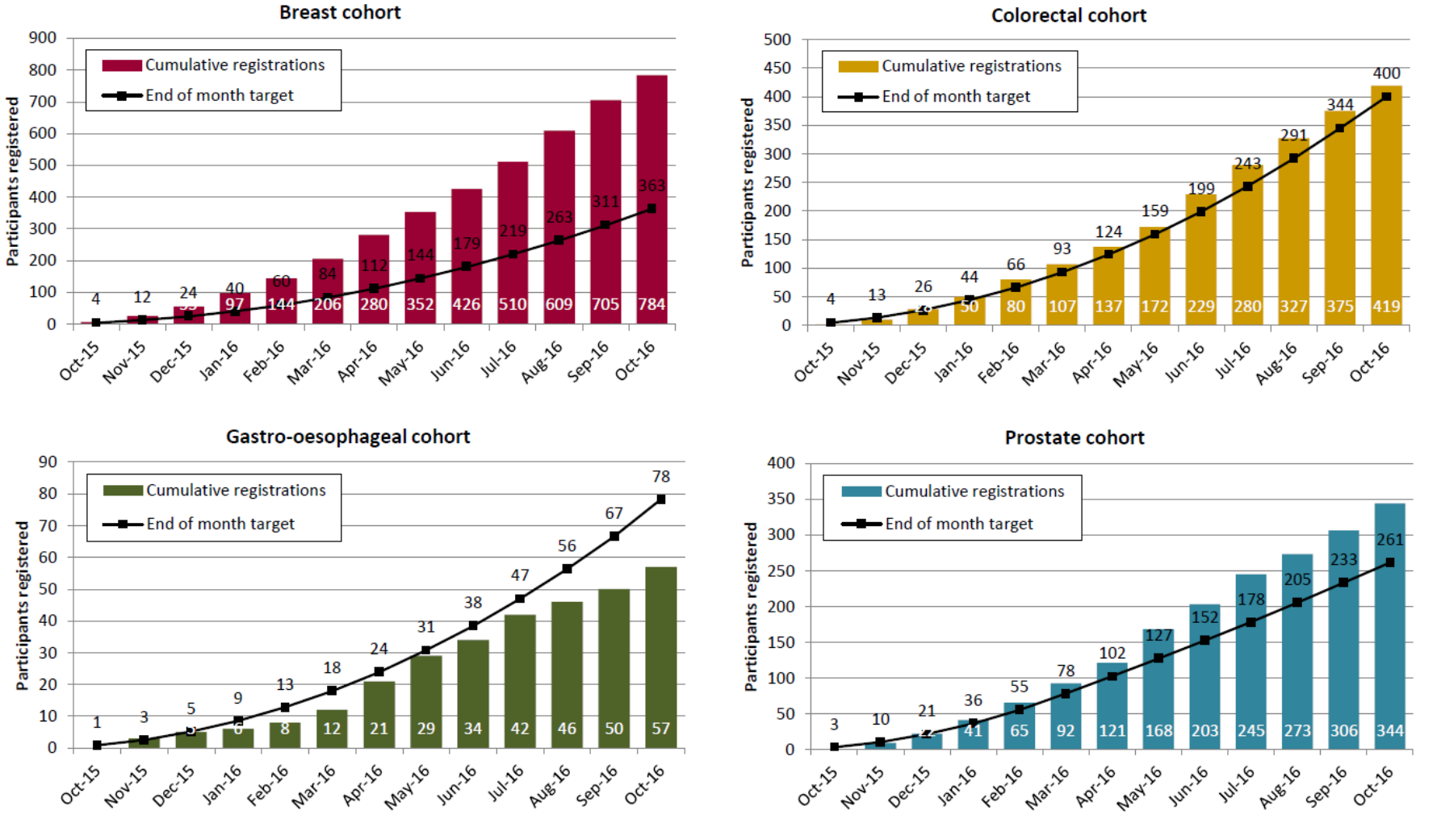
3.3.1 Recruitment at one year

After one year of the trial being open, 127 sites had registered at least one participant. A total of 1,605 participants had been registered, with recruitment ahead of projected targets in all but the gastro-oesophageal cohort. Table 3.1 shows, by cohort, recruitment after one year of the trial being open. The graphs in figure 3.1 show actual compared to projected recruitment rates.

Table 3.1 Add-Aspirin trial recruitment after one year, by cohort

Cohort	Target recruitment	Actual recruitment	Percentage of recruitment target achieved
Breast	363	785	216.3%
Colorectal	400	419	104.8%
Gastro- oesophageal	78	57	73.1%
Prostate	261	344	131.8%
Total	**1,102**	**1,605**	**145.6%**

Figure 3.1 Projected and actual recruitment in the first year of the add-Aspirin trial



3.3.2 Site initiation form data return

168 site initiation forms were completed, and contained data on 183 different sites across the UK (some forms contained data for a number of sites within a single NHS trust or board). The site initiation forms provide predicted recruitment rates for 96.5% of the individual cohorts recruiting at sites after one year of the trial being open. This allows a near comprehensive comparison of predicted to actual recruitment rates in the first year of the trial. Table 3.2 shows the number of recruiting sites with predicted recruitment figures available.

Table 3.2 Sites providing predicted recruitment rates by cohort

Number of sites	Breast cohort	Colorectal cohort	Gastro-oesophageal cohort	Prostate cohort
Sites providing predicted recruitment figures	155	160	118	142
Sites that recruited at least one participant	114	101	31	68
Recruiting sites with predictions available	109	99	29	66
Percentage of recruiting sites with predictions available	95.6%	98.0%	93.5%	97.1%
Percentage of recruiting sites with predictions available	**96.5%**			

3.3.3 Predicted recruitment

The highest predicted median monthly recruitment per site was in the prostate cohort, followed by the colorectal and breast cohorts, with the smallest median predicted recruitment in the gastro-oesophageal cohort (3, 2, 2, 1 participants per month at each site respectively). Table 3.3 summaries the predicted monthly recruitment by cohort.

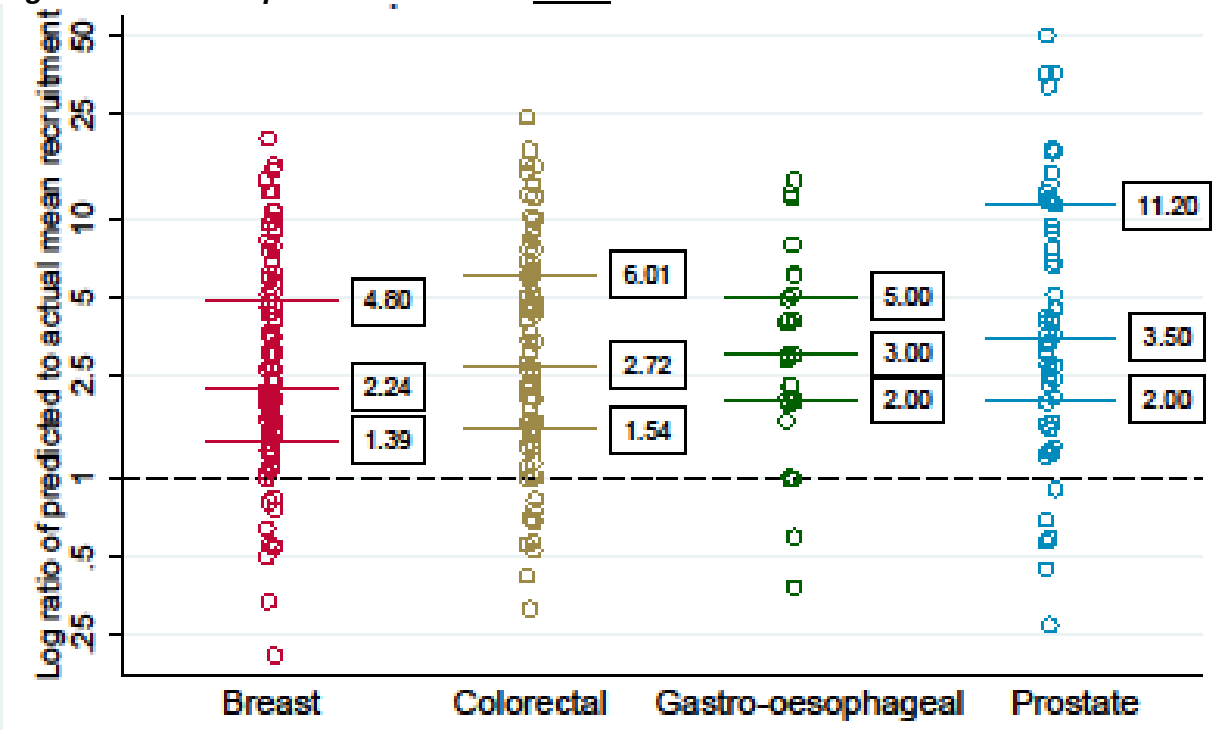
Table 3.3 Predicted monthly recruitment for each site by cohort

Monthly predicted recruitment	Breast cohort	Colorectal cohort	Gastro-oesophageal cohort	Prostate cohort
Min	0.33	0.25	0.17	0.17
Max	8.00	7.00	2.00	12.00
Median (IQR)	**2.00** (1.25-4.00)	**2.00** (1.00-3.00)	**1.00** (1.00-1.00)	**3.00** (1.00-4.00)

3.3.3.1 Comparison of mean monthly recruitment to predicted monthly recruitment

For each site, the mean monthly recruitment was calculated and compared to the predicted monthly recruitment in the form of a ratio. The dot plots provided in figure 3.2 show the ratio of the predicted to actual mean monthly recruitment for each site. A ratio below one suggests predicted recruitment is less than actual recruitment, and a ratio above one suggests predicted recruitment is greater than actual recruitment.

Figure 3.2 Ratio of predicted to actual <u>mean</u> recruitment at each site



Footnotes: *Solid lines and numbers indicate the median and IQR.*
A ratio below one suggests predicted recruitment is less than actual mean recruitment, and a ratio above one suggests predicted recruitment is greater than actual mean recruitment.

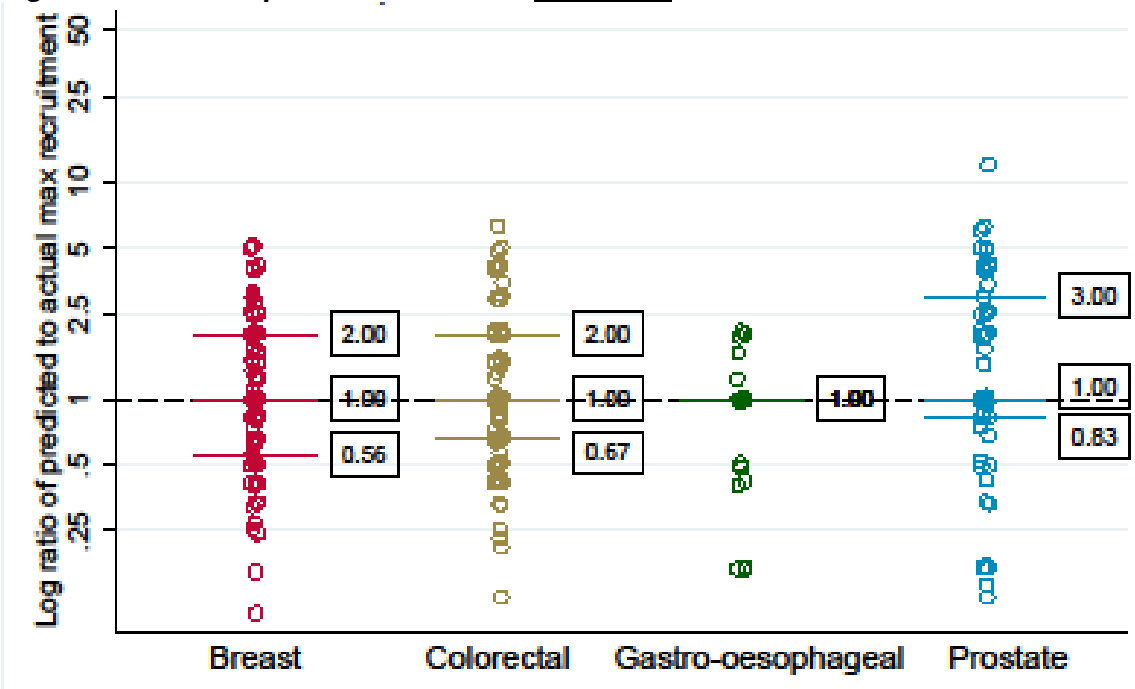
The median ratio of predicted to actual recruitment was 2.24, 2.27, 3.00 and 3.50 for the breast, colorectal, gastro-oesophageal and prostate cohorts respectively. It could be that sites over-estimate their ability to recruit in order to increase the attractiveness as a potential site to open a trial. Recruitment to the prostate cohort was the most over-estimated (median 3.5 fold). The reason for this is uncertain, but could be due to the fact that patients with prostate cancer are, on average, older than those in the other cohorts, and therefore may already be taking aspirin, or have co-morbidities, which would exclude them from the trial, and this may not have been taken into account in site recruitment predictions. Another consideration is that treatment

with radical radiotherapy and radical prostatectomy is conducted by different departments (clinical oncology and surgical departments respectively) and a number of different individuals would need to have been consulted to obtain an accurate prediction.

3.3.3.2 Comparison of maximum monthly recruitment to predicted monthly recruitment

In order to better understand why predicted recruitment figures significantly overestimate actual recruitment figures, I hypothesise that sites may be providing over-optimistic figures based on a maximum (or best possible) monthly recruitment. To investigate this, the actual maximum monthly recruitment for each site was compared to the predicted monthly recruitment in the form of a ratio for each site. The dot plots provided in figure 3.3 show the ratio of the predicted to actual maximum monthly recruitment for each site. A ratio below one suggests predicted recruitment is less than actual maximum recruitment, and a ratio above one suggests predicted recruitment is greater than actual maximum recruitment.

Figure 3.3 Ratio of predicted to actual <u>maximum</u> recruitment at each site



Footnotes: Solid lines and numbers indicate the median and IQR.
A ratio below one suggests predicted recruitment is less than actual maximum recruitment, and a ratio above one suggests predicted recruitment is greater than actual maximum recruitment.

The median ratio of actual maximum to predicted monthly recruitment was found to be equal to one for all cohorts, suggesting that when sites are asked to predict average monthly recruitment, the value provided more accurately reflects the maximum possible monthly value.

3.3.3.3 Trial recruitment timelines based on site predictions alone

The original trial recruitment projections anticipated it would take between three and a half and six years for each cohort to reach their total trial recruitment targets. Based on the total predicted recruitment from the 127 UK sites that had recruited at least one participant to the trial, the breast, colorectal and prostate cohorts would reach their overall recruitment target within one year, and the gastro-oesophageal cohort within two years. Recruitment predictions from potential sites are often used to advise trial recruitment projections and targets, however this highlights how site predictions would have been over-optimistic. Table 3.4 shows the time taken for recruitment targets to be reached if the total predicted recruitment represented actual recruitment rates.

Table 3.4 Time to reach overall recruitment targets based on site predictions alone

	Breast cohort	Colorectal cohort	Gastro-oesophageal cohort	Prostate cohort
Site predicted recruitment per month *(n)*	271.9	226.8	97.7	246.8
Site predicted recruitment per year *(n)*	3262.5	3201.4	1164.8	2961.0
Original recruitment target at one year *(n)*	363	400	78	261
Total trial recruitment target (UK and India) *(n)*	2600	3100	2100	2120
Time to reach total trial recruitment target based on original recruitment projections	42 months	42 months	66 months	60 months
Time to reach total trial recruitment target based on site predicted recruitment	**9.4 months**	**11.8 months**	**21.9 months**	**8.7 months**

3.4 Discussion

The Add-Aspirin trial opened ten months after originally intended due to a number of contractual delays, however at one year, the trial has exceeded its overall target recruitment by 46% (503 participants), which goes some way to catch up with the original recruitment targets had the trial opened on time. Many of the sites providing predicted recruitment rates

had not recruited a participant at one year (44, 61, 89 and 76 sites for the breast, colorectal, gastro-oesophageal and prostate cohorts respectively). This suggests there is the potential to improve recruitment rates further if the reason that these sites have not recruited can be established and addressed (as discussed in chapter 2).

3.4.1 Accuracy of site predicted recruitment rates and recommendations for improvement

Sites were found to overestimate how many patients they can recruit per month by a median of 220-350% depending on tumour type (figure 3.2). The median time from site opening to recruiting its first participant (to any cohort), was 43 days (see chapter 2 section 2.3.1), which could suggest that sites under-estimate the time taken to reach maximum recruitment capacity, which could also account for over-estimated monthly recruitment rates.

Predicted recruitment from sites for the Add-Aspirin trial was found to be very similar to maximum actual monthly recruitment (median ratio of 1.00 for each cohort, figure 3.3), suggesting that sites could be basing predicted recruitment on a best possible monthly figure. Selecting an anticipated recruitment rate by asking for a lowest and highest monthly recruitment prediction, and using the average may be a more accurate method, and would benefit from validation in future trials.

Given that anticipated recruitment projections underestimated actual recruitment figures in three out of four cohorts, more accurate predictions may have been obtained by using the method of obtaining site predictions as suggested above in combination with data from previous similar trials.

Chapter 4. An evaluation of the first 500 participants entering the Add-Aspirin trial and the utility of the run-in period

4.1 Introduction

4.1.1 Characteristics of patients entering the trial

The eligibility criteria for each cohort of the Add-Aspirin trial have been chosen to optimise safety, and strike a balance between selecting patients representative of the wider population, whilst having sufficient likelihood of a measurable treatment effect within the time-frame of the trial. It is important to examine baseline characteristics of patients actually enrolling in the trial to check that they match the characteristics on which prognostic predictions and sample size calculations were made. Identifying where particular characteristics are under-represented allows targeted recruitment to redress differences, or if necessary, allows re-assessment of the sample sizes required to adequately power the trial.

4.1.2 The Add-Aspirin trial run-in period

Maintaining adherence and minimising premature discontinuation of trial treatment represents a significant challenge to the Add-Aspirin trial. Data have consistently shown that long-term treatment with aspirin is required for the anti-cancer effects of aspirin to become identifiable, (a minimum of two years (124), and up to 5-10 years (92, 95)), therefore the trial requires participants to take trial treatment daily for at least five years. Long-term adherence is known to decline according to the length of time that adjuvant therapy is given (125), with one study investigating long-term persistence with adjuvant tamoxifen for breast cancer suggesting that only 49% of users completed the intended five years of treatment (126). Furthermore, the participants in an adjuvant trial will often be otherwise healthy and asymptomatic, and adherence is likely to be lower than for those with ongoing symptoms (127). Five years of treatment also extends exposure to potential toxicities.

To address these challenges, the trial design incorporates an active run-in period, where after registration, but prior to randomisation, all participants take 100mg aspirin (one tablet per day) in an open-label manner for a period of approximately eight weeks. At the end of the run-in period, the participant's tolerance of aspirin and adherence to daily treatment is assessed. This approach aims to identify those individuals who are unlikely to be able to tolerate aspirin, as well as those who are less likely to adhere to the protocol treatment schedule (128). This strategy has also been used successfully in other aspirin trials (129, 130). The run-in period provides an early opportunity to assess feasibility, in terms of early toxicity, recruitment and patient acceptability. An initial assessment of how the run-in period is working in practice will advise whether any amendments to the process could be made as the trial progresses.

4.1.3 Timing of trial registration across cohorts

Whilst the four tumour cohorts in Add-Aspirin are individually powered, there are a number of outcome measures that combine data across all tumour cohorts. These include a co-primary endpoint of overall survival across all four cohorts at 15 years after the first randomisation, and secondary outcome measures, including cardiovascular events, thromboembolism, toxicity, cognition and functional capacity. The trial was designed such that the timing of registration is aligned where possible across the four cohorts, however there is variation around when registration occurs with respect to adjuvant chemotherapy. All participants must receive the standard primary therapy with curative intent for their cancer before trial entry. An assessment of how the timing of entry criteria are being applied will allow any adjustments to be made to the criteria to both better align the cohorts, and facilitate recruitment by making the timing of entry criteria as practical as possible.

4.1.4 Recording concomitant medication

It is increasingly recognised that commonly taken drugs, for example vitamin D, bisphosphonates, metformin and statins may have anti-cancer effects (chapter 1). Utilising data from randomised trials to inform the design of further studies, as was the case for aspirin, is potentially insightful, but collecting, and subsequently analysing, such data can be

challenging. The CRF forms for the Add-Aspirin trial were designed to collect concomitant medications at baseline and throughout the trial (appendix D). An analysis of this initial data with respect to metformin use during the run-in period is potentially useful to optimise how concomitant medication data is collected and provide a baseline for metformin use in this population, and advise the feasibility and design of future trials (see chapter 5). The objectives of this chapter are summarised below.

4.1.5 Objectives

1. To examine the baseline characteristics to check that the patients entering the trial are as expected. This is to ensure participants are representative of the wider population and the trial is powered as expected.
2. To make an initial assessment of the design and utility of the run-in period. An overview of how this is working in practice will advise whether any amendments to the process are necessary.
3. To make an assessment of when the run-in period is initiated across the four tumour cohorts to see if any adjustments are necessary as the trial progresses.
4. To establish the pattern of metformin use in the Add-Aspirin trial to inform the potential design of a new metformin arm, and optimise the way concomitant medication data is collected in this and similar trials.

4.2 Methods

4.2.1 Run-in period design

The run-in period was designed so that all participants take 100mg aspirin (one tablet per day) in an open-label manner for a period of approximately eight weeks to assess adherence and tolerance (as described in chapter 2). To determine adherence during the run-in period, three different sources of adherence data were obtained to allow a more accurate assessment. These included a participant diary card, the return of used tablet blister packs, and a participant interview at an end of run-in assessment. Participants were suitable for randomisation if they had taken at least 80% of their run-in treatment, as judged by trial staff performing the end of run-in assessment, by considering all sources of adherence data available.

To be eligible for randomisation, participants must also have not experienced any aspirin-related severe toxicity (defined as greater than grade 3 CTCAE v4 (Common Terminology Criteria for Adverse Events)), or any grade of active gastrointestinal ulceration or bleeding, tinnitus, macular degeneration, intracranial bleeding or hypersensitivity to aspirin. Participants developing any of these toxicities were required to permanently discontinue aspirin immediately and will not be eligible for the trial.

An extension to the run-in period was incorporated into the design to avoid unnecessarily exclusion participants who had a valid reason for inadequate adherence (e.g. unforeseen social circumstances) or the cause was temporary (e.g. due to toxicity resulting from concomitant treatment which has subsequently stopped, or a non-recurrent unrelated event). Where agreed by the coordinating trial unit, the run-in period would normally be extended by four or eight weeks, after which adherence and toxicity would be reassessed in the same way. Only one extension was permitted per participant. Those participants identified as suitable for further study participation, remained eligible, and were willing to continue in the trial then re-confirmed their consent to participate before being randomised.

4.2.2 Timing of trial registration

The timing of entry criteria was designed so that aspirin can be started at the earliest opportunity to maximise the potential anti-cancer benefits, whilst starting at a time when it was considered safe to do so, and was unlikely to compromise the curative intent of standard primary treatment.

For patients who have undergone surgery without any adjuvant therapies, a minimum time period of six weeks before trial registration (and the start of the run-in period) was mandated to reduce the risk of post-operative bleeding complications. This follows standard surgical advice to recommend that patients refrain from strenuous physical activity for at least six weeks following major surgery to allow wound healing (131), and is consistent with evidence that wound tensile strength increases rapidly until six weeks after an operation (132).

For patients who have received radical chemoradiotherapy or radical radiotherapy, the run-in period was only permitted to start once this treatment had been completed. This is based on the rationale that if complications of aspirin therapy develop during radical chemoradiotherapy or radiotherapy, there is the potential for this to interrupt, and therefore compromise, the quality of treatment.

For patients receiving adjuvant chemotherapy, there was debate across the tumour cohorts as to whether the aspirin run-in period could be started before this had finished. For the colorectal and gastro-oesophageal cohorts it was agreed that if platelet counts after two cycles of chemotherapy remained greater than 100 $x10^9$/L on day one of each cycle then the run-in period could be started at that point, or later if preferred. For the breast cohort, there were concerns about chemotherapy related dyspepsia and the aspirin run-in was only allowed to start once adjuvant chemotherapy was finished.

The time interval during which registration was allowed was restricted to six weeks to ensure that trial treatment was started promptly, but some flexibility was allowed for individual cases

where an acceptable reason was provided to the coordinating trial unit. The design for the timing of entry criteria for each cohort is described in figures 4.1 to 4.4.

4.2.3 Data collection

Cohort specific registration CRF forms (appendix D) were designed to collect information on the treatment pathway taken by each participant from cancer diagnosis onwards, and the timing of trial registration with respect to the treatment pathway taken. Data on the timing of the treatment pathway taken was summarised using the median and interquartile range which was selected to minimise the influence of extreme outlying values which may be due to CRF completion errors.

Baseline and follow-up CRF forms were designed to collect concomitant medications at baseline and throughout the trial. Six drugs/classes of medications (metformin, statins, vitamin D, bisphosphonates, proton pump inhibitors and H2 (histamine receptor 2) antagonists) were selected for more detailed data collection because of emerging evidence of their anti-cancer activity (chapter 1, section 1.4). Data collected includes dose and frequency information, which could be used to inform the design of further studies, as was the case for aspirin. Data on all other medications were collected using a free-text. This analysis focuses on the collection of information on metformin. An end of run-in CRF form was designed to collect data on adherence from diary cards, a participant interview, and returned tablet blister packs. CRF forms also collected data on the toxicities experienced and whether the patient was randomised in the trial, and the reason if they were not randomised.

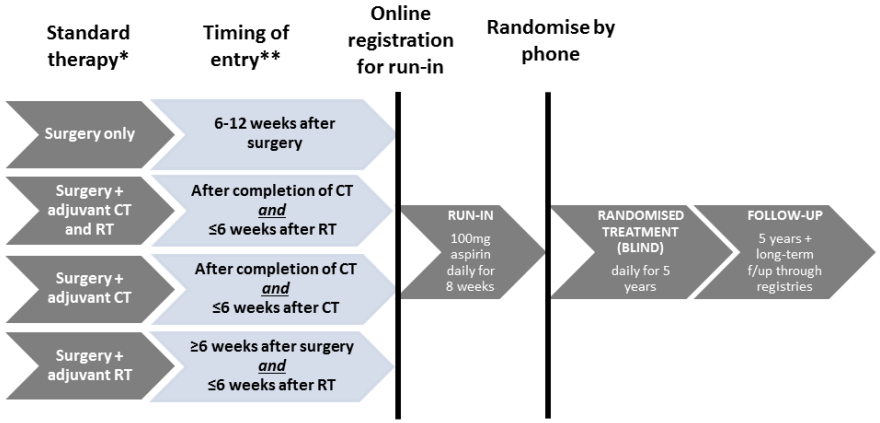
4.2.4 Data extraction

Data was extracted from the trial database for all participants registered up to and including the 500th participant for whom end of run-in data had been received. This means that data was also extracted to include those participants registered during that time for whom a registration or an end of run-in CRF form was outstanding (an additional 104 participants). This was done because CRF return rate was better in those who were randomised than in those who were not, and so by including all participants, regardless of whether a registration or end of run-in

CRF form is available, an unbiased representation of the randomisation rates is obtained. Tables summarising baseline characteristics, adherence during the run-in period, toxicity during the run-in period and concomitant metformin use are just based on the first 500 participants with registration and end of run-in data available.

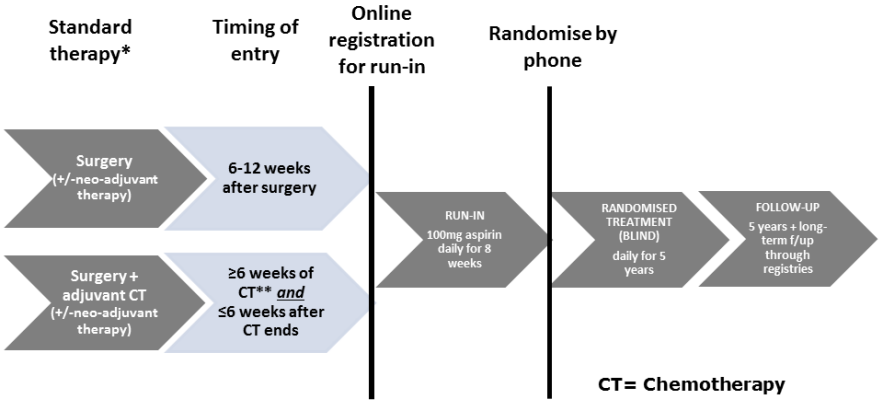
The date of randomisation for all included participants was extracted from the trial randomisation server. Permission for data release was sought from the Add-Aspirin Trial Management Groups (TMGs) for each cohort, the Trial Steering Committee (TSC) and Independent Data Monitoring Committee (IDMC).

Figure 4.1 Breast cohort timing of entry flow diagram.



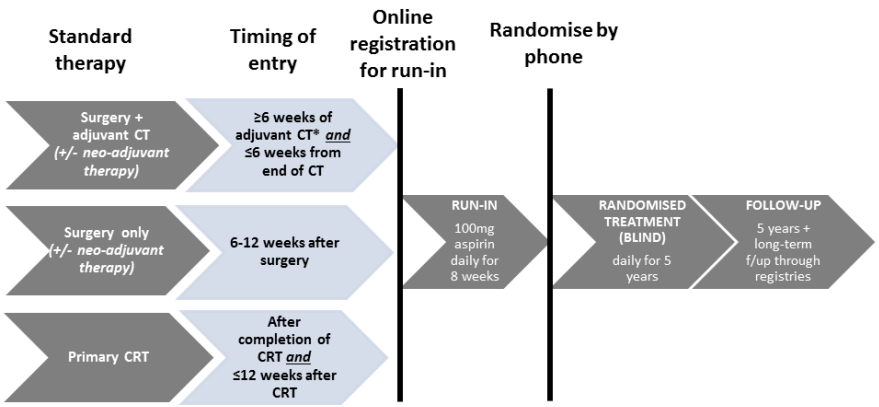
* Neo-adjuvant therapy is permitted and does not alter timing of trial registration
**Adjuvant endocrine, radiotherapy or HER-2 based therapy can be ongoing at trial registration
MRC CTU @ UCL
CT= Chemotherapy, RT= Radiotherapy

Figure 4.2 Colorectal cohort timing of entry flow diagram.



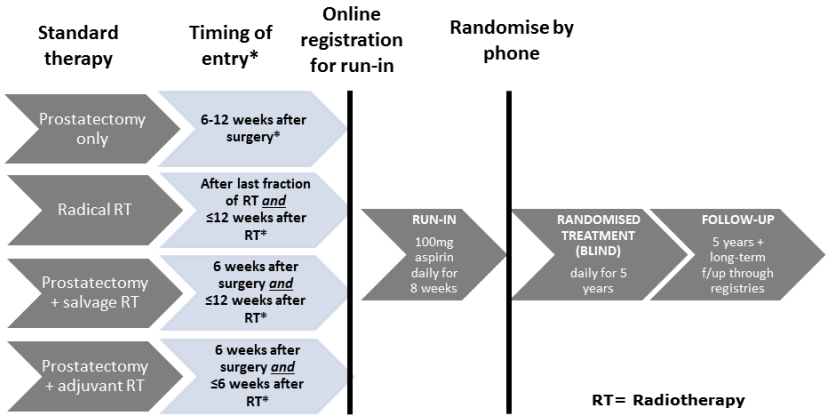
* Neo-adjuvant therapy is permitted and does not alter timing of trial registration
**If registration takes place whilst chemotherapy is ongoing platelet count should be ≥100 x 10^9/L on day 1 of each preceding cycle

Figure 4.3 Gastro-oesophageal cohort timing of entry flow diagram.



* If registration takes place whilst chemotherapy is ongoing, platelet count should be >100 x 109/L on day 1 of each preceding cycle.
CT= Chemotherapy, RT= Radiotherapy, CRT= Chemoradiotherapy
MRC CTU @ UCL

Figure 4.4 Prostate cohort timing of entry flow diagram.



MRC CTU @ UCL
* Adjuvant androgen deprivation therapy and adjuvant or salvage radiotherapy can be ongoing at trial registration

4.3 Results

4.3.1 Patient characteristics at registration

Tables 4.1 to 4.4 summarise the key characteristics at registration for the first 500 trial participants with registration and end-of run-in data, by cohort.

Table 4.1 Characteristics at registration— breast cohort

		n	**%**
Gender	Male	2	<1%
(n=252)	Female	250	>99%
Age at registration	Median (IQR)	53 (47 - 62)	
(n=252)	Range	27 - 87	
Primary surgery	Mastectomy	103	41%
(n=252)	Breast Conserving surgery	149	59%
Morphological type	Invasive lobular	34	13%
(n=252)	Invasive ductal	195	77%
	Mixed invasive lobular/ ductal	10	4%
	Missing	13	5%
Tumour grade	Well	20	8%
(n=252)	Moderate	88	35%
	Poor	126	50%
	Missing	18	7%
Lymphovascular	Yes	108	43%
invasion	No	126	50%
(n=252)	Unknown	18	7%
Pathological stage*	IA	42	17%
(TNM 7th edition)	IB	14	6%
(n=252)	IIA	60	24%
	IIB	67	27%
	IIIA	52	21%
	IIIB	1	<1%
	IIIC	16	6%
HER-2 / ER status	HER-2 negative / ER negative	60	24%
(n=252)	HER-2 negative / ER positive	126	50%
	HER-2 positive / ER negative	24	10%
	HER-2 positive / ER positive	38	15%
	Missing	4	2%
Chemotherapy received	No chemotherapy	14	6%
(n=252)	Adjuvant	181	72%
	Neo-adjuvant	56	22%
	Both neo-adjuvant and adjuvant	1	<1%
Hormone therapy	Not to be received	5	3%
(ER / PR positive patients)	Adjuvant	161	94%
(n=171)	Neo-adjuvant	1	<1%
	Both	3	2%
	Missing	1	<1%

*Footnote: *Neo-adjuvant chemotherapy resulted in the inclusion of some patients with very early T stage.*

Data on the baseline characteristics of breast cohort participants are generally consistent with data on populations studied in other adjuvant breast cancer trials (133-135), and there is no comparable national audit data available. Notably, the percentage of patients receiving neoadjuvant chemotherapy is higher than in the AZURE trial, (6% in AZURE vs 22% in Add-

Aspirin), which has similar eligibility criteria (133). This may reflect a recent trend towards the greater use of neoadjuvant chemotherapy for early stage breast cancer (136). Additionally, the proportion of ER positive patients entering the Add-Aspirin trial (65%) is consistently lower than in similar studies (78% in AZURE trial (133), and 69% in the TACT trial (134)), which may slightly alter the anticipated event rate.

Table 4.2 Characteristics at registration— colorectal cohort

		n	**%**
Gender ***(n=125)***	Male	70	56%
	Female	55	44%
Age at registration ***(n=125)***	Median (IQR)	61 (54 - 69)	
	Range	32 - 86	
Site of main tumour ***(n=125)***	Proximal Colon	38	30%
	Distal colon	52	42%
	Rectum	35	28%
Synchronous site of disease ***(n=125)***	No	114	91%
	Yes	7	6%
	Missing	4	3%
Type of surgery (rectal cancer only) ***(n=35)***	Abdominal perineal resection	4	11%
	Anterior Resection	30	86%
	Extralevator abdominoperineal excision	1	3%
Emergency operation ***(n=125)***	No	107	86%
	Yes	18	14%
Resection of liver metastases (R0) ***(n=125)***	No	122	98%
	Yes	3	2%
Pathological stage* (TNM 5th edition) ***(n=125)***	0	1	<1%
	I	2	2%
	IIA	23	18%
	IIB	9	7%
	IIIA	11	9%
	IIIB	58	46%
	IIIC	18	14%
	IV	3	2%
Differentiation grade ***(n=125)***	Well	12	10%
	Moderate	100	80%
	Poor	9	7%
	Missing	4	3%
Lymphovascular invasion ***(n=125)***	Present	39	31%
	Absent	42	34%
	Unknown	43	34%
	Missing	1	<1%

*Footnote: *Neo-adjuvant chemotherapy resulted in the inclusion of some patients with very early T stage.*

Data on the baseline characteristics of colorectal cohort participants are in keeping with data on populations studied in other adjuvant colorectal cancer trials (137, 138) and the National Bowel Cancer Audit Annual Report 2016 (139). However, one noticeable difference is that National Bowel Cancer Audit data suggests that in the UK, 9.5% of patients with potentially operable colorectal cancer (non-metastatic or resectable liver metastases) undergo a liver

resection for metastatic disease (139, 140), however only 2% of participants registered for Add-Aspirin had undergone resection of liver metastases. It is possible that this difference reflects concerns at site about the potential toxicity of aspirin in those who have undergone liver surgery. However, the proportion of participants who underwent emergency surgery was similar to that identified in UK colorectal cancer registration databases (16.1% (141)), suggesting investigators are not deterred from entering patients who may have a more complex surgical pathway. It is also possible that investigators are not aware of the eligibility of this group.

Table 4.3 Characteristics at registration— gastro-oesophageal cohort

		n	**%**
Gender	Male	13	68%
(n=19)	Female	6	32%
Age at registration	Median (IQR)	67 (56 - 72)	
(n=19)	Range	30 - 77	
Site of disease	Upper third of oesophagus	0	0%
(n=19)	Middle third of oesophagus	4	21%
	Lower third of oesophagus	9	47%
	Siewert type I	1	5%
	Siewert type II	0	0%
	Siewert type III	0	0%
	Fundus of stomach	2	11%
	Body of stomach	3	16%
Histology	Adenocarcinoma	13	68%
(n=19)	Squamous	6	32%
Differentiation grade	Moderate	5	26%
(n=19)	Poor	11	58%
	Missing	3	16%
Lymphovascular invasion	Yes	6	32%
(n=19)	No	4	21%
	Unknown	8	42%
	Missing	1	5%
Primary therapy	Surgery	14	74%
(n=19)	Chemoradiotherapy	5	26%
Surgical procedure	Transhiatal oesophagectomy	1	7%
(n=14)	Transthoracic oesophagectomy	3	21%
	Oesophagogastrectomy	2	14%
	Total gastrectomy	1	7%
	Sub-total gastrectomy	4	29%
	Other	3	21%
Surgical technique	Open operation	12	86%
(n=14)	Missing	2	14%

Interpretation of the baseline characteristics of gastro-oesophageal cohort participants are limited by the number of participants (*n=19*). Once more data is available, this can be compared to data from the National Oesophago-Gastric Cancer Audit Annual Report 2016 (142).

Table 4.4 Characteristics at registration— prostate cohort

		n %
Age at registration *(n=104)*	Median (IQR)	68 (62 - 73)
	Range	46 - 82
D'Amico risk classification *(n=104)*	Intermediate Risk	45 43%
	High Risk	56 54%
	Missing	3 3%
Pre-treatment stage (TNM 7th edition) *(n=104)*	IIA	23 22%
	IIB	51 49%
	III	29 28%
	Missing	1 <1%
Gleason score * *(n=104)*	3+3	10 10%
	3+4	43 41%
	3+5	4 4%
	4+3	25 24%
	4+4	11 11%
	4+5	9 9%
	5+4	2 2%
Hormone therapy *(n=104)*	None	40 38%
	Neo-adjuvant only	17 16%
	Adjuvant only	8 8%
	Neo-adjuvant and adjuvant	37 36%
	Missing	2 2%
Total duration of planned Hormone therapy *(n=62)*	Less than 6 months	20 32%
	1 year	6 10%
	2 years	13 21%
	3 years	22 35%
	Missing	1 2%
Primary treatment type *(n=104)*	Prostatectomy	39 38%
	Radical radiotherapy	65 62%
PARTICIPANTS UNDERGOING SURGERY ONLY *(n=39)*		
Surgery type *(n=39)*	Open radical prostatectomy	7 18%
	Laparoscopic radical prostatectomy	9 23%
	Robotic radical prostatectomy	23 59%
Resection margin clear (R0) *(n=39)*	No	9 23%
	Yes	29 74%
	Missing	1 3%
Salvage radiotherapy following prostatectomy *(n=39)*	No	33 85%
	Yes	5 13%
	Missing	1 3%

** Second score is maximum of the secondary pattern and tertiary pattern (if performed)*

Data on the baseline characteristics of prostate cohort participants are generally consistent with data from the National Prostate Cancer Audit Annual Report 2016 (143) and similar trials in patients undergoing radical prostatectomy (144), and radical radiotherapy (145). Notable differences include the proportion of patients undergoing a prostatectomy compared to radical chemoradiotherapy. 46.3% (2525/5452) of the men having radical treatment in England in 2016 had a prostatectomy, whereas 38% of participants had a prostatectomy in the Add-Aspirin trial. There are fewer surgeons (who manage the prostatectomy pathway) have registered as cohort leads than oncologists (who manage the radical radiotherapy pathway)

which could account for this difference, and might be addressed by promoting the trial amongst surgeons.

4.3.2 Run-in period

4.3.2.1 Duration of the run-in period

The duration of the initial run-in period, and details of run-in extensions, for the first 500 participants for whom registration and end of run-in data are available, is summarised in table 4.5.

Table 4.5 Duration of the run-in period

	Breast (*n=252*)	Colorectal (*n=125*)	Gastro-oesophageal (*n=19*)	Prostate (*n=104*)	**Total (*n=500*)**
Length of initial run-in period (days)					
n	248	123	19	103	**493**
Median (IQR)	56 (53-59)	56 (54-60)	56 (52-58)	56 (52-60)	**56 (53-59)**
Range	0 - 79	14 - 79	10 - 63	1 - 97	**0 - 97**
Missing	4	2	0	1	**7**
<40 days	13 5%	4 3%	1 5%	6 6%	**24 5%**
40-49 days	16 6%	4 3%	1 5%	7 7%	**28 6%**
50-59 days	159 63%	84 67%	15 79%	64 62%	**322 64%**
60-69 days	54 21%	28 22%	2 11%	23 22%	**107 21%**
70+ days	6 2%	3 2%	0 0%	3 3%	**12 2%**
Missing	4 2%	2 2%	0 0%	1 <1%	**7 1%**
RUN-IN PERIOD EXTENSIONS					
Run-in extension approved	0	4	0	2	**6**
Extension length					
4 week	0	4	0	1	**5**
8 week	0	0	0	1	**1**

The median duration of the run-in period was 56 days for all cohorts, which was exactly equal to the recommended duration. Some flexibility was permitted (+/-14 days) to fit with patient and appointment availability, and 91% of run-in period durations fell within that +/-14 day window. The duration fell short of 40 days in 5% (*n=24*) of participants. This was due to the run-in period being discontinued early for 23 participants, and data on the remaining participant is incomplete. For 2% (*n=12*) of participants, the duration of the run-in period was 70 days or over. Restricting eligibility for randomisation to a run-in duration of 56 +/-14 days would make the way adherence and tolerance is assessed more consistent.

A run-in period extension was only awarded six times (1.2% of participants). Two four week extensions were awarded so that aspirin could be withheld to allow for stoma reversal (both

participants were subsequently randomised). Two four week extensions were awarded to allow optimisation of blood pressure control in participants identified as hypertensive (one participant was randomised, the other was not). One four week extension was awarded to allow temporary NSAID treatment for pain (with subsequent randomisation). One eight week run-in period extension was awarded because the participant developed bleeding from haemorrhoids (who subsequently developed grade 2 tinnitus and was not randomised). Overall 67% of run-in extensions led to randomisation.

4.3.2.2 Adherence during the run-in period

Three sources of adherence data were available for 55.8% of participants (*n=279*), with two sources available for 22.0% (*n=110*), one source for 11.2%. No adherence data was available for 11% (*n=55)*, none of whom were randomised. Adherence during the run-in period from each of the three available sources (participant interview, blister packs and participant diary card) is summarised in figure 4.6. This data is based on the initial run-in attempt, rather than any extension.

Table 4.6 Adherence during the run-in period

	Breast (*n=252*)		Colorectal (*n=125*)		Gastro-oesophageal (*n=19*)		Prostate (*n=104*)		**Total (*n=500*)**	
Availability of adherence data										
n	252		125		19		104		**500**	
Participant reported	217	86%	115	92%	15	79%	90	87%	**437**	**87%**
Blister packs	156	62%	81	65%	9	47%	57	55%	**303**	**61%**
Diary card	184	73%	102	82%	13	68%	74	71%	**373**	**75%**
Run-in adherence based on participant interview										
n	217		115		15		90		**437**	
100%	138	64%	78	68%	10	67%	66	73%	**292**	**67%**
90% to <100%	54	25%	29	25%	3	20%	17	19%	**103**	**24%**
80% to <90%	12	6%	5	4%	1	7%	1	1%	**19**	**4%**
<80%	13	6%	3	3%	1	7%	6	7%	**23**	**5%**
Run-in adherence based on blister packs										
n	156		81		9		57		**303**	
100%	98	63%	49	60%	7	78%	43	75%	**197**	**65%**
90% to <100%	34	22%	23	28%	1	11%	10	18%	**68**	**22%**
80% to <90%	10	6%	4	5%	1	11%	1	2%	**16**	**5%**
<80%	14	9%	5	6%	0	0%	3	5%	**22**	**7%**
Run-in adherence based on diary card										
n	184		102		13		74		**373**	
100%	117	64%	69	68%	10	77%	52	70%	**248**	**66%**
90% to <100%	44	24%	23	23%	2	15%	19	26%	**88**	**24%**
80% to <90%	10	5%	5	5%	1	8%	1	1%	**17**	**5%**
<80%	13	7%	5	5%	0	0%	2	3%	**20**	**5%**

Participant interview, diary card and blister pack based adherence data was available for 87%, 75% and 61% of participants respectively. Overall, only 5% of participants had less than 80% adherence, (the cut-off for acceptable adherence in the run-in period) and this was similar across the three sources of adherence data. There were no notable differences in adherence data return between the breast, colorectal and prostate cohorts, and meaningful comparison with the gastro-oesophageal cohort was limited by small numbers. Tables 4.7 to 4.9 show a comparison of adherence sources.

Table 4.7 Comparison of adherence sources across cohorts

	Breast (*n=252*)		Colorectal (*n=125*)		Gastro-oesophageal (*n=19*)		Prostate (*n=104*)		**Total (*n=500*)**	
Comparison of adherence from participant interview and blister packs										
n	155		81		9		55		**300**	
Identical adherence from both sources	128	83%	71	88%	7	78%	50	91%	**256**	**85%**
Different but within +/-5%	14	9%	8	10%	2	22%	3	5%	**27**	**9%**
Different but within +/-10%	6	4%	0	0%	0	0%	1	2%	**7**	**2%**
Different by +/-10% or more	7	5%	2	2%	0	0%	1	2%	**10**	**3%**
Comparison of adherence from participant interview and diary card										
n	181		101		11		72		**365**	
Identical adherence from both sources	168	93%	90	89%	11	100%	67	93%	**336**	**92%**
Different but within +/-5%	7	4%	6	6%	0	0%	2	3%	**15**	**4%**
Different but within +/-10%	4	2%	2	2%	0	0%	3	4%	**9**	**2%**
Different by +/-10% or more	2	1%	3	3%	0	0%	0	0%	**5**	**1%**
Comparison of adherence from blister packs and diary card										
n	143		78		8		53		**282**	
Identical adherence from both sources	120	84%	64	82%	7	88%	45	85%	**236**	**84%**
Different but within +/-5%	9	6%	8	10%	1	13%	4	8%	**22**	**8%**
Different but within +/-10%	7	5%	1	1%	0	0%	3	6%	**11**	**4%**
Different by +/-10% or more	7	5%	5	6%	0	0%	1	2%	**13**	**5%**

Footnote: Based on those participants for whom percentage adherence was calculable.

Table 4.8 Statistical comparison of adherence sources

Adherence comparison	Participants	Correlation coefficient	p-value
Participant interview vs. blister packs	300	0.75	<0.0001
Participant interview vs. diary card	365	0.91	<0.0001
Blister packs vs. diary card	282	0.67	<0.0001

Footnotes: The correlation coefficient is a value between -1 and 1 which represents the strength of association between two variables, with 1 representing perfect "agreement", -1 representing perfect "disagreement" and 0 representing no correlation at all. The p-values given are a test of the hypothesis that there is no correlation.

Table 4.9 Comparison of level of reported adherence between sources

	n	%
Adherence based on participant interview vs blister packs		
n	300	
Identical adherence	256	85%
Blisters better than participant reported	18	6%
Participant reported better than blisters	26	9%
Adherence based on participant interview vs diary card		
n	365	
Identical adherence	336	92%
Diary card better than participant reported	6	2%
Participant reported better than diary card	23	6%
Adherence based on blister packs vs diary card		
n	282	
Identical adherence	236	84%
Diary card better than blisters	23	8%
Blisters better than diary card	23	8%
TOTAL	**500**	

To investigate consistency between the three adherence data sources, a correlation coefficient between each was calculated and the results are available in table 4.8. This shows a significant correlation between each of the adherence sources. The highest level of correlation in adherence data was found between the participant interview and diary card, suggesting participants may be using the diary card to inform verbally reported adherence at interview, rather than their used blister packs. There is no evidence of any systematic over, or under-reporting of adherence between sources (table 4.9). Given these findings, to simply the run-in period process, the blister pack assessment of adherence could be removed which would reduce the burden to participants and site staff.

4.3.2.3 Patient reported reasons for missed doses

At the end of run-in assessment, 32% of participants reported missing doses. The most frequent reason given was forgetting doses (57%) followed by toxicity (19%). The explanations given were similar for each tumour cohort, with the exception of the gastro-oesophageal cohort, where toxicity was given as a reason more frequently (43%), however the number of participants was too small for meaningful interpretation (*n=3*). The reasons reported for missing doses are shown in table 4.10.

Table 4.10 Patient reported reasons for missed doses

	Breast (*n=252*)	Colorectal (*n=125*)	Gastro-oesophageal (*n=19*)	Prostate (*n=104*)	**Total (*n=500*)**
Any doses missed					
No	161 64%	86 69%	12 63%	72 69%	**331 66%**
Yes	87 35%	35 28%	7 37%	30 29%	**159 32%**
Missing	4 2%	4 3%	0 0%	2 2%	**10 2%**
Reasons for missed doses (more than one reason allowed)					
n	87	35	7	30	**159**
Due to toxicity	18 21%	5 14%	3 43%	4 13%	**30 19%**
Due to other reasons	76 87%	34 97%	6 86%	27 90%	**143 90%**
Main other reason for missed doses (one reason per participant)					
Forgotten doses of aspirin	47 54%	24 69%	2 29%	18 60%	**91 57%**
Patient choice not to take aspirin	3 3%	1 3%	0 0%	3 10%	**7 4%**
Lost aspirin tablets	1 1%	0 0%	0 0%	0 0%	**1 <1%**
Other reason for missed doses	25 53%	9 26%	4 57%	6 20%	**44 28%**

4.3.2.4 Toxicity reported during the run-in period

In order to fully illustrate patient safety during the run-in period, any toxicity reported during the run-in period or any extension was included in the analysis. Table 4.11 shows all toxicities reported during the run-in period.

Table 4.11 Toxicity reported during the run-in period

Toxicity	CTCAE v4 grade	Breast (*n=252*)	Colorectal (*n=125*)	Gastro-oesophageal (*n=19*)	Prostate (*n=104*)	**Total (*n=500*)**
Allergic reaction to aspirin	Grade 1-2	0 (0%)	1 (<1%)	0 (0%)	0 (0%)	**1 (<1%)**
Anaemia	Grade 1-2	4 (2%)	5 (4%)	1 (5%)	7 (7%)	**17 (3%)**
Bleeding gums	Grade 1-2	8 (3%)	5 (4%)	0 (0%)	1 (<1%)	**14 (3%)**
Bruising	Grade 1-2	31 (12%)	7 (6%)	1 (5%)	6 (5%)	**45 (9%)**
Dyspepsia	Grade 1-2	31 (12%)	14 (11%)	2 (11%)	15 (14%)	**62 (12%)**
	Grade 3-4	0 (0%)	1 (<1%)	0 (0%)	0 (0%)	**1 (<1%)**
Haematuria	Grade 1-2	1 (<1%)	0 (0%)	0 (0%)	0 (0%)	**1 (<1%)**
Lower gastrointestinal bleeding	Grade 1-2	0 (0%)	1 (<1%)	1 (5%)	0 (0%)	**2 (<1%)**
Nose bleed (epistaxis)	Grade 1-2	10 (4%)	10 (8%)	0 (0%)	4 (4%)	**24 (5%)**
Tinnitus	Grade 1-2	5 (2%)	4 (3%)	3 (16%)	2 (2%)	**14 (3%)**

The majority of toxicities reported were grade 1 or 2, with only a single grade 3 toxicity (dyspepsia in a colorectal cohort participant). Only six participants (1.2%) experienced toxicities that mandated permanent discontinuation of the run-in period. These included grade 3 dyspepsia (*n=1*), grade 2 allergic reaction to aspirin (*n=1*), grade 2 tinnitus *(n=3),* and grade 1 lower gastrointestinal bleeding (*n=1*). Randomisation was permitted for one participant

reporting grade 1 lower gastrointestinal bleeding after a central review of clinical information establishing that the bleeding had been from the mucocutaneous junction of a stoma which had subsequently healed and was therefore low risk. Nine participants reported grade 1 tinnitus, however it was unestablished whether the onset of the tinnitus pre-dated the run-in period, and randomisation went ahead. A protocol amendment was subsequently made to only mandate permanent discontinuation in new cases or worsening tinnitus (grade 2 or above), and grade 3 or 4 lower gastrointestinal bleeding.

In the breast cohort, bruising (12%) was more frequent than in the other cohorts (5-6%). There is data to suggest that bruising is more common in women taking aspirin than men (146) which could account for this observation. In the colorectal cohort, epistaxis (8%, *n=10*) was reported more frequently than in the other cohorts (0-4%). Chemotherapy is also a common cause for epistaxis, and all 10 participants developing nose-bleeds had adjuvant chemotherapy, five of whom had ongoing chemotherapy at the time, which may explain this finding. In the gastro-oesophageal cohort, tinnitus (16%) was reported more frequently than in the other cohorts (2-5%). Whilst small numbers (*n=3*) limit interpretation, this finding does advocate further monitoring, as this is the only cohort who receive a cisplatin containing chemotherapy regimen, which is commonly associated with the development of tinnitus. In the prostate cohort, anaemia (7%, *n=7*) was reported more frequently than in the other cohorts (2-5%). Subclinical rectal and urinary blood loss are a known complication of radical prostate cancer treatment (147), and the contribution of aspirin requires further investigation in the randomised phase.

4.3.2.5 Run-in period outcomes

The outcomes of the run-in period are based on all participants registered up to and including the 500th participant for whom end of run-in data has been received, including those registered during that time where an end of run-in form has not been received, but are expected to have completed the run-in period (an extra 104 participants). This is to avoid biasing results based on data return, which is expected to be lower for non-randomised participants.

88% (*n=534/604*), of the participants registered, were randomised at the time of data extraction. There were 18 participants without CRF data confirming non-randomisation,

however sufficient time had passed to make randomisation impossible, and as such, those participants were included in the analysis as non-randomised. The proportion of participants going on to be randomised following the run-in period is similar in all four cohorts, and slightly below the 90% that was anticipated at the start of the trial (88%, 91%, 74%, and 89% in the breast, colorectal, gastro-oesophageal and prostate cohorts respectively). Table 4.12 summarises the run-in period outcomes and the reasons reported for non-randomisation.

Table 4.12 Run-in period outcomes

	Breast (*n=304*)		Colorectal (*n=145*)		Gastro-oesophageal (*n=23*)		Prostate (*n=132*)		**Total (*n=604*)**	
Run-in outcome										
n	304		145		23		132		**604**	
Not-randomised	36	12%	13	9%	6	26%	15	11%	**70**	**12%**
Randomised	268	88%	132	91%	17	74%	117	89%	**534**	**88%**
Reason for non-randomisation (more than one reason may apply)										
n	36		13		6		15		**70**	
Participant choice	19		4		1		4		**28**	
Toxicity or adverse event	15		2		3		6		**26**	
Inadequate adherence to protocol treatment	4		2		0		0		**6**	
Usage of non-permitted medication	1		1		0		2		**4**	
Other reason	4		4		3		4		**15**	
No reason given	1		1		0		0		**2**	
Missing	9		4		1		4		**18**	
Combinations of reasons given for non-randomisation:										
n	25		5		4		6		**40**	
Toxicity only	3	12%	1	20%	2	50%	2	33%	**8**	**20%**
Participant choice only	8	32%	3	60%	0	0%	0	0%	**11**	**28%**
Toxicity and participant choice	9	36%	1	20%	0	0%	4	67%	**14**	**35%**
Other combination of reasons	5	20%	0	0%	2	50%	0	0%	**7**	**18%**

Toxicity was given as the reason for non-randomisation for 26 participants. The nature of the toxicity experienced only mandated non-randomisation for six participants (table 4.11), therefore for the remainder (*n=20*), it is assumed that the toxicity experienced made it unacceptable to them to continue. This is consistent with the finding that both toxicity and participant choice were given as the reason for non-randomisation jointly (*n=14*), more frequently than they were given individually (toxicity alone *n=8*, participant choice alone *n=11*). Overall, the most frequent reason for non-randomisation was participant choice (n=28). It seems likely that this group would be at highest risk of discontinuing the trial prematurely, and as such, their non-randomisation prevents this happening during the main trial and fulfils one

of the main objectives of the run-in period. This CRF question has now been re-written to ask the main reason for non-randomisation, as the ability to select more than one reason doesn't reveal which is most important.

4.3.3 Timing of trial registration

4.3.3.1 Treatment pathway and early and late registrations by cohort

An analysis of the treatment pathway taken by participants, in relation to the timing of their registration, was conducted for the first 500 participants for whom end of run-in data had been received. The treatment pathway taken, and the number or registrations occurring outside the timing of entry window are shown in table 4.13.

Table 4.13 Timing of trial registration according to cohort and pathway

Cohort	Pathway*	N	Timing of entry criteria	Early registrations		Late registrations	
				N (%)	Min—max days early	N (%)	Min—max days late
Breast *(n=252)*	Surgery	4 (2%)	6-12 weeks after surgery	**0 (0%)**	-	**1 (25%)**	1
	Surgery + adjuvant CT	21 (8%)	After completion of CT *And* ≤6 weeks after CT	**0 (0%)**	-	**2 (10%)**	5-9
	Surgery + adjuvant RT	66 (26%)	≥6 weeks after surgery *And* ≤6 weeks after RT	**2 (3%)**	1-9	**3 (5%)**	1-7
	Surgery + adjuvant CT + adjuvant RT	161 (64%)	After completion of CT *And* ≤6 weeks after RT	**0 (0%)**	-	**5 (3%)**	2-13
Colorectal *(n=125)*	Surgery	12 (10%)	6-12 weeks after surgery	**0 (0%)**	-	**0 (0%)**	-
	Surgery + adjuvant CT	113 (90%)	≥6 weeks of CT *and* ≤6 weeks after CT ends	**3 (3%)**	1	**14 (12%)**	3-22
Gastro-oesophageal *(n=19)*	Primary CRT	5 (26%)	After completion of CRT *and* ≤12 weeks after CRT	**0 (0%)**	-	**0 (0%)**	-
	Surgery	10 (53%)	6-12 weeks after surgery	**0 (0%)**	-	**3 (30%)**	36-102***
	Surgery + adjuvant CT	4 (21%)	≥6 weeks of adjuvant CT *and* ≤6 weeks from end of CT	**1 (25%)**	22	**0 (0%)**	-
Prostate† *(n=90)***	Radical RT	51** (57%)	After last fraction of RT *and* ≤12 weeks after RT	**0 (0%)**	-	**2 (4%)**	2-6
	Prostatectomy	34 (38%)	6-12 weeks after surgery	**0 (0%)**	-	**1 (3%)**	10
	Prostatectomy + adjuvant RT	0	6 weeks after surgery *and* ≤6 weeks after RT	**-**	-	**-**	-
	Prostatectomy + salvage RT	5 (6%)	6 weeks after surgery *and* ≤12 weeks after RT	**0 (0%)**	-	**0 (0%)**	-

*Footnotes: *+/- neo-adjuvant therapy, ** an additional 9 patients have a missing radiotherapy end date and are therefore not included. *** possible data error. † an additional two patients were not included as the primary therapy was unclear. CT=chemotherapy, RT=radiotherapy, CRT=chemoradiotherapy.*

Across all tumour cohorts, there were very few participants registered before the timing of entry window (*1.2%, n=6*), however there were a more late registrations (*6%, n=31*). The Add-

Aspirin protocol was written to be pragmatic, recognising that not all clinical scenarios can be covered, and sites were directed to contact the trial team directly for discussion.

For patients undergoing surgery alone (in any tumour cohort), the last permitted point of registration was normally 12 weeks after completion of surgery. Late registrations for patients undergoing surgery alone in the breast and gastro-oesophageal cohorts were higher than anticipated (25% and 30% respectively), however this was not common in the prostate and colorectal cohorts (3% and 0% respectively). Interpretation is limited due to small numbers, but it is possible that the timing of entry window aligns poorly with the follow-up schedule for visits in breast and gastro-oesophageal cancer in some trusts, and thus requires further monitoring as the trial progresses.

For patients undergoing adjuvant chemotherapy without radiotherapy, the latest point of registration is six weeks after the final day of the final cycle of chemotherapy given. In the breast and colorectal cohorts, 10%, 12% were registered late respectively. It is possible that late registrations occur because additional time is required to recover from chemotherapy related toxicity in some patients.

The latest point of registration for pathways involving radiotherapy was 12 weeks after radical radiotherapy, and six weeks after adjuvant radiotherapy. There were very few late registrations in these pathways, suggesting the current window of trial entry is feasible.

4.3.3.2 Timing of registration for participants receiving adjuvant therapies

For some cohorts there is flexibility around which point in the treatment pathway patients register and start the run-in period. The timing of registration for participants receiving adjuvant therapies is summarised below in table 4.14.

Table 4.14 Timing of registration for participants receiving adjuvant therapies

Cohort	Pathway*	N	Adjuvant CT			Adjuvant RT			Salvage RT			Unclear
			Before*	During	After	Before	During	After	Before	During	After	
Breast (*n=248*)	Surgery + adjuvant CT	21			21 (100%)							0
	Surgery + adjuvant RT	66				9 (14%)	15 (23%)	41 (62%)				1 (2%)
	Surgery + adjuvant CT + adjuvant RT	161			160 (99%)	15 (9%)	27 (17%)	119 (74%)				1 (CT) (<1%)
Colorectal (*n=113*)	Surgery + adjuvant CT	113	2* (2%)	35 (31%)	75 (66%)							1 (<1%)
Gastro-oesophageal (*n=4*)	Surgery + adjuvant CT	4		2 (50%)	2 (50%)							0
Prostate (*n=5*)	Prostatectomy + adjuvant RT	0				-	-	-				-
	Prostatectomy + salvage RT	5								4 (80%)	1 (20%)	0

**Early registrations, CT=chemotherapy, RT=radiotherapy, CRT=chemoradiotherapy*

In the breast cohort, there was flexibility around the timing of registration with respect to delivery of adjuvant radiotherapy. The options to register before, during or after radiotherapy were all utilised, suggesting this approach was acceptable to participants and investigators.

In the colorectal cohort, participants were able to register whilst chemotherapy was ongoing (after two cycles with acceptable blood platelet counts), or once it had finished (and up to six weeks later). 31% of participants registered whilst chemotherapy was ongoing, 66% once chemotherapy was finished (2% registered early). There were initial concerns about the risk of chemotherapy induced dyspepsia when aspirin was given concomitantly. 14 participants developed dyspepsia in the colorectal cohort during the run-in period. The proportion developing dyspepsia who registered during chemotherapy (14%, 5/5 grade 1) was similar to the proportion who developed dyspepsia registering once chemotherapy had finished (12%, 8/9 grade 1, 1/9 grade 3). These findings could be used to reassure investigators of the risks of chemotherapy induced dyspepsia.

4.3.4 Metformin use at registration

There was a high level of data return on metformin use at trial registration (96%). At the time of registration, 4% of participants were using metformin overall, and the median total daily metformin dose reported was 1,000mg (IQR 625-1,650mg). Metformin use was twice as prevalent in the prostate compared to the breast cohort. This is likely to be a reflection of the increasing prevalence of DM with age, and the greater average age of those with prostate compared to breast cancer. Data on concomitant metformin use at the time of registration is summarised in table 4.15.

Table 4.15 Metformin use at registration

	Breast	Colorectal	Gastro-oesophageal	Prostate	Total
Participants in main analysis	252	125	19	104	500
Participants with metformin data	**241**	**120**	**19**	**101**	**481**
Current or previous metformin use at registration					
No	233 97%	115 96%	18 95%	95 94%	461 96%
Yes	8 3%	5 4%	1 5%	6 6%	20 4%
When was Metformin last taken?					
n	8	5	1	6	20
Within last week	8 100%	5 100%	1 100%	6 100%	20 100%
Frequency of use					
n	8	5	1	6	20
Daily	8 100%	5 100%	1 100%	6 100%	20 100%
Duration of use					
n	8	5	1	6	20
Less than or equal to 6 months	1 13%	1 20%	0 0%	2 33%	4 20%
Greater than 6 months	7 88%	4 80%	1 100%	4 67%	16 80%
Total daily dose (mg)					
n	7	5	1	5	18
Median	1000	2000	2000	500	1000
IQR	500 - 1500	1000 - 2000	2000 - 2000	500 - 1000	500 - 1700
Range	500 - 1700	1000 - 2500	2000 - 2000	500 - 1500	500 - 2500
Total daily dose (mg)					
n	8	5	1	6	20
500	2 25%	0 0%	0 0%	3 50%	5 25%
1000	3 38%	2 40%	0 0%	1 17%	6 30%
1500	1 13%	0 0%	0 0%	1 17%	2 10%
1700	1 13%	0 0%	0 0%	0 0%	1 5%
2000	0 0%	2 40%	1 100%	0 0%	3 15%
2500	0 0%	1 20%	0 0%	0 0%	1 5%
Missing	1 13%	0 0%	0 0%	1 17%	2 10%
TOTAL	**241**	**120**	**19**	**101**	**481**

4.4 Discussion

4.4.1 Baseline characteristics

Overall the characteristics of patients registering for the Add-Aspirin trial were similar to population data from national audits, and trials with similar eligibility criteria, however there were some notable differences, including a lower proportion, in the colorectal cohort, of participants with resection of liver metastases, and in the prostate cohort, of participants who had undergone a prostatectomy. This information can be used to direct recruitment strategies toward these under-represented groups, with the aim of making the trial representative of the wider population, and to ensure expected event rates are met to ensure the trial is powered as anticipated.

4.4.2 Design and utility of the run-in period

Overall, 88% of participants registered for the run-in period were randomised. The run-in period was effective in identifying 5% of participants who had less than 80% adherence, and 1.2% of participants who developed severe aspirin related toxicities, thus preventing their randomisation. Participants were also identified who choose not to be randomised, and who developed toxicities that were unacceptable to them, but where discontinuation was not mandated. This, along with the acceptability of a run-in period design, as demonstrated in chapter two, recommends continuation of the run-in period as the trial progresses.

The use of a run-in period extension was only used by 1.2% of participants, was useful in identifying a number of participants as suitable, who would otherwise had missed out unnecessarily. The duration of the run-in period was effective but restricting eligibility for randomisation to a run-in duration of 56 +/-14 days would make the way adherence and tolerance is assessed more consistent. Establishing accurate adherence rates is challenging, however the methods used showed a high level of concordance. The interview and diary card have the potential to be more prone to reporting and/or recall bias, which would favour the use of blister packs as a more objective measure of adherence, however there was no systematic over, or under-reporting of adherence between sources identified. The collection of used

blister packs didn't appear to provide any additional adherence information, and the data return rate was lower than the other two adherence sources, therefore discontinuing data collection from used blister packs is advocated.

A number of early signals suggest there may be patterns of toxicity for individual cohorts, including, epistaxis in those who have had chemotherapy, tinnitus in the gastro-oesophageal cohort (possibly related to cisplatin use), anaemia in the prostate cohort and bruising in the breast cohort. Further monitoring of these toxicities as the trial progresses is recommended.

4.4.3 Timing of registration

For most treatment pathways, there were very few early or late registrations suggesting that registering patients within the current timing of entry criteria was feasible. Exceptions include those who undergo surgery alone, and those having adjuvant chemotherapy (without radiotherapy), where a notable proportion of patients register late. For those undergoing surgery alone, a survey of cohort leads who are surgeons could help identify the underlying reasons, and potential corrective strategies. For those undergoing adjuvant chemotherapy (without radiotherapy), directing sites to contact the central trial team to discuss the clinical circumstances or reason underlying the request for late registration could help identify the underlying reasons, and potential corrective strategies. This is preferable to extending the registration window, which will reduce the alignment with other treatment pathways.

Around a third of the colorectal cohort participants started the active run-in period whilst chemotherapy was ongoing, demonstrating the acceptability of this approach. No significant increase in the risk of chemotherapy induced dyspepsia when aspirin was given concomitantly with chemotherapy was demonstrated. In the breast cohort, it is currently mandated that the run-in period is initiated once chemotherapy is complete. Giving the option of starting the run-in period whilst chemotherapy is ongoing would align the cohorts more closely, and help with treatment/timing recommendations in the future should the use of aspirin in this setting be shown to be beneficial, and should be considered.

4.4.4 Metformin use in Add-Aspirin trial participants

Metformin use in participants in the Add-Aspirin trial was only 4% overall, and there was only slight variation in its use between tumour cohorts. This is useful in estimating the number of patients with these tumour types that would not be eligible for a metformin trial (because of existing metformin use). It also shows the feasibility and utility of collecting detailed data on key concomitant medications, however, given the time consuming nature of collecting such data, the number of concomitant medications needs to be as selective as possible. The evidence supporting the adjuvant use of metformin, and how it may inform the design of such a trial is explored in the next chapter.

4.5 Conclusion

Overall, 88% of participants were randomised which is in-keeping with the expected non-randomisation rate of 10%.The rate of serious bleeding complications is low, but the number of patient-years examined in this analysis is too small to make inferences on bleeding risk. This data confirms the viability of the trial but suggests some minor protocol amendments that may increase recruitment further and facilitate the conduct of the trial.

Chapter 5. A systematic review and meta-analysis of Metformin as an adjuvant treatment for cancer

5.1 Introduction

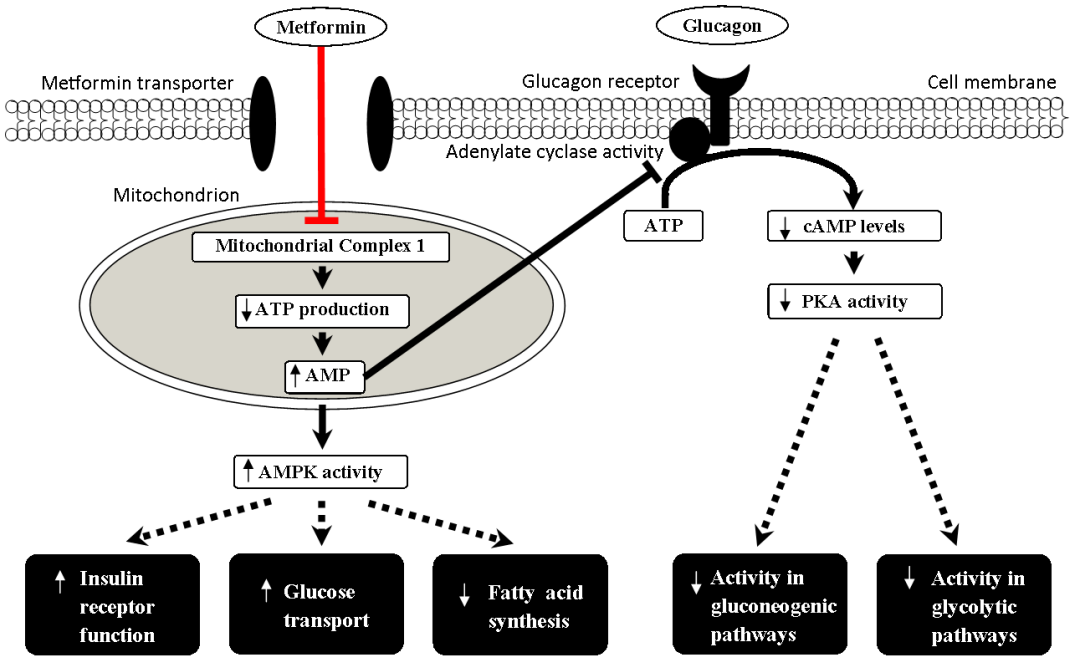
The oral drug metformin is the current first-line choice for the treatment of DM (148), and with an estimated 420 million diagnoses globally (149), metformin is now one of the most frequently prescribed generic medicines worldwide (150). Biguanides (a group of compounds of which metformin is one) were originally developed from Galegine, one of the active ingredients of the plant *Galega Officinalis*, also known as French Lilac, which is a traditional medicine used since the medieval era as a treatment of polyuria associated with DM (151). Metformin was first synthesised by chemists in 1922 (152), and discovered to induce hypoglycaemia in animals in 1929, but was overlooked as a potential treatment for DM until the 1950's (153) because of the greater glucose lowering properties of other biguanides like phenformin and buformin. It became available as a therapy for DM in the UK in 1958, but was not commonly used until the 1990's, initially due to the preference for insulin and other biguanides, and later, due to concerns about biguanide associated lactic acidosis (153), however further research established that metformin has a superior safety profile (153). It wasn't until 1995 that metformin was approved in the US by the FDA for the treatment of DM.

5.1.1 Mechanism of action of metformin in type II Diabetes Mellitus

In those with DM, metformin is thought to act mainly by addressing insulin resistance in the liver, through suppression of hepatic glucose production (gluconeogenesis) and opposing the hyperglycaemic effects of the hormone, glucagon. Metformin enters hepatocytes through solute transporters in the cell membrane (mostly organic cation transporter-1 (OCT1) (154)). Metformin then transiently inhibits the enzyme, Mitochondrial Complex I, which disrupts the electron transport chain (155) and reduces the availability of cellular energy by reducing levels of adenosine triphosphate (ATP) and increasing levels of its counterpart, adenosine monophosphate (AMP). The rise in levels of AMP promotes the activity of the adenosine monophosphate activated protein kinase (AMPK) pathway (156), which inhibit the cellular

mechanisms that perform gluconeogenesis and fatty acid synthesis (157). A additional consequence of the rise in AMP levels is disruption glucagon signalling. Under normal conditions, when the glucagon receptor is bound by glucagon, adenylate cyclase converts ATP to cyclic AMP (cAMP), which, in turn, increases protein kinase A (PKA) activity, resulting in increased glycolysis and gluconeogenesis. By increasing AMP levels, metformin inhibits glucagon induced formation of cAMP and thus reduces glycolysis and gluconeogenesis. Figure 5.1 describes the molecular pathway in hepatocytes by which metformin reduces circulatory blood glucose, increases insulin sensitivity, and improves DM associated dyslipidaemia.

Figure 5.1 The molecular pathway by which metformin lowers glucose production by hepatocytes

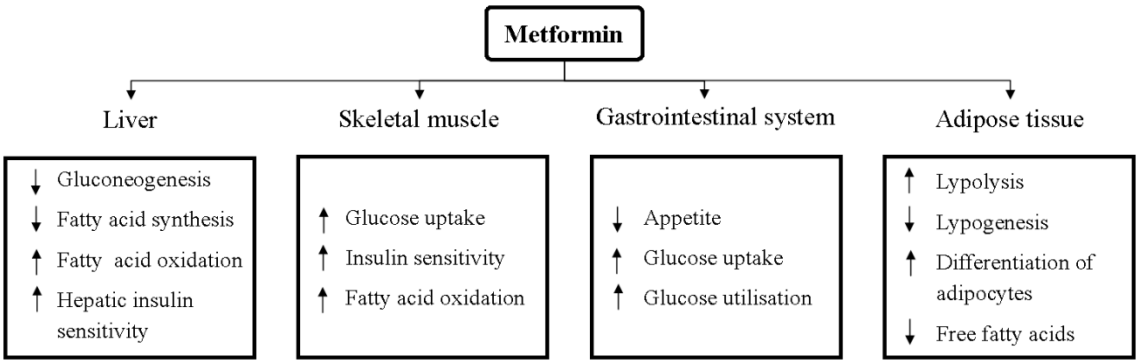


ATP= adenosine triphosphate, AMP= adenosine monophosphate , AMPK= AMP activated protein kinase, PKA= protein kinase A, cAMP= cyclic AMP.

Metformin is also proposed to have a number of additional mechanisms by which it counteracts the metabolic dysregulation seen in DM. In skeletal muscle, *in-vivo* studies show that increased AMPK signalling increases activity and translocation of glucose transporters to the cell membrane, particularly GLUT4, resulting in increased glucose uptake *(158, 159)*. In adipocytes, *in-vivo* studies show that raised AMPK activity increases glucagon-like peptide-1

(GLP1) and leptin levels (160), leading to reduced appetite (161). In studies in people without DM, metformin has been shown to reduce leptin levels, and as a consequence reduce centripetal adiposity, cholesterol levels and body weight (162). In the gastrointestinal tract, both animal and human studies have also shown that metformin increases lactate production in gastrointestinal cells suggesting an increase in anaerobic glucose metabolism (163, 164), however, this could also be a consequence of increased gastro-intestinal glucose uptake, which can be seen on Positron Emission Tomography (PET) imaging, where gastro-intestinal uptake of a 18-F-fluoro-deoxy glucose (a radiolabelled glucose analogue) is increased by metformin (165). Figure 5.2 describes the effects of metformin on glucose, insulin and lipid metabolism in different organ systems.

Figure 5.2 The effects of metformin on glucose, insulin and lipid metabolism



It has also been suggested that metformin may have beneficial effects for individuals with metabolic syndrome, a set of disorders which include raised fasting glucose, dyslipidaemia, high blood pressure, and central obesity (166). Metformin has also been investigated as a treatment for cardiovascular disease, non-alcoholic fatty liver disease and infertility in women with polycystic ovarian syndrome, (which are all associated with metabolic syndrome) however its use in these conditions remains controversial (167-169).

In 2004, the discovery that the tumour suppressor gene, liver kidney B1 (LKB1), is an upstream regulator of AMPK (170) inspired the first observational study investigating the anti-cancer effects of metformin. A large case-control study in Tayside, Scotland (*n=11,876*),

metformin was associated 23% reduction in the risk of developing cancer (OR 0.77, 95% CI 0.64-0.92), and a greater protective effect was observed with increasing duration of exposure to metformin and the number of prescriptions dispensed (171). This prompted an increase in research into the anti-cancer effects of metformin over the next decade.

5.1.2 Anti-cancer mechanistic hypothesis and *in-vitro* and *in-vivo* evidence

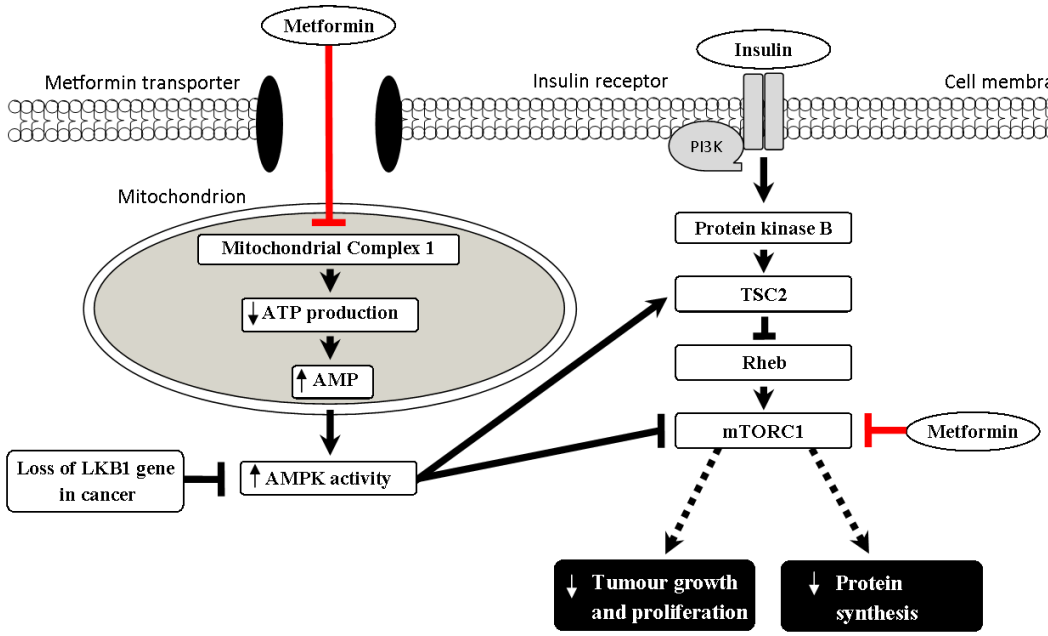
It has been proposed that that the anti-cancer properties of metformin result from both direct effects on cancer cells, and indirect systemic effects on the host (172). Many of the proposed indirect anti-cancer effects are a consequence of the metformin-induced metabolic changes, such as lower circulating glucose levels and increased insulin sensitivity, as described in the previous section. For example, mouse models have shown that high energy diets increase tumour growth (173), and metformin reduces glucose consumption in breast cancer xenographs (174). Metformin, by virtue of its glucose-lowering properties, could indirectly reduce the availability of energy to cancer cells thus reducing cancer proliferation and growth. However, contrary to this hypothesis, there is currently no evidence that other DM treatments, which also lower circulating glucose levels, have anti-cancer effects, in-fact a limited number of studies have suggested that sulphonylurea based DM therapies increase cancer incidence (175), and an observational study has also suggested that pioglitazone (one of the thiazolidinedione class of DM therapies), could increase the risk of bladder cancer (176), although this has not been substantiated in other studies (177, 178).

Unlike other treatments for DM, metformin is associated with a reduction in insulin levels. The ability of metformin to lower circulating insulin has also been proposed to underlie the anti-cancer effects of metformin, and while treatment with exogenous insulin is not known to adversely affect cancer outcomes (179), high levels of serum c-peptide (a marker of increased endogenous insulin secretion) have been shown to be associated with poor breast and prostate cancer outcomes (180, 181), suggesting endogenous insulin reduction may have a role in the anti-cancer effects of metformin. Another potential mechanism is the anti-inflammatory effects of metformin, where human studies have shown that it reduces tumour

necrosis factor (TNF-α) and interleukin 6 (IL-6) levels (182), Inflammatory cytokines including TNF-α and IL-6 are promoters of tumourogenesis (183). Metformin-induced systemic metabolic changes are a plausible anti-cancer mechanism, however translational studies aligned with phase III trials are needed if the contribution of indirect mechanisms are to be better understood.

Metformin may also have direct effects on cancer cells, though inhibition mediators in phosphatidylinositol-3-kinase/mammalian target of rapamycin (PI3K/mTOR) signalling, and LKB1 signalling, which are key oncogenic pathways. Both pathways are inhibited through activation of the AMPK pathway (184, 185), and also through direct inhibition of mTOR complex 1 (mTORC1) by metformin (186). Components of the PI3K/mTOR signalling pathway are mutated, amplified or translocated more than those in any other oncogenic pathway (187). LKB1 is a tumour suppressor gene, loss of which is associated with a number of epithelial malignancies, most commonly colorectal cancer as part of Peutz-Jeghers syndrome (a condition where there is significantly increased risk of intestinal polyp formation leading to colorectal cancer), but also breast, ovarian, liver and lung cancer (188). Figure 5.3 describes the proposed mechanism for the direct effects of metformin on cancer cells.

Figure 5.3 Direct anti-cancer effects of metformin



PI3K= phosphatidylinositol-3-kinase, TSC2= tuberous sclerosis complex 2, Rheb= RAS homologue enriched in brain, mTORC1= mammalian target of rapamycin complex 1, ATP= adenosine triphosphate, AMP= adenosine monophosphate, AMPK= AMP activated protein kinase, LKB1= liver kinase B1.

There is evidence that metformin has anti-cancer activity *in-vitro*. Metformin inhibits the growth and survival of cancer in a number of different cancer cell-lines (184, 189-193), however the metformin concentrations used (5-20mmol/L) are not achievable in humans (where serum concentrations are usually ≤0.5mmol/L (194)). It has been hypothesised that the requirement for supra-physiological concentrations of metformin to show that anti-cancer effects could be a consequence of the supra-physiological glucose concentrations used in cell cultures (189).

In-vivo models have studied the effect of metformin in HER-2 transgenic mice with mammary tumours (195) and prostate cancer xenograft mice (195), and found reductions in tumour growth in both. Evidence has also emerged in humans from window of opportunity studies, where the tissue is examined before and after metformin exposure. Pre-operative metformin use has been shown to reduce Ki-67 protein expression (a marker of tumour proliferation) in breast cancer (196, 197), and to reduce the precancerous changes (aberrant crypt foci) which are thought to precede the development of colorectal cancer (198). Most recently, a randomised phase III trial of non-DM patients showed that 250mg of metformin daily (a low dose compared to standard dose range of 1-2g daily in those with DM) was effective in the

chemoprevention of metachronous colorectal adenomas or polyps when compared with placebo (199).

5.1.3 Objectives

Objectives:

1. To conduct a systematic review and meta-analysis of randomised and non-randomised studies to investigate the effect of metformin use compared with non-use on recurrence-free survival (RFS), overall survival (OS) and cancer-specific survival (CSS) for individual tumour types in the adjuvant setting.
2. To assess whether there is sufficient evidence to undertake a phase III trial of metformin in the adjuvant setting, and possibly as an additional intervention to the Add-Aspirin trial.

5.2 Methods

All methods for this systematic review and meta-analysis were outlined in a protocol prospectively registered with PROSPERO, an International prospective register of systematic reviews and meta-analyses (identifier CRD42015020519, also available in appendix E). A freely available permanent record of the study protocol reduces the opportunity for reporting bias by enabling comparison of the completed review with what was planned in the protocol.

Reporting follows PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines(200). PRISMA guidelines aim to improve the quality of reporting for systematic reviews and meta-analyses by describing the minimum set of information that should be reported.

5.2.1 Eligibility criteria

Eligible studies were those that met the following criteria:

- Participants over 16 years old
- Data was available for participants with a potentially curable solid tumour

  (defined as those either undergoing radical therapy with curative intent, or those with an early stage cancer where cure is normally the objective of standard treatment)
- Data was available for individual tumour types
- Intervention was metformin use and the comparator individuals who were not using metformin
- Data is reported on at least one of RFS, CSS or OS for individual tumour types
- HR and CI are reported or could be calculated
- Randomised controlled trials or non-randomised studies (including observational, cohort and case-control studies) were eligible

5.2.2 Search strategy

Electronic searches of databases (MEDLINE, EMBASE, Cochrane Central Register of Controlled Trials), clinical trial registries (clinicaltrials.gov, ISRCTN and EU Clinical Trials Register) and conference proceedings (American Society of Clinical Oncology, and European Society of Medical Oncology) were conducted. All sources were searched from inception until the 31st May 2015 (conference abstracts 2005-2015). Bibliographies of the reports of all identified studies and review articles were hand-searched for further potentially eligible studies. A search strategy was designed to identify all randomised and non-randomised studies (including case-control, cohort, cross-sectional, longitudinal, retrospective and prospective observational studies) where a biguanide was the intervention investigated. Further details of the search strategy are available in appendix E.

5.2.3 Study selection

All retrieved studies were assessed for eligibility and, when sufficient information was not available from the title and/or abstract, the full-text publication or (for conference abstracts) the associated poster or presentation was acquired and where this was not available, the study author was contacted. For studies with multiple publications, or where there was overlap in the patients studied, the most recent publication was chosen. No study was excluded for weakness of study design or quality. For the purpose of analysis, studies presenting data separately by tumour type were treated as separate studies. Articles were grouped by cancer type according to the site of origin and histology.

5.2.4 Data items and collection

Data on patient characteristics, interventions and outcomes were extracted for all studies into a pre-designed table. These were cross-checked by a second independent reviewer (Fay Cafferty) and any disagreements were resolved by consensus. Where the necessary data was not available in published material, missing information was requested from the study author. A list of data extracted is available in appendix E. Studies were evaluated to determine whether they accounted for potential confounding factors [body mass index (BMI), age, gender, cancer-specific prognostic factors and the use of other anti-DM medications], either by demonstrating that there was no significant difference in their distribution between treatment groups or by inclusion in multivariable analyses. In order to minimise the potential for confounding by DM status, where the comparator included both non-DM patients and DM non-metformin users, data based on a DM non-metformin comparator was extracted in preference. Where a time-varying covariate was used to model treatment effect, the most conservative HR was selected. Where reported, the HR after adjustment for potential confounding factors was extracted in preference to an unadjusted value.

The Newcastle-Ottawa quality assessment scale for cohort studies (NOS) (201) was used to evaluate methodological quality. The NOS is a scoring system developed to assess the quality of non-randomised studies for use in systematic reviews. It judges studies from three

perspectives; the selection of the study groups, the comparability of the groups, and the ascertainment of the outcome of interest. Since all eligible studies were of cohort design, this scoring system was used for all studies (study scoring is presented in appendix F).

5.2.5 Statistical Analysis

The primary outcome of the study was RFS and secondary outcomes were OS and CSS. These are all time-to-event outcomes are most appropriately analysed using HRs, which take into account of the number and timing of events, and the time until last follow-up for each patient who has not experienced an event. Odds ratios (ORs) or relative risks (RRs) do not take account of when outcome events occur, only the total number of events, and therefore do not account for the maturity of data, or the length of follow-up. Combining OR and/or RR from different studies would therefore provide a summary statistic that is both unreliable and difficult to interpret. HRs and associated statistics were either extracted directly from the study reports, or estimated from the Kaplan-Meier curves by dividing up the curve into a number of time intervals to give a representation of event rates over time and calculating a HR using a computational spreadsheet using published methods (202-204).

Where sufficient data were available on outcomes for individual cancer types, a meta-analysis was conducted with a primary outcome of RFS and secondary outcomes of OS and CSS. HRs were combined across trials using a fixed-effect model. A fixed-effect model was chosen over a random-effects model because the former weights trials by their size and allows statistical heterogeneity to be tested, whereas the latter weights trials by both size and heterogeneity which, with small numbers of studies, can mean small trials have the same weight as large trials. With the fixed-effect model, where heterogenity is detected, this can be explored, or the reasons for it investigated. Heterogeneity was assessed using the chi-squared (χ^2) test and the I^2 statistic. A random-effects model (DerSimonian and Laird) (205) was used to assess whether the results were robust to the choice of model. Probability values were two-sided with $p<0.05$ considered of statistical significance.

Analyses were pre-planned to explore whether the size or the direction of the effect of metformin therapy varied according to specific study or patient characteristics, including: DM status of the comparator group (with and without non-DM patients in the comparator group); prostate cancer primary treatment type (prostatectomy or radical radiotherapy) and study design. The resulting HR estimates from study group analyses were compared using the χ^2 test for interaction.

We also planned to explore the impact of metformin dose/exposure on the outcomes described above but insufficient data was available. Sensitivity analyses were conducted for the primary outcome of RFS. This was carried out according to study quality (restricted to studies with a NOS score greater than, or equal to the median); publication type (restricted to studies where a full publication was available); setting (restricted to hospital based studies); follow-up (restriction of follow-up less than three years); and by the potential confounding factors accounted for (restricted to studies that adjusted for BMI, age, gender, cancer-specific prognostic factors and other DM medications).

An additional unplanned exploratory analysis was also conducted according to whether the study was from a western (North America or Europe) or non-western population after a wide geographical distribution of studies was noted. Study group and sensitivity analyses were only conducted where study numbers were sufficient to be meaningful (at least two studies were available in each group). Statistical analyses were carried out using STATA version 14.

5.3 Results

Using the search terms described 7,670 reports and conference abstracts were identified and screened. 23 full publications and four conference abstracts were identified that met the eligibility criteria, comprising of 24,178 participants (206-231). In-order to identify all relevant studies investigating metformin, the search strategy also identified studies using the term “biguanide”, “neoplasm” or a related term, and included cancer prevention studies, which sometimes contain RFS, OS and CSS as secondary outcomes. This identified a large number

of articles (6,210) the majority of which were excluded based on their title and/or abstract alone. Following full text review, a further 80 articles were excluded, and after qualitative synthesis (data extraction), a further nine studies were excluded. The PRISMA study selection diagram is shown in figure 5.4.

All studies identified were retrospective cohort studies except for one prospective cohort study embedded in a clinical trial (209). The majority of identified studies examined the effect of metformin in one of four tumour types; prostate, colorectal, breast and urothelial cancer (transitional cell carcinoma of bladder, kidney or urinary tract) which, therefore, represent the main focus of this analysis. 17 studies were based in North America, four in Asia, two in Europe and four included patients from more than one continent. 23 studies were hospital based and four were population based. A summary of the main characteristics for studies of breast, colorectal and prostate cancer is presented in table 5.1, and a table of study characteristics for other cancer types is presented in table 5.2.

Figure 5.4 PRISMA study selection diagram

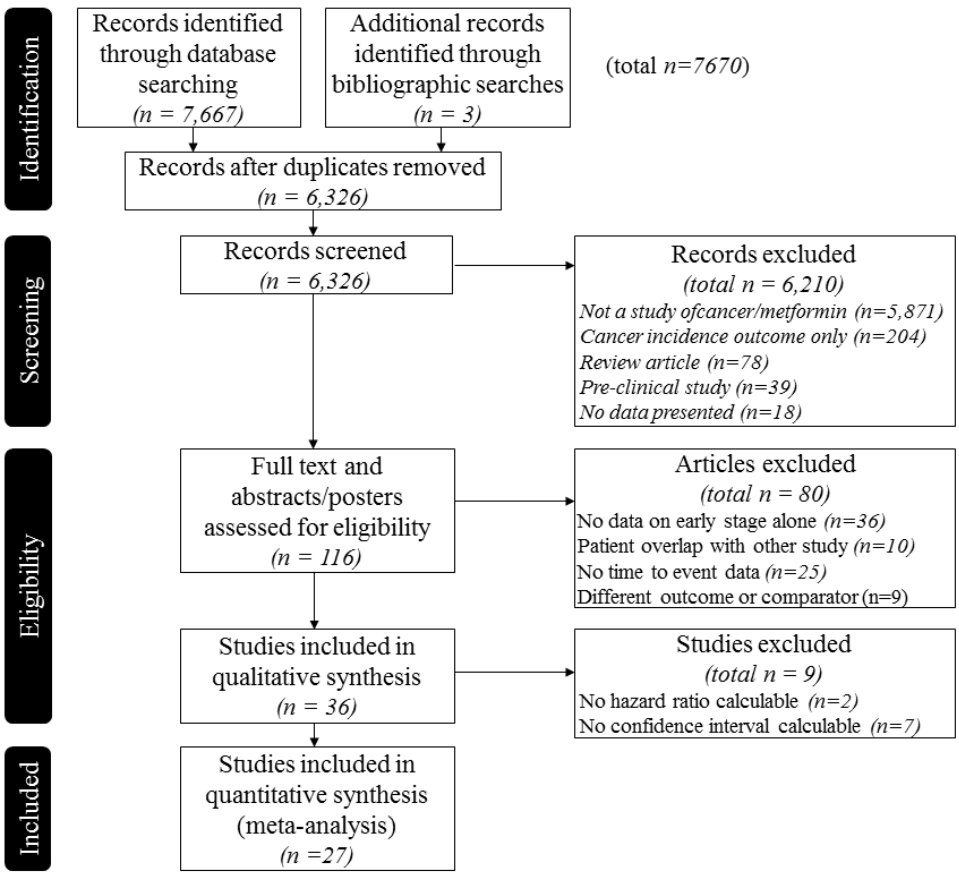


Table 5.1 Main study characteristics: Colorectal, prostate and breast cancer

Tumour group	Study author	Patient characteristics		Study characteristics				Comparator DM status		Outcomes			Definition of metformin exposure	Median follow-up (months)	Potential confounders (R=reported & not significant, M=included in multivariate model, x=not assessed, or significant but not adjusted for)					NOS score
		Treatment	Tumour stage / other restrictions	Sample size *(met/total)*	Article type	Study location	Setting (H=Hospital, P=Population)	DM	Non-DM	RFS	OS	CSS			BMI	Age	Sex	Cancer specific variables	Other DM meds	
Colorectal adenocarcinoma	Spillane (206)	Not specified	I-III	*207/315*	Full	Ireland	P	✓	X	X	✓	✓	In year before diagnosis	46	X	M	M	M	M	7
	Lee, GE (207)	Not specified	II-III	*223/356*	Abstract	Singapore	H	✓	X	✓	✓	X	At diagnosis	78	X	M	X	M	X	5
	Lee, JH (208)	Not specified	III[b]	*96/220*	Full	Korea	H	✓	X	X	✓	✓	>6m exposure	41	M[c]	M[c]	M[c]	M[c]	M[c]	8
	Singh (209)	Not specified	III /colon only	*115/267*	Abstract	USA & Canada	H	✓	X	✓	✓	X	Before randomisation	Not given	X	M	M	M	X	5
	Zanders (210)	Not specified	I-III	*512/778*	Full	The Netherlands	P	✓	X	X	✓	X	Cumulative exposure	41	X	M	M	M	M	7
Prostate Adenocarcinoma	Allott (211)	Prostatectomy	Localised	*155/369*	Full	USA	H	✓	X	✓	X	✓	At surgery	59/73[a]	M	M	n/a	M	X	8
	Kaushik (212)	Prostatectomy	Localised	*323/885*	Full	USA	H	✓	X	✓	✓	X	In 3months before surgery	61	M	M	n/a	M	R	7
	Rieken WJU (232)	Prostatectomy	Localised	*287/6486*	Full	USA & Europe	H	X	✓	✓	X	X	At surgery	25	X	M	n/a	M	n/a	6
	Spratt (214)	Radical radiotherapy	Localised	*157/319*	Full	USA	H	✓	X	✓	✓	✓	At diagnosis or after radiotherapy	104	R	M	n/a	M	R	8
	Margel (215)	Prostatectomy or radical radiotherapy	Localised[b]/ ≥66 years old	*Total 955*	Full	Canada	P	✓	X	X	✓	✓	Cumulative exposure	56	X	M	n/a	M	M	8
	Zannella (216)	Radical radiotherapy	Localised	*114/504*	Full	Canada	H	✓	✓	✓	X	X	At time of radiotherapy	82	X	R	n/a	M	X	5
	Danzig (217)	Prostatectomy	Localised	*98/767*	Full	USA	H	✓	X	✓	X	X	At surgery	27	X	M	n/a	M	X	6
	Taira (218)	Brachytherapy	Localised	*126/2298*	Full	USA	H	✓	✓	X	✓	X	Diagnosis to 3months after brachytherapy	100	M	M	n/a	M	X	7
Breast adenocarcinoma	Oppong (219)	Adjuvant chemo	I-III	*76/141*	Full	USA	H	✓	X	✓	✓	X	Diagnosis to 6 months after	87	R	M	n/a	M	M	8
	Bayraktar (220)	Adjuvant chemo	I-III / triple negative	*63/130*	Full	USA	H	✓	X	✓	✓	X	During adjuvant chemo	62	M[d]	M	n/a	M	R	8
	Lega (221)	Breast cancer surgery	Infer I-III / ≥66 years	*868/1774*	Full	Canada	P	✓	X	X	✓	✓	Cumulative exposure	54	X	M	n/a	M	M	6

Abbreviations: NOS= Newcastle-Ottawa Quality Assessment Scale for Cohort Studies, BMI= body mass index, met= metformin, N/A= not applicable, [a]=metformin/non-metformin, [b]=data from sub-analysis, [c]=main analysis only, [d]= adjustment for body weight, RFS=recurrence-free survival, OS=overall survival, CSS=cancer-specific survival

Table 5.2. Main study characteristics: Other cancer types

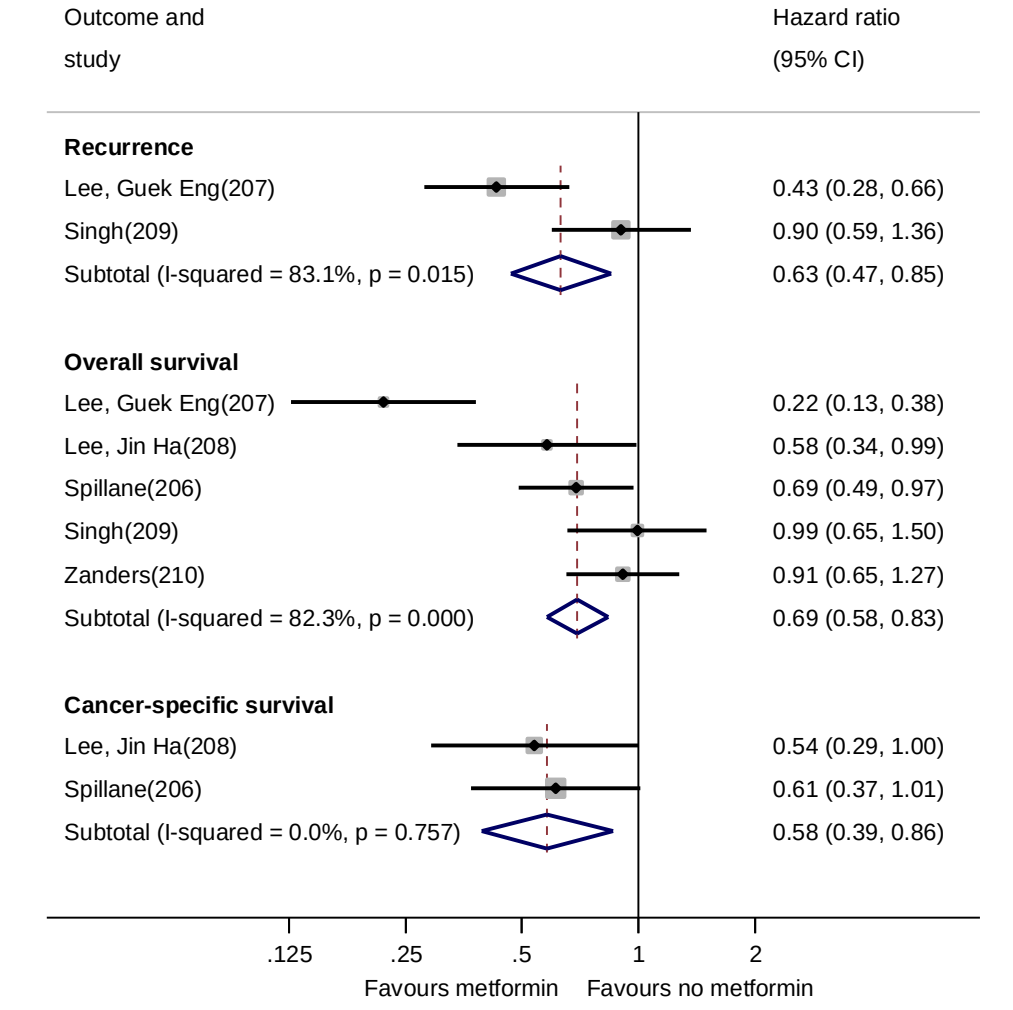
Tumour group	Study author	Patient characteristics		Study characteristics				Comparator DM status		Outcomes			Definition of metformin exposure	Median follow-up (months)	Potential confounders (R=reported & not significant, M=included in multivariate model X=not assessed, or significant but not adjusted for)					NOS Score
		Treatment	Tumour stage / other restriction	Sample size (*met/total*)	Article type	Study location	Setting (H=hospital, P=population)	DM	Non-DM	RFS	OS	CSS			BMI	Age	Sex	Cancer specific	Other DM meds	
Urothelial carcinoma	Rieken BJU (222)	TURBT	pTa-pT1 N0 M0 /urothelial carcinoma of bladder (NMI)	*43/1035*	Full	USA & Europe	H	X	✓	✓	✓	X	At surgery	64	X	M	R	M	n/a	8
	Rieken UO (223)	Radical surgery	M0 /invasive urothelial carcinoma of bladder	*80/1382*	Full	USA & Europe	H	X	✓	✓	✓	✓	At diagnosis	34	M	M	M	M	n/a	8
	Rieken EJS (213)	Radical surgery	M0 / Upper tract urothelial carcinoma	*194/2330*	Full	USA, Europe & Japan	H	X	✓	✓	✓	✓	At surgery	36	X	M	M	M	n/a	6
Head &neck (squamous cell carcinoma)	Kwon (224)	curative surgery or radiotherapy	No distant metastases	*99/1072*	Full	Korea	H	X	✓	✓	✓	✓	Ever exposure	65	M	M	R	M	n/a	8
	Thompson (225)	Not specified	Disease free at 3m / oral-oropharynx	*33/78*	Full	USA	H	✓	X	✓	X	X	Diagnosis to relapse	44	X	R	R	R	X	5
Renal cell carcinoma	Hakimi (226)	partial/radical nephrectomy	T2-T3 N0 M0	*55/784*	Full	USA	H	✓	✓	✓	X	✓	At surgery	41	M	M	R	M	X	6
	Psutka (227)	partial/radical nephrectomy	Localised	*83/200*	Full	USA	H	✓	X	✓	✓	✓	In 90 days before surgery	97	R	M	R	M	X	8
Pancreatic adenocarcinoma	Ambe (228)	Radical surgery	Resectable	*19/44*	Abstract	USA	H	✓	X	X	✓	X	At surgery	Not given	R	R	R	R	X	7
Non-small cell lung carcinoma	Fortune-Greeley (229)	Not specified	data on stage I-II	*Not given*	Abstract	USA	H	✓	X	X	✓	X	Not given	Not given	M	M	X	M	X	6
Endometrial cancer	Ko (230)	Not specified	I-IV (RFS data extracted)	*200/363*	Full	USA	H	✓	X	✓	X	X	At diagnosis	33	R	M	n/a	M	R	8
Gastric cancer	Lee, CK (231)	Gastrectomy	I-III	*132/326*	Full	Korea	H	✓	X	✓	✓	✓	Cumulative exposure	74	M	M	M	M	M	9

Abbreviations: NOS= Newcastle-Ottawa Quality Assessment Scale for Cohort Studies, BMI= body mass index, met= metformin, N/A= not applicable, NMI=Non-muscle invasive, TURBT=Transurethral resection of bladder tumour, RFS=recurrence-free survival, OS=overall survival, CSS=cancer-specific survival

5.3.1 Colorectal cancer

Five eligible studies were identified, with a total of 1,936 colorectal cancer patients. RFS was assessed in two studies (623 patients), OS in all five studies (1,936 patients), and CSS in two studies (535 patients). Overall, metformin use appeared to demonstrate significant improvements in RFS (HR 0.63, CI 0.47-0.85), OS (HR 0.69, CI 0.58-0.83) and CSS (HR 0.58, CI 0.39-0.86) (figure 5.5), although there was variation between the results of the individual studies for RFS (I^2=83.1%, *p=0.015*) and OS (I^2=82.3 *p<0.001*). When the random effects model was applied, the benefits seen for both OS (HR 0.62, CI 0.40-0.97) and CSS (HR 0.58, CI 0.39-0.86) remained but there was no longer a significant benefit of metformin on RFS (HR 0.62, CI 0.30-1.29). In an unplanned exploratory analysis that grouped studies with western and non-western populations separately we found there was a significant interaction between the effect of metformin on OS and the population studied (χ^2 = 14.31, *p<0.001*). In studies in non-western populations, there was a highly significant benefit of metformin on OS (HR 0.36, CI 0.25-0.53) however there was evidence of heterogeneity (I^2=85.8% *p*=0.013). In studies with western populations, only a trend towards a significant effect was identified (OS HR 0.84, CI 0.68-1.03) with no clear evidence of heterogeneity (I^2=4.6% *p*=0.350). In planned sensitivity analyses, there appeared to be a larger relative benefit of metformin on OS when analyses were restricted to studies that had follow-up of greater than three years (HR 0.64, CI 0.52-0.78). Insufficient data were available to explore the impact of dose or duration on the effect of metformin. Details of study group and sensitivity analyses for all tumour types are available in table 5.4.

Figure 5.5 Meta-analysis of early stage colorectal cancer outcomes according to metformin use

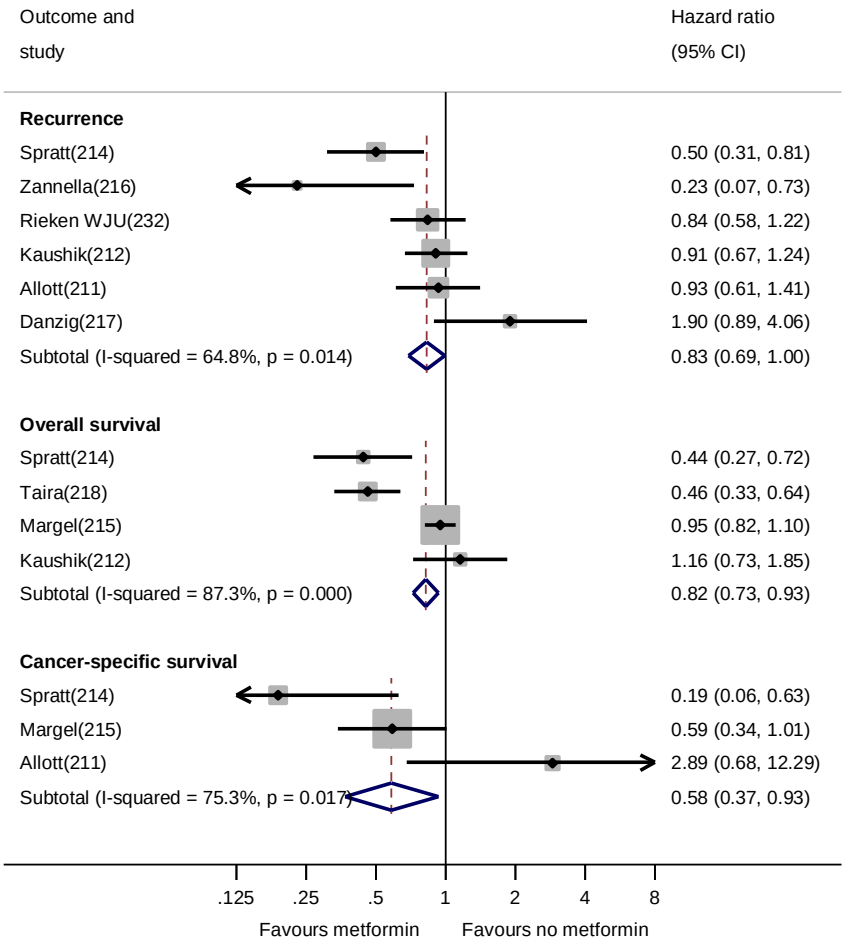


5.3.2 Prostate cancer

Eight eligible studies were identified, with a total of 12,583 prostate cancer patients. RFS was assessed in six studies (9,330 patients), OS in four studies (4,457 patients), and CSS in three studies (1,643 patients). Metformin use demonstrated a borderline significant improvement in RFS (HR 0.83, CI 0.69-1.00), and significant improvements in OS (HR 0.82, CI 0.73-0.93) and CSS (HR 0.58, CI 0.37-0.93) (figure 4.6), however the relationship was inconsistent across studies (RFS I^2=64.8%, *p=0.014*; OS I^2=87.3% *p<0.001*; CSS I^2=75.3% *p=0.017*), which was reflected when the random effects model was applied (RFS HR 0.80, CI 0.57-1.13; OS 0.69, CI 0.44-1.10; CSS 0.64, CI 0.19-2.12). In a pre-specified analysis, there was significant interaction between the effect of metformin and the primary treatment type on RFS (χ^2 test for interaction 9.03, *p=0.003*). For patients receiving radical radiotherapy (214, 216) there was a

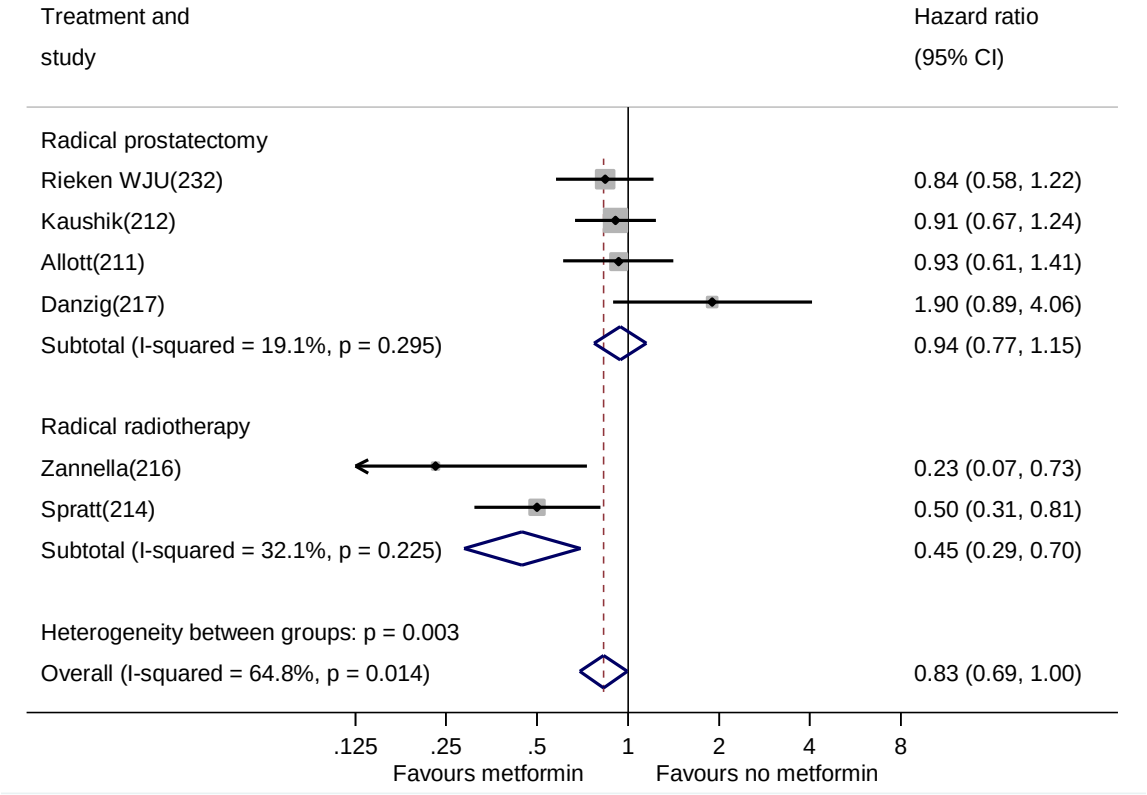
significant benefit from metformin (HR 0.45, CI 0.29-0.70), whereas no significant benefit was seen for patients who underwent radical prostatectomy (HR 0.94, CI 0.77-1.15) (figure 5.7). Only a single study was able to provide data on OS and CSS in those having radical radiotherapy, however significant improvements were seen in both (OS 0.44, CI 0.27-0.72; CSS 0.19, CI 0.06-0.63) (214). There was no evidence of an interaction between the effect of metformin on RFS and the presence or absence of non-DM patients in the comparator group (χ^2 = 0.49, *p=0.48*). In planned sensitivity analyses, there appeared to be a larger relative benefit of metformin on RFS when analyses were restricted to studies that had a follow-up of greater than three years (HR 0.77 CI 0.62-0.96) or considered other DM medications in their analysis (HR 0.79 CI 0.64-0.98), however, insufficient data were available to explore the impact of dose or duration on the effect of metformin.

Figure 5.6 Meta-analysis of early stage prostate outcomes according to metformin use



Footnotes: (a) Hazard ratios for Taira et.al. (218) were estimated from Kaplan Meier curves and summary statistics using published methods (202-204).

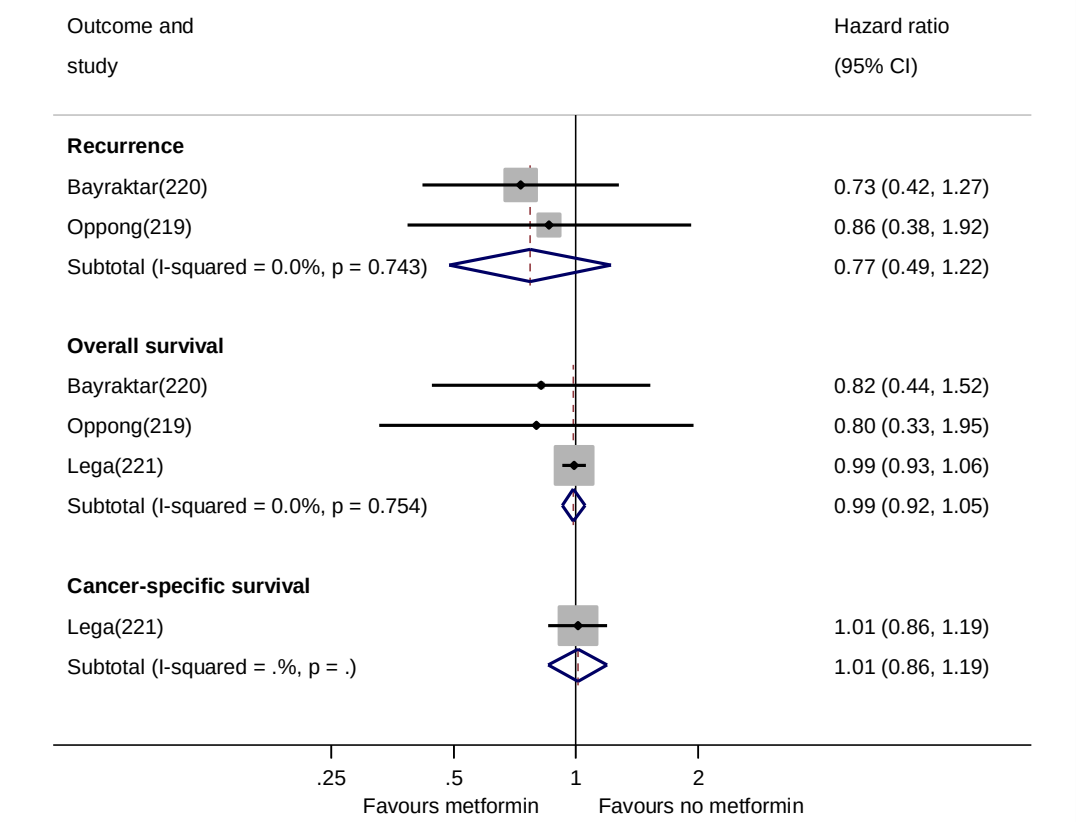
Figure 5.7 Meta-analysis of prostate cancer recurrence-free survival according to metformin use by primary treatment



5.3.3 Breast cancer

Three eligible studies were identified, with a total of 2,045 breast cancer patients. RFS was assessed in two studies containing 271 patients and OS in all three studies (2,045 patients). Metformin demonstrated a trend towards improvement in RFS (HR 0.77, CI 0.49-1.22) (figure 5.8), however no effect was seen in OS (HR 0.99, CI 0.92-1.05). There was no evidence of variation between the results of the studies either for RFS (I^2=0.0% *p=0.74*) or OS (I^2=0.0% *p=0.75*). As CSS was only available for one study containing 1,774 patients, no meta-analysis was possible for this outcome, however in this study, metformin did not appear to have an impact on CSS (HR 1.01, CI 0.86-1.19). There were insufficient study numbers for any meaningful study group or sensitivity analyses.

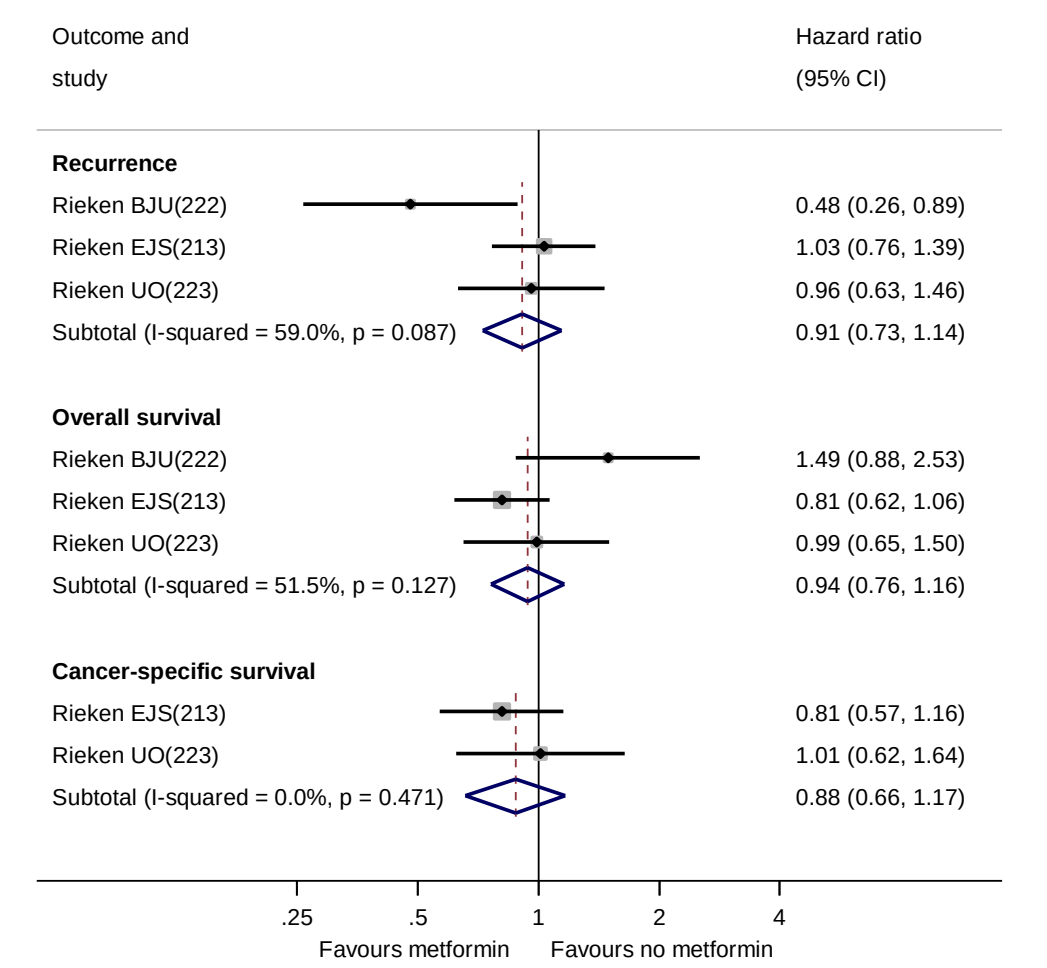
Figure 5.8 Meta-analysis of early stage breast cancer outcomes according to metformin use



5.3.4 Urothelial Cancer

Three studies were identified and included patients with upper tract urothelial carcinoma, and urothelial carcinoma of the bladder. RFS and OS was assessed in all three studies including 4,747 patients. Two studies assessed CSS including 3,712 patients. There was no clear evidence that metformin improved either RFS (HR 0.91, CI 0.73-1.14), OS (HR 0.94, CI 0.76-1.16) or CSS (HR 0.88, CI 0.66-1.17) (figure 5.9). Although there was some evidence of inconsistency between the results of studies for both RFS (I^2=59.0% *p=0.087*) and OS (I^2-51.5% *p=0.127*), the results did not change significantly when the random-effects model was applied (RFS HR 0.84, CI 0.57-1.24; OS HR 1.00, CI 0.72-1.39; CSS HR 0.88 CI 0.66-1.17). There were insufficient study numbers for any meaningful study group or sensitivity analyses.

Figure 5.9 Meta-analysis of early stage urothelial cancer outcomes according to metformin use



5.3.5 Other cancer types

There were insufficient studies identified to warrant meta-analyses for other cancer types, the findings of which are presented in table 5.3. In head and neck cancer, a positive trend towards improved RFS and CSS was seen in one study (224), but there was no effect on OS. However, the second study identified showed a potential detriment of metformin use on RFS(225). In renal cell carcinoma, two studies were identified, both showing a non-significant inverse relationship with metformin use and RFS, and no significant benefit in OS or CSS. Single studies were identified showing a significant improvement in OS in lung cancer, RFS and OS in endometrial cancer and RFS, OS and CSS in gastric cancer. A small single study in pancreatic cancer did not suggest any effect of metformin, however this study had a very small sample size.

Table 5.3 Early stage cancer outcomes by metformin use for tumour types with limited numbers of studies

Tumour group	Study author	Sample size	Recurrence-free survival HR (95% CI)	Overall survival HR (95% CI)	Cancer-specific survival HR (95% CI)
Head and neck	Kwon (224)	*1072*	0.76 (0.49-1.21)	0.95 (0.59-1.50)	0.79 (0.42-1.50)
	Thompson (225)	*78*	1.26 (0.62-2.56)	-	-
Renal cell carcinoma	Hakimi (226)	*784*	1.22 (0.66-2.27)	-	0.76 (0.21-2.70)
	Psutka (227)	*200*	1.07 (0.61-1.88)	0.74 (0.48-1.15)	0.83 (0.41-1.67)
Pancreas	Ambe (228)	*44*	-	0.54 (0.16-1.68)	-
Lung	Fortune (229)	*Not given by stage*	-	0.85 (0.77-0.93)	-
Endometrial	Ko (230)	*363*	0.56 (0.34-0.91)	0.43 (0.24-0.77)	-
Gastric	Lee, CK (231) [(a)]	*326*	0.86 (0.80-0.94)	0.87 (0.80-0.95)	0.87 (0.78-0.96)

Footnote: (a)HR for each 6 months of metformin use

5.3.6 Duration and dose

The impact of different exposures to metformin on early-stage cancer outcomes is examined in some of the identified studies, however limited data, and differences in the methods used to investigate exposure, preclude any study-group analyses. In colorectal cancer, Spillane et al. (206) conducted additional analyses on dose intensity and found survival benefits for high-intensity metformin users not using other diabetic therapies (CSS HR 0.44, CI 0.20-0.95; OS HR 0.41, CI 0.24-0.70), but no significant benefits were identified in other sub-groups. In gastric cancer, Lee et al. (231) found that increased cumulative duration of metformin use improved cancer-specific and all-cause mortality. Single studies in colorectal (210) and prostate cancer (211) also investigated the impact of different exposures to metformin but found no significant associations.

Table 5.4 Sub group and sensitivity analyses

Restriction	Study group	RFS	OS	CSS
Colorectal cancer				
All	**HR**	Lee GE, Singh HR=0.63 (0.47, 0.85), *p=0.002*	Lee GE, Lee GH, Spillane, Singh, Zanders HR=0.69 (0.58, 0.83), *p<0.001*	Lee JH, Spillane HR=0.58 (0.39, 0.86), *p=0.006*
NOS<7 (median)	**HR**	x	Lee GH, Spillane, Zanders HR=0.75 (0.61, 0.94), *p=0.011* (hetero *p=0.301*)	x
Without abstracts	**HR**	x	Lee GH, Spillane, Zanders HR=0.75 (0.61, 0.94), *p=0.011* (hetero *p=0.301*)	x
Without western patients (not North America, Europe)	**Western population**	x	Western (Spillane, Singh, Zanders): HR=0.839 (0.682, 1.033), *p=0.097* (hetero *p=0.350*)	x
	Non-western population	x	Non-West (Lee GE, Lee GH): HR=0.362 (0.247, 0.531), *p<0.001* (hetero *p=0.013*)	x
	Interaction	x	Chi-squared=14.31, *p<0.001*	x
Without population setting	**HR**	x	Lee GE, Lee GH, Singh Hr=0.58 (0.43, 0.76), *p<0.001* (hetero *p<0.001*	x
<36 months	**HR**	x	Lee GE, Lee GH, Spillane, Zanders HR=0.64 (0.52, 0.78), *p<0.001* (hetero *p<0.001*)	x
Sex	**HR**	x	Lee GH, Spillane, Singh, Zanders HR=0.80 (0.66, 0.97), *p=0.023* (hetero *p=0.295*)	x
Other DM Meds	**HR**	x	Lee GH, Spillane, Zanders HR=0.75 (0.61, 0.94), *p=0.011* (hetero *p=0.301*)	x
Prostate cancer				
All	**HR**	Spratt, Zannella, Rieken WJU, Kaushik, Allott, Danzig HR=0.83 (0.69, 1.00), *p=0.044*	Spratt, Taira, Margel, Kaushik HR=0.82 (0.73, 0.94), *p=0.003*	Spratt, Margel, Allott HR=0.58 (0.37, 0.93), *p=0.023*
Comparator group	**DM only control**	Spratt, Kaushik, Allott, Danzig HR=0.863 (0.698, 1.066), *p=0.171* (hetero *p=0.025*)	x	x
	Mixed or non-DM control	(Zannella, Rieken WJU) HR=0.744 (0.522, 1.060), *p=0.102* (hetero *p=0.037*)	x	x
	Interaction	Chi-squared = 0.49, p=0.483	x	x
NOS<7 (median)	**HR**	Spratt, Kaushik, Allott HR=0.807 (0.647, 1.006), *p=0.057* (hetero *p=0.089*)	x	x
Without population setting	**HR**	x	Spratt, Taira, Kaushik HR=0.577 (0.455, 0.731), *p<0.001* (hetero *p=0.003*)	Spratt, Allott HR=0.570 (0.227, 1.431), *p=0.231* (hetero *p=0.004*)
<36 months	**HR**	Spratt, Zannella, Kaushik, Allott HR=0.772 (0.622, 0.959), *p=0.019* (hetero *p=0.027*)	x	x
BMI	**HR**	Spratt, Kaushik, Allott, HR=0.807 (0.647, 1.006), *p=0,057* (hetero *p=0.089*)	Spratt, Taira, Kaushik HR=0.577 (0.455, 0.731), *p<0.001* (hetero *p=0.003*)	Spratt, Allott HR=0.570 (0.227, 1.431), p=0.231 (hetero *p=0.004*)
Other DM Meds	**HR**	Spratt, Rieken WJU, Kaushik HR=0.788 (0.637, 0.975), *p=0.028* (hetero *p=0.111*)	Spratt, Margel, Kaushik HR=0.910 (0.793, 1.044), *p=0.178* (hetero *p=0.008*)	Spratt, Margel HR=0.487 (0.299, 0.795) (hetero *p=0.089*)

Footnotes: Study group analyses are only presented where there are two or more studies in each group. X= insufficient study numbers for study group analysis. Sensitivity analyses are only presented where there are two or more studies after restriction. The fixed effect model is used for all analyses.

Table 5.4 continued.

Restriction	Study group	RFS	OS	CSS
Breast cancer				
All	**HR**	Bayraktar, Oppong HR=0.77 (0.49, 1.22), *p=0.263*	Bayraktar, Oppong, Lega HR=0.99 (0.93, 1.05)	Lega HR=1.01 (0.86, 1.19), *p=0.907*
NOS<7 (median)	**HR**	x	Bayraktar, Oppong HR=0.81 (0.49, 1.35), *p=0.426* (hetero *p=0.964*	x
Without population setting	**HR**	x	Bayraktar, Oppong HR=0.81 (0.49, 1.35), *p=0.426* (hetero *p=0.964*	x
BMI	**HR**	x	Bayraktar, Oppong HR=0.81 (0.49, 1.35), *p=0.426* (hetero *p=0.964*	x
Urothelial cancer				
All	**HR**	Reiken BJU, Reiken EJS, Reiken UO HR=0.91 (0.73, 1.14), *p=0.414*	Reiken BJU, Reiken EJS, Reiken UO HR=0.94 (0.76, 1.16), *p=0.549*	Reiken EJS, Reiken UO HR=0.88 (0.66, 1.17), *p=0.361*
NOS<7 (median)	**HR**	Reiken BJU, Reiken UO HR=0.77 (0.54, 1.09), *p=0.140* (hetero *p=0.068*)	Reiken BJU, Reiken UO HR=1.16 (0.84, 1.61), *p=0.378* (hetero *p=0.234*)	x
<36 months	**HR**	Reiken BJU, Reiken EJS HR=0.89 (0.68, 1.17), *p=0.397* (hetero *p=0.029*)	Reiken BJU, Reiken EJS HR=0.92 (0.72, 1.17), *p=0.506* (hetero *p=0.044*)	x

Footnotes: Study group analyses are only presented where there are two or more studies in each group. X= insufficient study numbers for study group analysis. Sensitivity analyses are only presented where there are two or more studies after restriction. The fixed effect model is used for all analyses.

5.4 Discussion

This analysis suggests that metformin could be a useful adjuvant agent, particularly in colorectal and prostate cancer. The number of studies identified for each tumour type is likely to reflect the incidence and demographics of the disease, particularly the likelihood of presentation with early stage disease and a diagnosis of DM, for example, a large proportion of patents colorectal and prostate cancer present at an early stage of disease, this is also the case for breast cancer patients, but presentation is often a younger age where DM is less prevalent.

The variation in the adjuvant effects of metformin according to tumour type could be explained by differences in both patient characteristics and tumour biology. As outlined in the introduction to this chapter, the effect of metformin on AMPK signalling has been hypothesised to be a major pathway through which metformin exerts its anti-cancer effects (185). AMPK signalling dysregulation is also associated with metabolic syndrome (156). Metabolic syndrome is also known to increase the risk of developing some cancers, particularly colorectal cancer (233), where it is also associated with poorer recurrence and survival outcomes (234). In addition metabolic syndrome is known to develop as a consequence of androgen deprivation therapy in men with prostate cancer (235). Metformin may improve OS by reducing the number of cardiovascular deaths associated with metabolic syndrome, however the improvements in RFS and CSS identified suggest an anti-cancer effect. In prostate cancer the study group analysis suggests that the beneficial effects of metformin use could be limited to those undergoing radical radiotherapy. The AMPK pathway is known to play a role in regulating cellular responses to radiotherapy (236) and studies in xenograft mice models suggest that metformin can improve tumour oxygenation and therefore radiation response (216).

The limitations of this meta-analysis include the inherent weaknesses of observational data, particularly potential measurement errors in the exposure to metformin, and variation in the definition of metformin use, and the risk of time related biases (237). For example, if metformin was started at a time after diagnosis of cancer, there will not be any deaths during that period

(otherwise they would not have been recorded as starting metformin), which prevents early deaths being recorded in metformin users who started after cancer diagnosis. This could bias results and underestimate the risk of death in metformin users (representing immortal time bias). A high degree of variation between the results of studies was observed for a number of the outcomes investigated in most of the cancer types. Sensitivity analyses were designed to explore possible reasons for this to inform future observational and clinical trial design, however only a small number of analyses were possible due to insufficient study numbers.

For both prostate and colorectal cancer, the relative effect size appeared to increase for studies with follow-up of three years or greater, highlighting the importance of ensuring adequate duration of follow-up in future studies. Similarities have been seen in studies of aspirin, where greater benefits have been seen with longer follow-up (90, 92, 93). A limited number of studies investigated the relation with frequency, dose and duration of metformin in early stage cancer, however findings are inconsistent and further research is required to better understand this relationship.

Previous studies have suggested that a diagnosis of DM has a negative impact on cancer outcomes (238, 239), in an analysis of 97 prospective cohort studies, patients with DM had a 25% increased risk of death from cancer, HR 1.25 (95% CI, 1.19-1.31), compared to those without (240). Therefore, inclusion of non-DM patients in comparator groups could underestimate the beneficial effect of metformin. Owing to insufficient study numbers, it was only possible to analyse the effect of the presence or absence of non-DM patients in the comparator group for RFS in prostate cancer, where no evidence for an effect was found.

5.4.1 Observational data in the cancer primary prevention and treatment setting

Previous meta-analyses have examined the role of metformin in the primary prevention of cancer, where it was found to significantly reduce overall cancer incidence, however findings were inconsistent when individual tumour types were considered (241-245) again suggesting analyses are best conduced for individual tumour types separately. Benefits in the primary

prevention, or advanced setting do not necessarily translate to utility in the adjuvant setting as the mechanism of action may be different. A systematic search using MEDLINE identified a number of meta-analyses which have investigated the effect of metformin use across all stages in a number of individual tumour types (246-253). Table 5.5 describes the results of previous meta-analyses on the effect of metformin on cancer outcomes across all stages for individual tumour types.

Table 5.5 Results from other meta-analyses of the effect of metformin across all stages for individual tumour types

Tumour type	Overall Survival Effect size (95% confidence interval)							
	Zhang (246) (relative risk)	Lega (247)	Yin (248)	Mei (249)	Raval (250)	Stopsack (251)	Yu (252)	Xu (253)
Breast	RR 0.70, (0.55-0.88)	HR 0.81, (0.64-1.04)	HR 0.94, (0.90-0.99)	x	x	x	x	HR 0.53, (0.39-0.71)
Colorectal	RR 0.70, (0.59-0.84)	HR 0.65, (0.56-0.76)	HR 0.65, (0.56-0.77)	HR 0.56, (0.41-0.77)	x	x	x	x
Prostate	x	HR 0.73, (0.51-1.06)	HR 0.68, (0.51-0.90)	x	HR 0.86, (0.67-1.10)	HR 0.88, (0.86-0.90)	HR 0.86, (0.64-1.14)	x
Tumour type	Cancer-specific survival Effect size (95% confidence interval)							
Breast	x	x	HR 0.88, (0.79-0.99)	x	x	x	x	HR 0.89, (0.79-1.00)
Colorectal	x	x	HR 0.66, (0.50-0.87)	HR 0.66, (0.50-0.87)	x	x	x	x
Prostate	x	x	x	x	HR 0.76, (0.43-1.33)	HR 0.76, (0.44-1.31)	x	x

Statistically significant benefit No statisticially signficiant benefit found

In colorectal cancer, four meta-analyses have examined the effect on OS (246-249), two of which also investigated colorectal CSS (248, 249). All meta-analyses identified significant improvements in these outcomes which is consistent with the findings of this study. For prostate cancer, findings are less consistent. Five meta-analyses have examined the effect of metformin on OS (247, 248, 250-252), two of which also investigated prostate CSS (250, 251). Only two meta-analyses identified a significant benefit in OS (248, 251), with no benefit identified in prostate CSS. This differs from the findings of this study where significant benefits in OS and prostate CSS were identified, which could suggest that metformin is better suited to the adjuvant setting for prostate cancer.

In breast cancer four meta-analyses examined OS (246-248, 253), two of which investigated breast CSS. Two meta-analyses identified a significant benefit in OS (246, 248, 253), the other approached significance (HR 0.81, CI 0.64-1.04) (247), and the two meta-analyses investigating breast CSS also showed significant improvements (248, 253). This differs from the findings of this study where no significant benefit in OS and breast CSS was identified. This could suggest that metformin may be effective in those with established breast cancer which is consistent with the findings of breast cancer window studies where direct anti-tumour effects have been identified (196, 197).

5.4.2 Randomised evidence

We systematically searched the clinicaltrials.gov database for phase III trials investigating the effect of metformin on cancer, and while no phase III trials have reported to date, a number were ongoing. In colorectal cancer, a phase III trial of metformin versus standard care assessing recurrence and survival in stage III disease is now in set-up in South Korea (NCT02614339). In prostate cancer, the Metformin Active Surveillance Trial (NCT01864096), an ongoing randomised, phase III trial of metformin versus placebo given before primary therapy is assessing time to progression in men with low risk prostate cancer. The STAMPEDE trial (NCT00268476), a multi-arm multi-stage randomised controlled trial investigating a number of agents in the treatment of hormone-naïve, high risk, localised and metastatic prostate cancer, is investigating whether the addition of metformin improves survival in this group.

In breast cancer, the results did not identify any meaningful benefit of metformin use in the adjuvant setting, however this could be due to the limited number of studies identified. Additional supporting data are available in the primary prevention and treatment setting (across all stages), where meta-analyses have shown a beneficial effect (246, 248, 253, 254). A randomised phase III trial of metformin versus placebo assessing recurrence and survival in early stage breast cancer has recently completed recruitment (MA-32, NCT01101438), and interim biomarker analysis has reported a small reduction in fasting insulin levels, C-reactive

protein (a marker of inflammation) and weight in the metformin arm (255), and the main results are awaited.

5.4.3 Safety data

Metformin has been in continuous clinical use for five decades, consequently, it has a well-defined safety profile and has been administered alongside most cancer treatments without the emergence of any important interactions. Common adverse effects associated with metformin in those with DM are almost entirely related to the gastrointestinal tract and include nausea, abdominal discomfort, and diarrhoea, but these side-effects are usually mild, transient and self-limiting. Animal studies suggest the gastrointestinal tract accumulates higher concentrations of metformin than any other tissue (256), which may account for this toxicity profile, (and may also explain the activity seen in those with colorectal cancer). These side-effects are not thought to compromise safety and are minimised by starting a low dose and slowly increasing.

Historically there have been concerns about the association between metformin and lactic acidosis, (build-up of lactate which is a life-threatening medical emergency). Recent meta-analyses have shown that the risk of lactic acidosis is very low when metformin is prescribed according to its contraindications, particularly renal dysfunction (creatinine clearance greater than 60 mL/min), or any acute conditions with the potential to alter renal function. The incidence of lactic acidosis with metformin use is approximately 9/100,000 patient years of metformin treatment (257).

The glucose lowering properties of metformin do not occur as a result of increased insulin levels, but rather, metformin is associated with a reduction in insulin levels, and consequently does not cause overt hypoglycaemia in patients without DM. Data on the toxicity profile of metformin in those without DM is already available from clinical trials investigating its role as a treatment for polycystic ovarian syndrome, metabolic syndrome, weight-loss and DM prevention and is thought to be similar to those with DM (181, 258, 259). A key consideration of metformin use in the adjuvant cancer setting is that metformin is generally discontinued

prior to, or at the time of CT (computed tomography) scans and restarted 48 hours later as iodinated contrast can cause renal failure, could cause lactic acidosis (260).

5.5 Incorporating metformin as an intervention in the Add-Aspirin trial

The Add-Aspirin trial has the potential to investigate additional agent(s) within the same platform, thus maximising the utilisation of participant and operational resources to evaluate further potential adjuvant anti-cancer treatment(s). Any potential additional intervention would need to have sufficient supporting evidence to justify its inclusion, and not impact on the safety or integrity of the original trial. The design of Add-Aspirin would need to be adapted such that its ability to answer its original research questions would not be compromised.

Of the four Add-Aspirin tumour cohorts, this meta-analysis suggests that metformin may be a useful adjuvant therapy in colorectal and prostate cancer. A treatment effect in breast and gastro-oesophageal cancer in the adjuvant setting has not been excluded, however the availability of supporting evidence at this time is limited. In the adjuvant breast cancer setting the findings of the MA-32 trial will provide further insight. The addition of a second intervention in some, but not all cohorts, or even different second interventions in individual cohorts, could be possible, provided impact on existing outcomes that span cohorts, (for example cardiovascular outcomes or combined OS at 15 years) are considered carefully.

5.5.1 Metformin dose and formulation

Data on the relationship between metformin dose and its anti-cancer effect is limited, however, when used as a treatment for DM, the dose of metformin is increased gradually over several weeks to minimise the risk of gastro-intestinal side effects and titrated according to glycaemic control up to 2000mg per day (148). The optimal schedule to maximise tolerability and minimise gastro-intestinal side-effects, where the treatment aim is not glycaemic control, is unknown, however, a common regimen used in a number of other trials of patients without DM (STAMPEDE trial, NCT00268476 and MA-32 trial NCT01101438) is 850mg once daily, increasing to 850mg twice daily if tolerated after 4-6 weeks, and reducing to 750mg then

500mg once daily where side-effects develop. Metformin is also available in an extended release formulation which would be advantageous in a randomised controlled trial as it allows once daily dosing, is thought to reduce gastro-intestinal toxicity (261, 262), and as such, could improve adherence. A major drawback is the price difference between extended release tablets and the immediate release formulation, at twelve pence compared to two pence per tablet (500mg) respectively (current NHS listing price) (112), which could be problematic without pharmaceutical industry support.

5.5.2 Eligibility and DM status

The Add-Aspirin trial includes patients both with and without DM. While some window studies have suggested an anti-cancer effect in individuals without DM, existing observational evidence (including this meta-analysis) is only available in those with DM. There is no evidence to suggest that metformin is not efficacious as an adjuvant cancer therapy in those without DM, however this remains an unproven assumption. Investigating metformin in a phase III trial in those with DM only is not feasible because randomising a patient with DM to not receive metformin, where it is currently indicated, or may become indicated, would be unethical. One approach could be to have a second randomisation to metformin for patients without DM only.

5.5.3 Safety considerations

Following prescribing information and current National Institute for Health and Care Excellence (NICE) guidelines, metformin would need to be interrupted for the following reasons:

- Deterioration of renal function below 30 ml/min/1.73m^2 (with an estimated glomerular filtration rate (eGFR) below 45 ml/min/1.73m^2, the dose of metformin should be reviewed)

- Any risk of tissue hypoxia, such as severe infection, shock, sepsis, acute heart failure, respiratory failure or hepatic impairment, or those who have recently had a myocardial infarction
- 48 hours before a scheduled surveillance CT scan or colonoscopies in the colorectal cohort, and CT scans investigating recurrence in both colorectal and prostate cohorts, and until renal function is confirmed as within normal limits afterwards

5.5.4 Potential biomarkers

High BMI at the time of diagnosis of many cancer types is associated with increased all-cause mortality (263). The influence of obesity on the anti-cancer effects of metformin are unknown, however, obesity is associated with chronic inflammation (264), and is one of the potential mechanisms for its anti-cancer effects and could represent an important biomarker.

The metabolic effects of metformin could differ in patients with an underlying impaired glucose tolerance. By performing a glucose tolerance test at baseline, as well as a glycosylated haemoglobin level (HbA1c) level, would allow this potential biomarker to be investigated. Stratification by BMI, glucose tolerance, and HbA1c would also ensure that this potential confounding factor is evenly distributed between arms.

5.5.5 Trial conduct methodology for incorporating metformin into the Add-Aspirin trial

Applying a run-in period to metformin could help select a population that is able to tolerate and adhere to metformin, particularly as both aspirin and metformin cause gastrointestinal side-effects. A run-in period would also allow the dose of metformin to be titrated to a tolerated dose. If a placebo-control for metformin was used, this would also need to be titrated up, making the use of a placebo-control impractical. There are no known interactions between aspirin and metformin and their mechanisms are thought to be independent, so metformin could be added as a three by two factorial design, with the following arms (table 5.6):

Table 5.6 Potential factorial design for the incorporation of metformin into the Add-Aspirin trial

Aspirin 300mg + metformin	Aspirin 300mg + Placebo (metformin)
Aspirin 100mg + metformin	Aspirin 100mg + Placebo (metformin)
Placebo (aspirin) + metformin	Placebo (aspirin) + Placebo (metformin)

The aim of this design would aim to answer the following questions:

1) The efficacy of aspirin
2) The efficacy of aspirin according to dose
3) The efficacy of metformin
4) The efficacy of metformin and aspirin together

There is a precedent for trials investigating aspirin alongside other interventions using a factorial design. In the Canadian transient ischaemic attack study aspirin with sulfinpyrazone were examined individually, together and with placebo (265) and the Physicians' Health Study compared aspirin and beta carotene individually, together and with placebo (266).

5.6 Conclusions

The findings of this meta-analysis support the concept of randomised clinical trials using metformin in the adjuvant setting, with the strongest supporting evidence in colorectal and prostate cancer, particularly those treated with radical radiotherapy. Such trials could also further the understanding of the relationships between cancer outcomes and the dose and duration of metformin. There are no ongoing adjuvant phase III trials of metformin in prostate cancer, or colorectal cancer in western populations. The addition of metformin as a second randomisation in the Add-Aspirin colorectal and prostate cancer cohorts has the potential to evaluate a second established medicine as an adjuvant cancer therapy.

Chapter 6: Conclusions and future work

A systematic review and meta-analysis found that adjuvant metformin use was associated with significant improvements in cancer outcomes for early stage colorectal and prostate cancer and phase III trials of metformin in these tumour types are advocated. Research into the design and conduct of the Add-Aspirin trial showed that opening a large phase III basket trial with an active run-in period is feasible, and identifies key challenges, as well as potential solutions which are now adopted into the design and delivery of the trial.

This chapter summarises the lessons learnt about both the early and later stages of the repurposing process from metformin and aspirin, which could be applied to overcome similar challenges in evaluating other established drugs as cancer therapies in the future. The potential barriers to implementing repurposed medicines into standard practice and future research directions are also discussed.

6.1 The early stages of the repurposing process

6.1.1 Pre-clinical data

For aspirin and metformin, some of the preliminary steps of the drug development pathway such as target identification, compound discovery and chemical optimisation (ADMET) (section 1.3) were not undertaken because their development pre-dated the molecular era of drug discovery. The absence of these steps has resulted in uncertainty surrounding mechanisms of action for both drugs (sections 1.5.1 and 5.1.2), and therefore optimal dosing. For example, the dose of aspirin required for an anti-platelet mechanism is likely to be less than that required for one based on direct tissue effects (51).

An unknown mechanism of action also results in difficulty identifying biomarkers for response and toxicity. For aspirin, proposed biomarkers involving a direct tissue effect include tumour COX-2 expression (71) and PIK3CA mutation status (102), and for an anti-platelet mechanism,

potential biomarkers such as urinary 11-dehydro-thromboxane B_2 (TBX2) levels are proposed (51). For metformin, there are several potential biomarkers that are dependent on a mechanism involving alterations in glucose metabolism (172), such as BMI, baseline glucose tolerance, HbA1c, and c-peptide levels, whereas others such as TNF-α and IL-6 (inflammatory cytokines) rely on an anti-inflammatory mechanism (182).

In-vitro and *in-vivo* studies on aspirin and metformin provide evidence supporting potential mechanisms of action, biomarkers, and possible resistance mechanisms, however many of the pre-clinical studies identified failed to appraise whether biologically relevant concentrations of the drug were used, and therefore if the effect could be tenable in humans. Mechanistic understanding from the early steps of de-novo drug development are likely to be more robust, than for older established medicines. Additional pre-clinical research, with a programme of mechanistic studies conducted in parallel to phase III trials will be an essential part of the repurposing process for other established medicines with potential anti-cancer effects.

6.1.2 Observational data

A large number of observational studies investigating the anti-cancer effects of aspirin and metformin were identified, with a greater number for aspirin. This is likely to reflect that aspirin use is more common than metformin use and that the anti-cancer effects of aspirin were proposed a longer time ago. It is very possible that other established medicines in non-cancer indications have anti-cancer activity. For newly emerging candidates for repurposing, particularly those in less common use, it will take longer for a sufficient number of adequately powered observational studies to accumulate for a robust systematic review and meta-analysis. Collaborative working by research groups across different populations, could be used to generate sufficient power to investigate the anti-cancer effects of less commonly used medications, particularly if common data collection fields and methods could be agreed.

Data on concomitant medication use collected within randomised clinical trials can provide an alternative source of observational evidence, providing reliable data on cancer outcomes

linked to prospective data on medication exposure. Collection of concomitant medication data was shown to be feasible for a limited number of medications in the Add-Aspirin trial (section 4.1.4 and 4.3.4). By establishing collaborations to standardise the way concomitant medication data is collected in clinical trials, this form of observational data could allow research into the anti-cancer properties of less commonly used medicines.

In this thesis the observational evidence supporting the anti-cancer effects of aspirin was analysed separately for the cancer prevention, adjuvant and advanced setting as the underlying mechanism, and therefore treatment effect, could differ between them. This principle was also applied in the methods of the systematic review and meta-analysis of metformin. In reality, there is overlap between treatment settings for each individual patient. For example, for an adjuvant cancer patient, there would be an adjuvant treatment effect, the potential to prevent second primary cancers and, in the event of cancer recurrence, the opportunity to delay disease progression. Outcomes such as OS and CSS have the potential to be influenced by drug activity in all three settings and therefore it is difficult to evaluate these separately in observational data. Phase III trials are needed to provide this information by incorporating trial outcomes in all treatment settings, for example, second primary cancer rates, RFS, and PFS (in the event of recurrence) can be examined in an adjuvant trial.

6.1.3 Randomised data

Conducting a systematic review and meta-analysis of metformin in the adjuvant cancer setting highlighted the inherent weakness of observational data, particularly the potential for measurement errors in treatment exposure. Randomised data on an established medicine in another indication can minimise the risk of these biases and was found to be the cornerstone of the evidence supporting the anti-cancer effects of aspirin (90, 95, 96). All the evidence supporting metformin use as an adjuvant therapy is based on observational rather than randomised data, and only in patients with DM. Unlike aspirin, data on cancer incidence and survival from randomised trials in other indications is unlikely to emerge because phase III trials evaluating metformin in DM involve short-term administration (often between twelve

weeks and one year). Trials of metformin in other indications like polycystic ovarian syndrome and infertility also involve short-term administration and are conducted in a young adult population where cancer incidence is low. Trialists working in disease areas other than cancer have the opportunity to incorporate the collection of cancer outcome data which could maximise the research opportunities each trial can offer. The creation of generic protocols for collecting such data could encourage this practice.

6.1.4 Selecting a dose

The modern drug development process provides data on the pharmacokinetics and tolerability of a new medicine and allows the optimal dose for investigation in late phase trials to be established. These data are not available for older drugs. For established medicines, existing data are based on doses selected for other indications, and as such data in the dose range needed to infer efficacy in the cancer setting may not exist. Additionally, the most favourable dose may be unestablished when there is mechanistic uncertainty. For aspirin, observational data can sometimes provide information on efficacy and tolerability, and its relation to dose and duration. However, for medications like metformin, the dose taken often varies for individual patients according to diabetic control and tolerability, therefore information on the relationships between dose, effect and toxicity is often unavailable. The approach taken by the Add-Aspirin trial was to randomise patients between two different doses of aspirin (100mg and 300mg) and placebo in a platform design. Dosing based randomisations within similar trials could be used to provide this information.

6.2 Later stages of the repurposing process

The Add-Aspirin trial provides an example of how an established medicine can be evaluated as an adjuvant cancer therapy in a phase III trial. During the set-up of the trial there were several barriers which were overcome. In the absence of commercial interest from the pharmaceutical industry, all financial support for the trial was obtained from governmental

(NIHR Health Technology Assessment Programme and the Medical Research Council) and charity sources (Cancer Research UK). Furthermore, significant cost-efficiencies were made by directly commissioning and managing many operational procedures normally dependent on industry support, for example drug packaging, labelling, blinding, distribution, supply management and unblinding provision. Cost and practical efficiencies were also incorporated into the trial through the use of a multi-tumour type (basket) design, each incorporating multiple dose randomisations.

This research finds that opening and conducting a phase III trial of an established medicine is achievable and provides recommendations for minor conduct modifications and protocol amendments summarised below.

6.2.1 Basket design

It was shown to be possible to successfully open and recruit to a trial with four tumour types in one protocol, with 66.7% of NHS trusts and boards in the UK having at least one recruiting site, and 81% of those recruiting in at least three out the four tumour cohorts at one year. Research sites recognised the efficiencies offered from a basket trial design particularly in terms of gaining approvals, staffing and data entry. A basket design could therefore help overcome the financial barriers of the later stages of the repurposing process for other similar agents by ensuring a broad evaluation in all suitable tumour types where sufficient evidence exists.

At sites, the main challenges from a basket design were identified as understaffing, and the unequal division of labour between tumour cohorts at sites. To overcome understaffing in future similar trials, a system created to share NIHR credit for research activity and support costs between hospital sites when patients change site for primary treatment, adjuvant therapy, and follow-up could be considered. Ensuring staff-time is divided evenly between tumour types could reduce the opening times and improve recruitment for individual cohorts. Future trials employing a basket design could consider contracts between sites and a sponsor

which include an agreement to ring-fence staff for each cohort and to reallocate staff-time to poorly recruiting cohorts where necessary.

6.2.2 Run-in period design

The design of the run-in period was well received by site staff and successfully implemented with 88% of the first 500 participants registered being successfully randomised after its completion. The run-in period was found to be successful in its objective to prevent randomisation of patients identified to have poor adherence or tolerance with short term aspirin use. These patients are unlikely to take regular aspirin long-term. Run-in period methodology could be a useful strategy for any trial of an agent that requires long-term adherence in-order for an anti-cancer effect to be identified.

The three methods employed to assess adherence exhibited high levels of concordance, however given the poor return of blister packs, adherence assessments could be limited to the use of a diary card and direct questioning of participants.

The existing evidence suggested that an eight-week period of aspirin use was unlikely to have a detectable treatment effect. Trials considering a run-in period would need to evaluate whether the drug under investigation had any short-term treatment effects before using this methodology. A change in side-effect profile when moving from the active run-in period to the randomised phase was not anticipated to result in unblinding treatment allocation to participants because aspirin is generally well tolerated, however the potential for unblinding needs to be considered for other agents.

6.2.3 Optimising recruitment

For a trial to be successful, recruitment milestones must be met, and for trials with a basket design, this needs to be achieved across all trial cohorts. To achieve this, anticipated recruitment projections must be as accurate as possible. This research explored how site recruitment predictions compare to actual recruitment rates and discovered that sites overestimate how many patients they can recruit per month by a median of 220-350%

depending on tumour type (section 3.3.3.1), and site recruitment predictions more accurately reflect their best month of recruitment (section 3.3.3.2). It is proposed that selecting an anticipated recruitment rate by asking for a lowest and highest monthly recruitment prediction and using the mean value may be a more accurate method.

The Add-Aspirin trial has adopted a strategy where participants who are already taking part in other trials are encouraged to co-enrol, as long as both trial teams agree, and no negative impact on safety or the results of either trial is anticipated. This approach was successfully implemented, with a total of forty trials agreeing co-enrolment in the first year. A trial conduct survey found that site staff believed co-enrolment improved recruitment to clinical trials and maximised participant choice, but identified concerns about the potential for confounding, additional patient burden and toxicity (section 2.3.12). This approach could be utilised in phase III trials of repurposed medicines to attract participants who might normally favour a trial of a new cancer therapy over an established drug, thus maximising the pool of potential participants. Co-enrolment is well suited to trials investigating established medicines which are proposed as adjuncts to other treatments as it allows them to be examined alongside new and future therapies. To promote co-enrolment amongst site-staff and patients, a frequently asked questions document is recommended to provide reassurance about the chance of any negative impact on trial results and any additional burden or risk of toxicity. Patient representative involvement was also found to be key to successfully negotiating co-enrolment agreements between trial teams.

This research proposes several strategies to increase recruitment of certain groups identified as under-represented in the trial. The gastro-oesophageal cohort failed to meet recruitment targets and the eligibility criteria have now been adjusted to include patients with positive resection margins and the timing of entry criteria for patients undergoing radical chemoradiotherapy from twelve to fourteen weeks. Other under-represented groups included patients in the colorectal cohort who underwent resection of liver metastases, and patients in

the prostate cohort who underwent a prostatectomy. The eligibility of these groups has now been highlighted to Investigators.

6.2.4 Future implementation of repurposed drugs

Once an established medicine has been shown to be efficacious in a phase III trial, there are likely to be barriers to adoption as part standard therapy. With late phase trials being conducted by several separate academic groups rather than a single pharmaceutical company, it is unclear who will take responsibility for taking licencing applications forward. Without the financial drive of industry, the source of funding for regulatory approvals is also uncertain. Governmental support and regulatory change may be needed to complete the final steps of the repurposing process.

Another potential barrier to implementation is patient perceptions about the efficacy of older medicines compared to newly developed therapies. In an era where treatments have become targeted to particular tumour subtypes, a therapy indicated in multiple tumour types could be seen as outmoded and be less trusted. Patient and public involvement in the creation of promotional material to increase awareness and highlight any potential benefits will be essential.

6.3 Future research directions

6.3.1 Metformin

Whilst this thesis provides evidence supporting a phase III trial of metformin in the adjuvant treatment of colorectal cancer and prostate cancer, there are no trials running in these tumour types at the present time. One major phase III trial (MA-32, NCT01101438) investigating metformin for the adjuvant treatment of breast cancer has completed recruitment, and another (IBIS 3, ISRCTN93764730)) has completed its feasibility phase. The findings of these trials will provide a randomised evaluation of the anti-cancer effects of metformin in patients without DM, and irrespective of tumour type investigated, will allow a fuller evaluation.

This research found that in prostate cancer, patients undergoing radical radiotherapy had a greater improvement in RFS (section 5.3.2). Any phase III trial in this setting should use randomisation by minimisation to ensure that primary treatment modalities are evenly distributed between arms, and sub-analyses are conducted to investigate these differences. Phase III trials in both western and non-western populations are also needed to investigate the potential differences in response to metformin identified in these populations (section 5.3.1).

Observational studies investigating the adjuvant effects of metformin have mostly been conducted in patients with breast, colorectal and prostate cancer. To examine the efficacy of metformin in less common tumour types, collaboration between research groups is needed to combine the observational data available from different populations.

6.3.2 Aspirin

The Add-Aspirin trial is currently recruiting and the last of the four cohorts is expected to complete recruitment in 2021. Sub-studies conducted in parallel to the main study have the potential to maximise the research value of the trial. Aspirin resistance is said to affect 25% of the population (267), and if the anti-cancer mechanism of aspirin relies on anti-platelet activity, then this has the potential to affect the power of the study. TBX2 is a product of platelet aggregation and is a marker of platelet activity (268). Urinary measurement of TBX2 in a sub-population at baseline and at different timepoints throughout the trial could investigate this as a potential biomarker, provide mechanistic insights and investigate the role of aspirin resistance. A collection of tumour and blood samples from trial participants has been created which will allow the investigation of several of the biomarkers proposed, for example tumour expression of COX-2 and HLA, and also PIK3CA and BRAF mutation status.

6.3.3 Trial methodology

Further research into the accuracy of anticipated recruitment rates is needed, particularly the proposal to investigate differences between the accuracy of predictions from different types of

cancer centres. The proposal to improve the accuracy of recruitment projections by collecting minimum and maximum monthly site recruitment predictions and using the mean value, needs prospective validation.

It is possible that adherence may diminish as participants continue to take trial treatment for up to 5 years. Investigation of adherence annually in a sub-group using the adherence methods used in the run-in period could be considered. Given the potential for placebo arm contamination, an assessment of participant blinding could also be considered at the completion of the trial. An analysis of the correlation between treatment adherence in the run-in period and trial visit attendance later in the trial is also proposed.

If sufficient evidence from phase III trials emerges to support the use of both aspirin and metformin in the adjuvant cancer setting, a poly-pill containing both aspirin and metformin could be considered. This could improve long-term adherence thought the use of a single pill and could be seen as a more innovative cancer therapy by patients. If developed in collaboration with the pharmaceutical industry it could also attract the financial and operational support necessary for further research and regulatory approval.

6.4 Summary

The repurposing of medicines for use in another treatment setting is very attractive, particularly because of its potential to generate low-cost and globally available therapies. Whilst this research highlights several advantages of repurposing, there will always be a need for new drug development, as all medicines must be discovered before they can be repurposed, and drug resistance means that new medicines will always be needed. This thesis provides a significant addition to the literature on the potential anti-cancer activity of metformin and how phase III trial methodology could be adapted for repurposing.

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Appendix B:

Add-Aspirin trial conduct survey

Add-Aspirin Trial Conduct Survey

We would be grateful if you could complete this short survey on the experiences that you have had of the Add-Aspirin trial at your site. This will allow us improve the conduct of this, and similar trials.
Please seek input from colleagues at your site as necessary. You can go back and edit your answers up until the survey is complete.

Answering all questions should take less than 10 minutes.

We thank you in advance for participating in this survey.

Add-Aspirin Trial Conduct Survey

* 1. Please select your job title/role

(please select the single option which most closely applies or select "other")

- ◯ Clinical trials nurse
- ◯ Clinical trials coordinator
- ◯ Clinical trials assistant
- ◯ Clinical trials sister
- ◯ Clinical trials practitioner
- ◯ Other (please specify)

* 2. Is your site open to the Add-Aspirin trial?

◯ Yes ◯ No

Add-Aspirin Trial Conduct Survey

* 3. Please select the reason(s) that your site has not opened to Add-Aspirin.
This will help us to identify any barriers we may be able to assist with in future.
(Select all that apply).

- [ ] Insufficient staff
- [ ] Lack of interest in study
- [ ] concerns about excess trial costs
- [ ] Unable to identify principal investigator(s)
- [ ] Competing trials
- [ ] Other (please specify)
- [ ] Lack of familiarity with a platform design
- [ ] Lack of familiarity with a run-in period design
- [ ] Concerns about differences in follow-up schedules
- [ ] Delay in sponsor opening site

Add-Aspirin Trial Conduct Survey

* 4. Please enter your four digit site number.
If you work at multiple sites please add additional site numbers below

Site number (four digits)

Additional site number (optional)

Additional site number (optional)

* 5. How many cohorts (tumour types) are open at your site?

◯ One ◯ Two ◯ Three ◯ Four

* 6. Please select the cohorts that are open at your site
(select all that apply).

- [ ] Breast cohort
- [ ] Colorectal cohort
- [ ] Gastro-oesophageal cohort
- [ ] Prostate cohort

* 7. If the **breast** cohort is **not** open at your site, please select the reason(s).
(If the **breast** cohort is open at your site, please select "N/A" only).

- [ ] N/A (breast cohort is open)
- [ ] Breast cancer patients are not seen at this site
- [ ] Insufficient staffing
- [ ] Unable to identify a Principal Investigator
- [ ] Other (please specify)
- [ ] Concerns about differences in follow-up schedules
- [ ] Competing trials
- [ ] Intention to open soon

* 8. If the **colorectal** cohort is **not** open at your site, please select the reason(s).
(If the **colorectal** cohort is open, please select "N/A" only.)

- [ ] N/A (colorectal cohort is open)
- [ ] Colorectal cancer patients are not seen at this site
- [ ] Insufficient staffing
- [ ] Unable to identify a Principal Investigator
- [ ] Other (please specify)
- [ ] Concerns about differences in follow-up schedules
- [ ] Competing trials
- [ ] Intention to open soon

* 9. If the **gastro-oesophageal** cohort is **not** open at your site, please select the reason(s).
(If the **gastro-oesophageal** cohort is open, please select "N/A" only.)

- [ ] N/A (gastro-oesophageal cohort is open)
- [ ] Gastro-oesophageal cancer patients are not seen at this site
- [ ] Insufficient staffing
- [ ] Unable to identify a Principal Investigator
- [ ] Other (please specify)
- [ ] Concerns about differences in follow-up schedules
- [ ] Competing trials
- [ ] Intention to open soon

* 10. If the **prostate** cohort is **not** open at your site, please select the reason(s).
(If the **prostate** cohort is open, please select "N/A" only.)

- [ ] N/A (prostate cohort is open)
- [ ] prostate cancer patients are not seen at this site
- [ ] Insufficient staffing
- [ ] Unable to identify a Principal Investigator
- [ ] Other (please specify)
- [ ] Concerns about differences in follow-up schedules
- [ ] Competing trials
- [ ] Intention to open soon

Add-Aspirin Trial Conduct Survey

Add-Aspirin has a platform design that spans four tumour types (cohorts) in one trial

* 11. Were there any efficiencies in **opening** Add-Aspirin as one trial in multiple tumour types (cohorts) compared to **opening** the same number of separate trials?

◯ Yes ◯ No

* 12. Please indicate how strongly you agree or disagree with the following statement.

*"**Opening** Add-Aspirin as one trial in multiple tumour types (cohorts) was more efficient than **opening** the same number of separate trials"*

Strongly agree	agree	neither agree or disagree	disagree	strongly disagree
◯	◯	◯	◯	◯

Add-Aspirin Trial Conduct Survey

* 13. Please indicate where efficiencies in **opening** Add-Aspirin as one trial in multiple tumour types (cohorts) occur (compared to opening the same number of separate trials).
(Select all that apply)

- [ ] Staffing
- [ ] Training
- [ ] Pharmacy set-up
- [ ] Other (please specify)
- [ ] Radiology departmental approval
- [ ] Pathology departmental approval
- [ ] Regulatory approval

Add-Aspirin Trial Conduct Survey

* 14. Please provide details of any reasons why it was less efficient to **open** Add-Aspirin as one trial in multiple tumour types, compared to **opening** the same number of separate trials (enter "N/A" if there were no inefficiencies identified).

Add-Aspirin Trial Conduct Survey

Copy of page: Add-Aspirin has a platform design that spans four tumour types (cohorts) in one trial

* 15. Were there any efficiencies in **running** Add-Aspirin as one trial in multiple tumour types, compared to **running** the same number of separate trials?

Yes No

* 16. Please indicate how strongly you agree or disagree with the following statement.

*"**Running** Add-Aspirin as one trial in multiple tumour types (cohorts) was more efficient than **running** the same number of separate trials"*

Strongly agree	agree	neither agree or disagree	disagree	strongly disagree
○	○	○	○	○

Add-Aspirin Trial Conduct Survey

* 17. Please indicate where efficiencies in **running** Add-Aspirin as one trial in multiple tumour types (cohorts) occur (compared to **running** the same number of separate trials).
(Select all that apply)

- [ ] Staffing
- [ ] One overarching protocol
- [ ] Data entry
- [ ] Familiarity with common trial processes
- [ ] Drug dispensing
- [ ] Other (please specify)

Add-Aspirin Trial Conduct Survey

* 18. Please provide details of any reasons why it was less efficient to**run** Add-Aspirin as one trial in multiple tumour types, rather than **running** the same number of separate trials.
(Please enter "N/A" if there were no inefficiencies identified).

Add-Aspirin Trial Conduct Survey

Add-Aspirin incorporates a run-in period design where all participants take 100mg of aspirin for eight weeks to select participants that are more likely to tolerate and adhere to trial treatment during the rest of the trial.

* 19. Please indicate how strongly you agree or disagree with the following statements.

	Strongly agree	Agree	Neither agree or disagree	Disagree	Strongly disagree
"Treatment adherence in the Add-Aspirin run-in period is likely to be a good predictor of treatment adherence in the rest of the trial (randomised phase)"	○	○	○	○	○
"Treatment tolerance in the Add-Aspirin run-in period is likely to be a good predictor of treatment tolerance in the main trial (randomised phase)"	○	○	○	○	○
"The Add-Aspirin run-in period is likely to reduce the number of participants who do not tolerate treatment during the main trial (randomised phase)"	○	○	○	○	○
"The Add-Aspirin run-in period is likely to reduce number of participants who do not adhere to treatment in the main trial (randomised phase)"	○	○	○	○	○
"The Add-Aspirin run-in period is likely to reduce number of participants who miss scheduled follow-up visits in the main trial (randomised phase)"	○	○	○	○	○

20. Please help us to understand why you selected the answers above (optional question):

ADD-ASPIRIN Add-Aspirin Trial Conduct Survey

* 21. The following are potential reasons that patients might give for not registering for Add-Aspirin. Based on your experiences (or that of your colleagues), please rank them in order of how frequently each reason has been given, (with the most common reason at number 1 etc). Please select "N/A" if a particular reason has not been expressed.

⠿	▲▼ Over the counter availability of aspirin (without a prescription)	☐ N/A
⠿	▲▼ Number of follow-up visits	☐ N/A
⠿	▲▼ Reluctance to be randomised to placebo	☐ N/A
⠿	▲▼ Need to take trial treatment for at least 5 years	☐ N/A
⠿	▲▼ Concerns about toxicity	☐ N/A

Add-Aspirin Trial Conduct Survey

* 22. Is your site open to the gastro-oesophageal cohort?

◯ Yes ◯ No

Add-Aspirin Trial Conduct Survey

* 23. Please select from the following list, the reason(s) which reduce(s) recruitment to the gastro-oesophageal cohort of Add-Aspirin at your site.

(Select all that apply)

- ☐ Gastro-oesophageal MDT is at a different site
- ☐ Workload from other Add-Aspirin tumour types
- ☐ Few patients meet eligibility criteria
- ☐ Trial registration timelines
- ☐ It is easier to recruit patients in other tumour types (cohorts) in Add-Aspirin
- ☐ Concerns about toxicity

Other (please specify)

Add-Aspirin Trial Conduct Survey

Individuals who are participating in other approved trials may also register (co-enrol) for the Add-Aspirin trial (providing they are otherwise eligible). By including individuals who are registered in other trials, we will be able to assess how well aspirin works when given in addition to both current and potentially future standard treatments.

* 24. Please indicate how strongly you agree or disagree with the following statements.

	Strongly agree	agree	neither agree or disagree	disagree	strongly disagree
"Allowing co-enrolment with other trials maximises participant choice."	○	○	○	○	○
"Allowing co-enrolment with other trials improves recruitment to clinical trials."	○	○	○	○	○

25. If you have any concerns you have about co-enrolment please describe them below (optional question).

Add-Aspirin Trial Conduct Survey

Add-Aspirin includes a short cognitive assessment called the MoCA blind. This is to investigate the hypothesis that aspirin protects against cognitive decline. It can be administered by any member of the trial team where the task is delegated by the local Principal Investigator.

* 26. Have you, or a colleague at your site (other than a clinician) administered the cognitive assessment (MoCA blind) questionnaire?

- ○ Yes
- ○ No

Add-Aspirin Trial Conduct Survey

* 27. How long, on average, did the MoCA blind questionnaire take to deliver?
(Drag the slider to the preferred position)

0 minutes 15 minutes 30 minutes

* 28. Please indicate how difficult the survey was to deliver (Select one)

Very easy	Easy	Average	Hard	Very hard
◯	◯	◯	◯	◯

* 29. Were you aware of the audio recording and video example of the questionnaire being administered on Add-Aspirin website?

- ◯ Yes
- ◯ No

Add-Aspirin Trial Conduct Survey

Once you click **"done"** below, the survey will finish and no further changes will be possible.
If you want to go back and make changes please click **"prev"** to edit any responses.

Thank-you for completing this survey.

Appendix C:

Add-Aspirin site initiation form

ADD-ASPIRIN

Initial Site Evaluation Form

Please complete this questionnaire and keep the original at the front of your site file.
Please send a signed and dated copy by email as an attachment to: mrcctu.add-aspirin@ucl.ac.uk.
Please return only 1 form from your site.

Site name and address:	**Principal Investigator name:**	
	Email:	**Phone:**
Clinical Research Network:	**Please list all other clinical sites where your patients might also be seen/followed up:**	

In order to facilitate the R&D approval process, each clinical site should name a Principal Investigator (PI) to take overall responsibility for the trial at site, as well as responsibility for their own tumour site-specific cohort(s). There should also be named co-PIs, responsible for each of the other tumour site-specific cohorts that a site will recruit to. PIs and co-PIs will be acknowledged in the relevant publications.

Which cohort(s) do you anticipate that you will recruit to?
It is hoped that the vast majority of sites will recruit to all four cohorts. If you are unable to identify co-PIs within your site, please contact the trial team (mrcctu.add-aspirin@ucl.ac.uk) who may be able to assist.

	Yes	No	If yes, please indicate which cohort(s) the PI is responsible for and provide co-PI name and email for the other cohorts	
			Name	Email
Breast	☐	☐		
Gastro-oesophageal	☐	☐		
Colorectal	☐	☐		
Prostate	☐	☐		

Please provide the following contact details in order to facilitate the set-up process:

	Name	Email	Phone
First point of contact			
Main contact for agreement between Trust and UCL			
Contact to transfer SSI form to on IRAS			
Main pharmacy contact			

Patient population: approximately how many eligible patients do you expect to recruit at your site per month (broken down by cohort)?		
	Approximately how many eligible patients do you see?	Approximately how many patients would you expect to recruit?
Breast		
Gastro-oesophageal		
Colorectal		
Prostate		

Investigator and Staff experience

Please note that PI and co-PIs for the trial must send a copy of their CV to MRC CTU before site activation. Please send the copies to the Add-Aspirin trial team either by fax (020 7670 4818) or email (mrcctu.add-aspirin@ucl.ac.uk). All staff who will be working on the trial are required to have up-to-date GCP training. It is the responsibility of the PI to ensure that this is the case.

	Yes	No	Details
Has your site participated in other MRC CTU studies? If yes, please provide brief details.	☐	☐	
Has your site participated in other academic studies? If yes, please provide brief details.	☐	☐	
Has your site participated in pharmaceutical company studies? If yes, please provide brief details.	☐	☐	
Has your site ever had any serious protocol breaches? If yes, provide details.	☐	☐	

Has your site ever had an audit or MHRA inspection? If yes, please give details of any major or critical findings that might be relevant to the Add-Aspirin trial.	☐	☐	

Pharmacy
Default initial drug delivery at set-up will be 70 boxes (approx. total size 495cm x 585cm x 135cm). Please contact the trial team (mrcctu.add-aspirin@ucl.ac.uk) if you anticipate any problems with receiving this quantity of trial drugs.

	Yes	No	Comments
Drug destruction policy: can your pharmacy routinely destroy returned drug? **Please note that we are only collecting returned drug for the run-in period, and not for the blinded part of the trial.**	☐	☐	
Do you accept IMP deliveries on Saturdays?	☐	☐	
Please provide the contact name, exact address and telephone number for IMP deliveries			
Please provide instructions for delivery including what to do if named contact is not available to receive delivery			

Signature:	Date:
Printed name:	Role:

Thank you very much for completing this form.

Appendix E:

Protocol for metformin systematic review and meta-analysis

A systematic review and meta-analysis of metformin as an adjuvant treatment for cancer

Christopher Coyle, Fay Cafferty, Claire Vale, Ruth Langley

Citation
Christopher Coyle, Fay Cafferty, Claire Vale, Ruth Langley. A systematic review and meta-analysis of metformin as an adjuvant treatment for cancer. PROSPERO 2015:CRD42015020519 Available from http://www.crd.york.ac.uk/PROSPERO_REBRANDING/display_record.asp?ID=CRD42015020519

Review question(s)
Aim: To systematically review the available evidence on cancer outcomes in patients receiving, compared to those not receiving, metformin.

Primary objective: To assess the effects of metformin on recurrence-free survival in the adjuvant setting by tumour group.

Secondary objective: To assess the effects of metformin for the treatment of cancer in terms of cancer specific and overall survival by tumour group.

Searches
Identification of studies

Electronic Databases:

• MEDLINE 1966-2015 will be searched to retrieve any RCT or non-RCT examining the effects of metformin cancer mortality or primary or secondary prevention of cancer

• EMBASE 1982-2015 will be searched to retrieve any RCT or non-RCT examining the effects of metformin on the primary or secondary prevention of cancer or cancer mortality.

• Cochrane Central Register of Controlled Trials (CENTRAL) will be searched for any meta-analysis or systematic review examining the effects of metformin on the primary or secondary prevention of cancer or cancer mortality.

Trial Registers:

ClinicalTrials.gov

Conference Proceedings:

Conference proceedings searched electronically:

Proceedings of the American Society of Clinical Oncology (ASCO) 2004-2015

Proceedings of the European Society of Medical Oncology (ESMO) 1990-2015

Proceedings of the European Cancer Conference Organization (ECCO) 1990-2015

Additional hand searches:

Bibliographies of the reports of all identified studies and review articles will be screened for further studies.

Types of study to be included
Inclusion criteria

Any randomised controlled trials (RCTs), non- RCTs (including epidemiologic studies, case control studies, cohort studies, cross-sectional studies and longitudinal studies).

Studies must include data on individual tumour groups.

Condition or domain being studied
The oral anti-diabetic medication Metformin.

Cancer and cancer outcomes, in the adjuvant settting

Participants/ population
Inclusion criteria

Adults with a diagnosis of any solid cancer type

Exclusion criteria

Age less than 16 years old

Intervention(s), exposure(s)
Inclusion criteria: metformin use

Comparator(s)/ control
Treatment comparisons:

• Metformin users compared to non-metformin users with a diagnosis of type II diabetes

• Metformin users with type II diabetes compared to non-metformin users without type II diabetes

Outcome(s)
Primary outcomes
Recurrence-free survival

Secondary outcomes
Overall survival

Cancer specific survival

Data extraction, (selection and coding)
Selection of studies

All relevant abstracts will be assessed for eligibility, and when search results cannot be rejected from the title and/or abstract, the full text publication will be obtained, where available. Any queries will be checked by a second independent reviewer and resolved by consensus.

Data extraction and management

Data on patient characteristics, interventions and outcomes will be extracted from publications and presentations into predesigned forms (appendix C) and cross-checked by a second independent reviewer. Any disagreements will be resolved by consensus.

Patient characteristics

Age

Sex

Diabetic status (intervention and control arm)

Treatment setting (e.g. adjuvant)

Clinical Stage

Patient ethnicity

Study Characteristics

country

Type of study (e.g. rct, case-control, retrospective cohort study)

Publication type (e.g. full, conference abstract)

Method of participant selection

Source data

Number of inclusions/exclusions

Median follow-up

Study statistical methods- confounding factors examined (age, stage, sex, BMI, smoking status, other anti-diabetic therapy use) and whether adjustment performed or multivariate analysis used

Risk of bias (e.g. time- related biases, adjustment for confounders)

Treatment characteristics

Control arm details (e.g. any additional agents)

Time-point of metformin exposure assessment

Outcomes

(Primary)

Recurrence-free survival

(Secondary)

Overall survival

Cancer specific survival

Other outcomes of interest

We also hope to explore the impact of metformin dose/exposure and clinicopathological characteristics on the outcomes described above and also examine data on metastasis free survival where data is available

Risk of bias (quality) assessment

Data will be extracted on the type of study and for potential sources of bias, for example whether particular potential confounding factors are accounted for (see trial group analysis plan). All studies will be individually assessed for bias.

Strategy for data synthesis
The primary analysis of recurrence free survival will be undertaken for patients who have undergone potentially curative treatment. Studies will be grouped by tumour type for analysis, and a meta-analysis will be performed where sufficient data exists for each tumour type. All eligible studies will be included in the analyses for secondary outcomes (overall survival and cancer specific survival) and meta-analysis performed as described above for each tumour type where possible.

For meta-analyses of time-to-event outcomes, such as recurrence-free survival and overall survival, the hazard ratio (HR) is the most appropriate statistic. Where available, the HR and associated statistics will be extracted directly from the trial reports. Where not reported, they will be estimated from Kaplan Meier curves or summary statistics using published methods (Parmar 1998, Tierney 2007, Williamson 2002). Where insufficient data are available, supplementary data may be sought directly from the trial investigators.

Analysis of subgroups or subsets
Trial group analyses

To explore whether any trial or patient characteristics have any impact on the size or the direction of the effect of metformin therapy, for the primary outcome of recurrence free survival, we also aim calculate HRs for groups of trials, providing sufficient data are available. The resulting HR estimates from the trial groups will be compared using the test for interaction. If no difference in the effect of treatment between groups of trials is found, interpretation will be based on results from all trials together, otherwise they will be based results from groups of trials.

Studies will be therefore be grouped according to:

•Treatment comparison (studies with diabetic compared to non-diabetic control groups)

•Study type (RCT, case-control study, retrospective cohort study).

•Secondary outcomes in patients who have undergone potentially curative treatment only

And for studies in prostate cancer only:

•Primary treatment type (prostatectomy or radical radiotherapy)

Heterogeneity will be assessed using the chi-square test for heterogeneity and the I-squared statistic. The random effects model will be used to establish the robustness of all results to the choice of model.

Contact details for further information
Dr Coyle

c.coyle@ucl.ac.uk

Organisational affiliation of the review
Medical Research Council Clinical Trials Unit at University College London (MRC CTU at UCL)

http://www.ctu.mrc.ac.uk

Review team
Dr Christopher Coyle, MRC CTU at UCL
Dr Fay Cafferty, MRC CTU at UCL
Dr Claire Vale, MRC CTU at UCL
Dr Ruth Langley, MRC CTU at UCL

Anticipated or actual start date
15 May 2015

Anticipated completion date
31 July 2015

Funding sources/sponsors
MRC CTU at UCL

Conflicts of interest
None known

Language
English

Country
England

Subject index terms status
Subject indexing assigned by CRD

Subject index terms
Adjuvants, Immunologic; Adjuvants, Pharmaceutic; Humans; Metformin; Neoplasms

Stage of review
Completed and published

Date of registration in PROSPERO
15 May 2015

Date of publication of this revision
13 December 2016

Details of final report/publication(s)
Coyle C, Cafferty FH, Vale C, Langley RE. Metformin as an adjuvant treatment for cancer: a systematic review and meta-analysis. Annals of Oncology. 2016 Sep 28.
http://annonc.oxfordjournals.org/content/early/2016/09/27/annonc.mdw410.full.pdf+html

DOI
10.15124/CRD42015020519

Stage of review at time of this submission	Started	Completed
Preliminary searches	Yes	Yes
Piloting of the study selection process	Yes	Yes
Formal screening of search results against eligibility criteria	Yes	Yes
Data extraction	Yes	Yes
Risk of bias (quality) assessment	Yes	Yes
Data analysis	Yes	Yes

Search Strategies

MEDLINE Search strategy
Interventions:
1. exp Biguanides/
2. exp Metformin/
3. Metformin.tw,ot.
4. Biguanide$.tw,ot.
5. or/1-4 (Combines all of the intervention hits)

Outcomes (+ disease setting)
6. exp Mortality/
7. mortality.tw,ot.
8. mortaliti$.tw,ot.
9. or/6-8 (combines all mortality studies)
10. exp Primary Prevention/
11. exp Secondary Prevention/
12. (prevention$ or prevent$).tw,ot.
13. exp Neoplasm/
14. (cancer$ or neoplasm$ or tumo?r$).tw,ot.
15. or/10-14 (combines all primary and secondary prevention studies)

Standard RCT search
16. exp Randomized Controlled Trials as topic/
17. Randomized Controlled Trial.pt.
18. exp Controlled Clinical Trials as topic/
19. Controlled Clinical Trial.pt.
20. exp Random Allocation/
21. exp Double-Blind Method/
22. exp Single-Blind Method/
23. or/16-22

Other Study types
24. Epidemiologic Studies/
25. exp Case-Control Studies/
26. exp Cohort Studies/
27. Cross-Sectional Studies/
28. (epidemiologic adj (study or studies)).ab,ti.
29. case control.ab,ti.
30. (cohort adj (study or studies)).ab,ti.
31. cross sectional.ab,ti.
32. cohort analy$.ab,ti.
33. (follow up adj (study or studies)).ab,ti.
34. longitudinal.ab,ti.
35. retrospective$.ab,ti.
36. prospective$.ab,ti.
37. (observ$ adj3 (study or studies)).ab,ti.
38. adverse effect?.ab,ti.
39. Or/24-38 (Non-RCTs)
40. exp "Review Literature as topic"/
41. exp Technology Assessment, Biomedical/
42. exp Meta-analysis as topic/
43. Meta-analysis.pt.
44. hta.tw,ot.
45. (health technology adj6 assessment$).tw,ot.
46. (meta analy$ or metaanaly$ or meta?analy$).tw,ot.
47. ((review$ or search$) adj10 (literature$ or medical database$ or medline or pubmed or embase or cochrane or cinahl or psycinfo or psyclit or healthstar or biosis or current content$ or systemat$)).tw,ot.
48. or/40-47 (Combines all reviews)
49. 23 or 39 (RCTs or non-RCTs)
50. 48 or 49 (RCTs or non-RCTs or reviews)
51. 5 and 15 and 50 (Interventions + prevention studies + RCTs and non-RCTs and reviews)

52. 5 and 9 and 50 (Interventions and mortality + RCTs and non-RCTs and reviews)
53. 51 or 52 (Combines prevention and mortality)
54. limit 53 to animals
55. limit 53 to humans
56. 54 not 55 (Animals not humans)
57. 53 not 56 (Prevention and mortality – all studies excluding animal studies)

EMBASE Search strategy
Interventions:
1. exp Biguanides/
2. exp Metformin/
3. Metformin.tw,ot.
4. Biguanide$.tw,ot.
5. or/1-4 (Combines all of the intervention hits)

Outcomes (+ disease setting)
6. exp Mortality/
7. mortality.tw,ot.
8. mortaliti$.tw,ot.
9. or/6-8 (combines all mortality studies)
10. exp Primary Prevention/
11. exp Secondary Prevention/
12. (prevention$ or prevent$).tw,ot.
13. exp Neoplasm/
14. (cancer$ or neoplasm$ or tumo?r$).tw,ot.
15. or/10-14 (combines all primary and secondary prevention studies)

Standard RCT search
16. randomi$.tw
17. placebo.mp
18. double-blind.tw
19. or/16-18

Other Study types
20. epidemiology/
21. exp case control study/
22. exp cohort analysis/
23. cross sectional study/
24. (epidemiologic adj (study or studies)).ab,ti.
25. case control.ab,ti.
26. (cohort adj (study or studies)).ab,ti.
27. cross sectional.ab,ti.
28. cohort analy$.ab,ti.
29. (follow up adj (study or studies)).ab,ti.
30. longitudinal.ab,ti.
31. retrospective$.ab,ti.
32. prospective$.ab,ti.
33. (observ$ adj3 (study or studies)).ab,ti.
34. adverse effect?.ab,ti.
35. Or/20-34 (Non-RCTs)
36. exp literature/
37. exp biomedical technology assessment/
38. exp meta-analysis/
39. meta-analysis.kw
40. hta.tw,ot.
41. (health technology adj6 assessment$).tw,ot.
42. (meta analy$ or metaanaly$ or meta?analy$).tw,ot.
43. ((review$ or search$) adj10 (literature$ or medical database$ or medline or pubmed or embase or cochrane or cinahl or psycinfo or psyclit or healthstar or biosis or current content$ or systemat$)).tw,ot.
44. or/36-43 (Combines all reviews)
45. 19 or 35 (RCTs or non-RCTs)
46. 44 or 45 (RCTs or non-RCTs or reviews)
47. 5 and 15 and 46 (Interventions + prevention studies + RCTs and non-RCTs and reviews)

48. 5 and 9 and 46 (Interventions and mortality + RCTs and non-RCTs and reviews)
49. 47 or 48 (Combines prevention and mortality)
50. limit 49 to animals
51. limit 49 to humans
52. 50 not 51 (Animals not humans)
53. 49 not 52 (Prevention and mortality – all studies excluding animal studies)

Cochrane search strategy
#1 "metformin"
#2 "neoplasm"
#1 and#2

ASCO and ESMO search strategy
Keyword search for "metformin"
Clinicaltrials.gov, ISRCTN and EU Clinical Trials Register search strategy
Search term "metformin" AND "cancer"
Restricted to trials with published results

Data extraction fields

Study characteristics

- Tumour group
- Tumour subtype/histopathology
- Treatment
- Other eligibility restrictions
- Stage
- Comparator diabetic status
- Study design
- Cancer outcomes reported
- Publication type
- Study time period
- Countries
- Clinical setting (population level, hospital, number of centres)
- Metformin exposure definition
- Data source

Participant characteristics

- Mean/median age of participants
- Median follow-up
- Gender (%male)
- Median BMI
- Proportion of metformin users (%)
- Number of metformin users (intervention group)
- Number of non-metformin users (comparator group)

Cancer outcome data

- End-point terminology used
- Hazard ratio
- 95% confidence interval
- p-value

Study confounding factors/biases

- Covariates adjusted for
- Assessment of specific potential confounders
- (BMI, age, gender,

Appendix F:

Newcastle-Ottawa scoring for metformin systematic review

NEWCASTLE - OTTAWA QUALITY ASSESSMENT SCALE
CASE CONTROL STUDIES

Note: A study can be awarded a maximum of one star for each numbered item within the Selection and Exposure categories. A maximum of two stars can be given for Comparability.

Selection

1) Is the case definition adequate?
   a) yes, with independent validation ✵
   b) yes, eg record linkage or based on self reports
   c) no description

2) Representativeness of the cases
   a) consecutive or obviously representative series of cases ✵
   b) potential for selection biases or not stated

3) Selection of Controls
   a) community controls ✵
   b) hospital controls
   c) no description

4) Definition of Controls
   a) no history of disease (endpoint) ✵
   b) no description of source

Comparability

1) Comparability of cases and controls on the basis of the design or analysis
   a) study controls for _____ (Select the most important factor.) ✵
   b) study controls for any additional factor ✵ (This criteria could be modified to indicate specific control for a second important factor.)

Exposure

1) Ascertainment of exposure
   a) secure record (eg surgical records) ✵
   b) structured interview where blind to case/control status ✵
   c) interview not blinded to case/control status
   d) written self report or medical record only
   e) no description

2) Same method of ascertainment for cases and controls
   a) yes ✵
   b) no

3) Non-Response rate
   a) same rate for both groups ✵
   b) non respondents described
   c) rate different and no designation

NEWCASTLE - OTTAWA QUALITY ASSESSMENT SCALE
COHORT STUDIES

Note: A study can be awarded a maximum of one star for each numbered item within the Selection and Outcome categories. A maximum of two stars can be given for Comparability

Selection

1) Representativeness of the exposed cohort
   a) truly representative of the average _____ (describe) in the community ✵
   b) somewhat representative of the average _____ in the community ✵
   c) selected group of users eg nurses, volunteers
   d) no description of the derivation of the cohort

2) Selection of the non exposed cohort
   a) drawn from the same community as the exposed cohort ✵
   b) drawn from a different source
   c) no description of the derivation of the non exposed cohort

3) Ascertainment of exposure
   a) secure record (eg surgical records) ✵
   b) structured interview ✵
   c) written self report
   d) no description

4) Demonstration that outcome of interest was not present at start of study
   a) yes ✵
   b) no

Comparability

1) Comparability of cohorts on the basis of the design or analysis
   a) study controls for _____ (select the most important factor) ✵
   b) study controls for any additional factor ✵ (This criteria could be modified to indicate specific control for a second important factor.)

Outcome

1) Assessment of outcome
   a) independent blind assessment ✵
   b) record linkage ✵
   c) self report
   d) no description

2) Was follow-up long enough for outcomes to occur
   a) yes (select an adequate follow up period for outcome of interest) ✵
   b) no

3) Adequacy of follow up of cohorts
   a) complete follow up - all subjects accounted for ✵
   b) subjects lost to follow up unlikely to introduce bias - small number lost - > ____ % (select an adequate %) follow up, or description provided of those lost) ✵
   c) follow up rate < ____% (select an adequate %) and no description of those lost
   d) no statement

Appendix G:

Publications

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ADD-ASPIRIN: A phase III, double-blind, placebo controlled, randomised trial assessing the effects of aspirin on disease recurrence and survival after primary therapy in common non-metastatic solid tumours

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ABSTRACT

Background: There is a considerable body of pre-clinical, epidemiological and randomised data to support the hypothesis that aspirin has the potential to be an effective adjuvant cancer therapy.
Methods: Add-Aspirin is a phase III, multi-centre, double-blind, placebo-controlled randomised trial with four parallel cohorts. Patients who have undergone potentially curative treatment for breast ($n = 3100$), colorectal ($n = 2600$), gastro-oesophageal ($n = 2100$) or prostate cancer ($n = 2120$) are registered into four tumour specific cohorts. All cohorts recruit in the United Kingdom, with the breast and gastro-oesophageal cohort also recruiting in India. Eligible participants first undertake an active run-in period where 100 mg aspirin is taken daily for approximately eight weeks. Participants who are able to adhere and tolerate aspirin then undergo a double-blind randomisation and are allocated in a 1:1:1 ratio to either 100 mg aspirin, 300 mg aspirin or a matched placebo to be taken daily for at least five years. Those participants ≥75 years old are only randomised to 100 mg aspirin or placebo due to increased toxicity risk.
Results: The primary outcome measures are invasive disease-free survival for the breast cohort, disease-free survival for the colorectal cohort, overall survival for the gastro-oesophageal cohort, and biochemical recurrence-free survival for the prostate cohort, with a co-primary outcome of overall survival across all cohorts. Secondary outcomes include adherence, toxicity including serious haemorrhage, cardiovascular events and some cohort specific measures.
Conclusions: The Add-Aspirin trial investigates whether regular aspirin use after standard therapy prevents recurrence and prolongs survival in participants with four non-metastatic common solid tumours.

1. Background and introduction

1.1. Rationale

The Add-Aspirin trial includes participants with breast, colorectal, gastro-oesophageal and prostate tumours which, together, accounts for approximately one third of all cancer cases and cancer deaths [1]. The selected disease sites are those for which (i) the evidence relating to potential benefit of aspirin is strongest; (ii) the potential impact is large (common cancers with large numbers of cases diagnosed at an early stage, or where outcomes of curative treatment are particularly poor); and (iii) recruitment is feasible. As a low-cost pharmaceutical, feasible to administer in both resource poor and rich countries, aspirin has the potential to significantly impact on cancer outcomes worldwide. This, combined with other possible health benefits (such as cardiovascular effects), means that aspirin warrants further investigation as an anti-cancer agent.

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[1] The Add-Aspirin investigators are detailed in Appendix A.

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1551-7144/

1.2. Supporting evidence

There have been well over 100 case-control and cohort studies investigating the use of aspirin and cancer risk [2]. A meta-analysis of such studies showed that aspirin use resulted in significant reductions in the risk of developing cancer, most notably in colorectal (relative risk (RR) 0.73, 95% confidence-interval (CI) 0.67–0.79), gastric (RR 0.67, CI 0.54–0.83), adenocarcinoma of the oesophagus/cardia (RR 0.67, CI 0.54–0.83), squamous cell carcinoma of the oesophagus (RR 0.64, CI 0.52–0.78), breast (RR 0.90, CI 0.85–0.95), and prostate cancer (RR 0.90, CI 0.85–0.98) [2]. Observational studies have also shown improvements in survival with aspirin use after a diagnosis of breast [3–5], colorectal [6–11], gastro-oesophageal [12,13] and prostate cancer [14–16].

Randomised data is available to indirectly substantiate these observations. A meta-analysis of individual participant data on cancer incidence in randomised trials (designed to investigate the effect of aspirin on vascular disease) show marked reductions in cancer incidence and cancer mortality associated with regular aspirin use (greater than three years) in both the short and long-term [17–20]. Similarly, long-term follow-up from the Women's Health Study, a randomised placebo-controlled trial designed to assess the effects of aspirin (100 mg on alternate days) in the primary prevention of cardiovascular disease and cancer, showed that allocation to aspirin reduced the incidence of colorectal cancer with ten years of follow-up (hazard ratio (HR) 0.80, CI 0.67–0.97) [21]. A recent meta-analysis of studies examining aspirin use after a cancer diagnosis has shown a significant reduction in cancer-specific mortality in colon cancer, but not in breast and prostate cancer, however significant heterogeneity between studies was identified [22].

Furthermore, the first randomised trial specifically designed to demonstrate that aspirin can prevent the development of cancer has shown that 600 mg of aspirin daily for up to four years prevents colorectal and other cancers associated with Lynch syndrome (a hereditary condition which predisposes to cancer development) HR 0.45, CI 0.26–0.79 [23].

The potential benefits of aspirin have to be weighed against the risk of adverse effects. A number of systematic reviews and meta-analyses have examined the potential risks of adverse events [24,25]. A recent review estimates that, depending on age and sex, regular aspirin use over a 15-year period would lead to an absolute increase in major bleeding events of between 0.16% and 0.81%. The authors conclude that prophylactic aspirin use for a minimum of five years at a dose of 75 mg–325 mg daily has a favourable benefit-harm profile [26]. For individuals treated for cancer, with a high risk of recurrent disease, the balance could be even more favourable.

2. Methods

2.1. Aims

The Add-Aspirin trial aims to assess whether regular aspirin use after standard potentially curative primary therapy can prevent recurrence and prolong survival in individuals with four common early stage solid tumours. Avoiding recurrent disease, subsequent treatment and the associated morbidity and mortality in these individuals is an important goal. Multicentre and international recruitment will allow assessment of the intervention in a range of settings, with the aim of demonstrating that implementation is both feasible and cost-effective across varying health care systems and in both the developing and developed world. A secondary aim is to assess the potential overall health benefits of aspirin for these individuals including cardiovascular outcomes.

2.2. Overview of design

The Add-Aspirin trial investigates the use of both 100 mg daily and 300 mg daily aspirin compared with matched placebo (double-blind) in each of four different tumour types, utilising an overarching protocol. Further details of the rationale for this design are provided in the discussion section of this article. Fig. 1 shows a summary schema for the trial.

2.3. Participants

Participants entering the Add-Aspirin trial have undergone potentially curative treatment (surgery or other radical treatment) for breast, colorectal, gastro-oesophageal or prostate cancer with standard neoadjuvant and/or adjuvant therapy if indicated, and may also have participated in any pre-approved trials and satisfy the eligibility criteria, summarised in Fig. 2.

2.4. Registration

The Add-Aspirin trial is open to centres in every Cancer Research Network (CRN) throughout the four devolved nations of the United Kingdom (UK) and will also recruit participants in India (other countries may join subsequently). Eligible participants who have provided consent and meet the timing of entry criteria are registered online (through the trial website, www.addaspirintrial.org). The timing of entry window has been designed so that aspirin can be started at the earliest opportunity to maximise the potential benefits, whilst starting at a time when it is considered safe to do so and unlikely to compromise the curative intent of standard primary treatment. Figures describing the timing of entry criteria for each cohort are available in Appendix B.

2.5. Run-in period

The Add-Aspirin trial incorporates a feasibility phase lasting approximately 2 1/2 years during which recruitment feasibility, treatment adherence and safety will be assessed. During the feasibility phase of the study, all participants are required to complete an active run-in period after registration but prior to randomisation where they take 100 mg aspirin daily (one tablet per day) in an open-label manner for a period of approximately eight weeks.

2.6. End of run-in period assessment

At the end of the run-in period, the participant's tolerance of aspirin and adherence to daily treatment will be assessed. This approach allows those individuals who are unlikely to be able to tolerate aspirin, as well as those who are unlikely to be able to adhere to the protocol treatment schedule, to be identified. Adherence will be assessed using a combination of a participant diary card, used blister packs and patient reported adherence. Participants will be suitable for randomisation if they have taken at least 80% of their run-in treatment and have not experienced any aspirin-related severe toxicity (defined as ≥grade 3 CTCAE v4), nor any grade of gastrointestinal bleeding, active gastrointestinal ulceration, new or worsening tinnitus, macular degeneration, intracranial bleeding or hypersensitivity to aspirin. If the investigator feels that the reason for inadequate adherence is temporary (for example, due to toxicity resulting from concomitant adjuvant treatment which has subsequently finished or a non-recurrent unrelated event), the run-in period may be extended by four or eight weeks to reassess adherence and toxicity subject to agreement from the central trial team. Those participants identified as suitable for further study participation, and who remain eligible and are willing to continue in the trial then re-confirm their consent to participate before being randomised.

2.7. Randomisation

Following assessment at the end of the run-in period, eligible participants in the UK are randomised by phone and, in India, via the trial website. Participants undergo a double-blind randomisation. Randomisation is performed separately within each tumour-specific

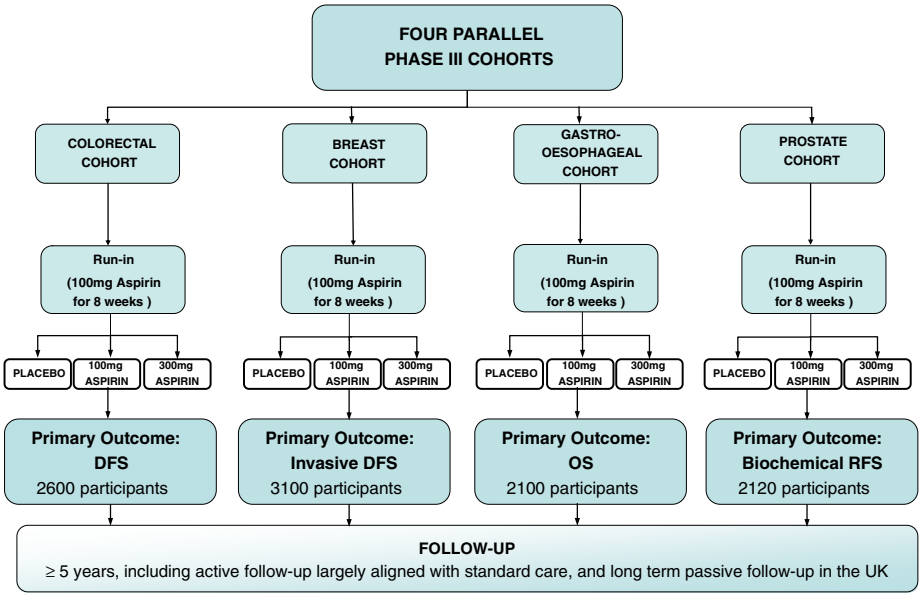

Fig. 1. Add-Aspirin trial schema. DFS = Disease free survival, OS = overall survival, RFS = recurrence free survival.

cohort and uses minimization algorithms based on key prognostic factors (dependent on tumour site), incorporating a random element. Within each tumour-specific cohort, participants who are below 75 years old are allocated in a 1:1:1 ratio to either 100 mg aspirin, 300 mg aspirin or a matched placebo. Participants who are 75 years old or over, are only allocated to either 100 mg aspirin or matched placebo as toxicity is thought to increase with age but the allocation ratio of 2:1 remains so that they have the same chance of receiving active

Breast cohort main inclusion criteria

Histologically confirmed invasive breast cancer.

Node positive or node negative with high risk features.

Surgery (R0 resection) with standard. neoadjuvant and/or adjuvant therapy where indicated.

Known HER2 and ER status.

Colorectal cohort main inclusion criteria

Stage II or III colon or rectum adenocarcinoma. (Patients with resected liver metastases are eligible*).

Surgery (R0 resection) with standard neo-adjuvant /adjuvant therapy where indicated.

CEA ≤1.5 X upper limit of normal.

Gastro-oesophageal cohort main inclusion criteria

Oesophageal or gastric adenocarcinoma or squamous cell carcinoma.

Previous therapy with curative intent:

Either: Surgery (R0 resection) with standard neo-adjuvant +/- adjuvant therapy,

or Primary chemoradiotherapy

Proton pump inhibitor is mandated for patients undergoing partial gastrectomy or oesophagectomy.

Prostate cohort main inclusion criteria

Histologically confirmed non-metastatic prostate adenocarcinoma (T1-3a, N0).

Intermediate or high risk as per D'Amico classification

Previous therapy with curative intent:

Either: Radical radiotherapy,

or Radical prostatectomy (+/- adjuvant radiotherapy)

or Salvage radiotherapy after radical prostatectomy

Common exclusion criteria

No clinical or radiological evidence of residual or distant disease.
(*Patients with colorectal cancer who have undergone resection of liver metastases with clear margins are eligible).

No current, regular use of aspirin, NSAIDs or anti-coagulants.

No pre-disposition to aspirin toxicity (e.g. active ulceration).

For the full eligibility criteria please see the protocol or www.addaspirintrial.org.

Fig. 2. Summary of eligibility criteria.

treatment as the other participants. The target randomisation figure is 9920 participants in the United Kingdom (UK) and India combined. Assuming that approximately 10% of participants will not be randomised following the run-in (for reasons relating either to toxicity or adherence), it is expected that 11,000 participants will be registered to begin the run-in period.

2.8. Follow-up

Patients are followed up at three-monthly intervals initially and then six-monthly. Adherence to treatment is verbally assessed at every follow-up visit. In the UK, trial treatment, and active follow-up, continues for at least five years after randomisation. Long-term passive follow-up data will be obtained from routinely-collected healthcare databases for at least ten further years. Indian participants will be actively followed-up for at least ten years after randomisation. For participants that are registered but do not go on to be randomised, active participation in the trial will end at that time. However, passive follow-up will continue via routinely-collected healthcare datasets where consent for this has been obtained. The trial assessment schedule for each cohort is aligned with standard practice where possible to ensure they can be implemented easily. This is balanced with the need to ensure appropriate monitoring of patients on trial treatment and assessment of outcome measures. The trial follow-up schedules are available in Appendix C.

2.9. Toxicity management

Participants that experience any aspirin-related severe toxicity (defined as ≥grade 3 Common Terminology Criteria for Adverse Events (CTCAE v4)) or any grade of gastrointestinal bleeding, active gastrointestinal ulceration, tinnitus, macular degeneration, intracranial bleeding or hypersensitivity to aspirin are required to permanently discontinue aspirin immediately.

For those who are asymptomatic, prophylactic measures to reduce the risk of gastrointestinal toxicity from aspirin (such as proton pump inhibitor (PPI) prophylaxis and helicobacter pylori eradication) are not routinely recommended in participants at low risk of gastrointestinal complications and so are not mandated in the Add-Aspirin trial protocol. However, PPI use for the duration of aspirin treatment is recommended for patients who have undergone oesophagectomy or partial gastrectomy and should also be considered for older patients (≥75 years), or any other participant who might be at increased risk of toxicity. Intracranial bleeding is a rare toxicity of aspirin, and hypertension can increase the risk. Those with poorly controlled hypertension have trial treatment withheld until their blood pressure is controlled. Further guidelines are available in the trial protocol.

Investigators are advised to manage toxicities under the assumption that the participant is receiving the highest possible dose of the active product (300 mg aspirin), without the need for unblinding, however where knowledge of treatment allocation would alter clinical management, unblinding is possible. Unblinding can be performed via an access-controlled system available through the trial website (www.addaspirintrial.org).

2.10. Sub-studies

The size and diversity of the Add-Aspirin cohort provides opportunity to address other secondary research questions and evaluate novel methodology. Aspirin has been proposed to have a number of health benefits beyond cancer, particularly in older people. To investigate the overall health benefits of aspirin, functional capacity is assessed using the Vulnerable Elders Survey (VES-13) [27]. This is performed for participants that are 65 years old or over at trial registration, and five years after randomisation and can be administered in person or over the telephone. The hypothesis that aspirin protects against cognitive decline is assessed using a short version of the Montreal Cognitive Assessment (the MoCA-blind). The MoCA-blind takes approximately 7 to 10 min to complete and is administered in all Add-Aspirin trial participants at registration, then again at one and five years after randomisation. No training is required to administer the questionnaire, which can be conducted in person, or over the telephone. A methodological sub-study will compare the quality and completeness of routinely-collected healthcare data with data collected within the trial, with the aim of assessing the suitability of passive follow-up data collection for investigating long-term primary and secondary outcome measures within the trial. This will be an early validation that will determine ongoing use of routinely collected data in the trial. A sub-study is planned to investigate methods of measuring adherence, including the collection of urine samples to measure thromboxane B2 (a direct measure of the effects of aspirin). Methodological sub-studies to improve site initiation and recruitment will also be undertaken.

2.11. Translational objectives

The Add-Aspirin trial incorporates a sample repository where a baseline blood sample and tumour sample are stored for future translational projects. The sample repository is jointly hosted by two institutions in the UK, Tayside Tissue Bank and the Wales Cancer Bank. In India, a baseline blood sample and tumour sample from selected sites will be stored at the Tata Memorial Centre biobank. A number of studies are expected to be initiated whilst the trial is ongoing (subject to funding), including studies to identify groups that will benefit most from aspirin use (for example, investigation of the role of tumour PIK3CA mutation status), and to investigate the mechanisms underlying the anti-cancer effects of aspirin, particularly effects on platelet function and the pro-thrombotic tumour microenvironment.

3. Results

3.1. Outcomes

Tumour site-specific primary analyses will take place 5–6 years after recruitment of the last participant for that cohort, with the exact timing based on the observed numbers of events. Primary and secondary outcome measures are available in Table 1. Overall survival is a secondary outcome measure in all cohorts except the gastro-oesophageal cohort, where it is the primary outcome. Overall survival will also be assessed as a co-primary outcome measure in all participants after 15 years. The longer follow-up and large sample size associated with this analysis will enable any long-term benefits of aspirin to be realised, including those unrelated to the primary cancer, for example the potential for prevention of deaths related to vascular events and second malignancies. Consideration of rates of serious toxicity (and particularly serious haemorrhage), as well as other secondary health outcomes, alongside the efficacy results will be particularly important in these analyses in order to provide an holistic assessment of the potential risks and benefits associated with different doses.

3.2. Statistical considerations

Primary analyses will compare outcomes for participants allocated to aspirin (100 mg and 300 mg arms combined) and participants allocated to placebo, regardless of the treatment received (i.e. intention-to-treat). The primary analyses will include both those participants <75 years who underwent the full randomisation and those ≥75 years who underwent randomisation between 100 mg aspirin or placebo only, but the dose effects of aspirin will be investigated only on those randomised between the two doses.

If an overall effect of aspirin vs. placebo is observed in the primary treatment comparison for one or more cohorts, a further analysis will be performed to investigate differences in efficacy according to aspirin

Table 1
Outcome measures.

Cohort	Primary outcome measures
Breast cancer	Invasive disease-free survival (IDFS) [28]
Colorectal cancer	Disease-free survival (DFS)
Gastro-oesophageal cancer	Overall survival
Prostate cancer	Biochemical recurrence-free survival (bRFS)
All cohorts combined	Overall survival
Cohort	Secondary outcome measures
All cohorts	Overall survival (except for gastro-oesophageal cohort)
	Adherence
	Toxicity
	Serious haemorrhage CTCAE (v4) grade 3 or greater
	Serious vascular events
	Thrombotic events
	Diabetes and associated complications
	Second malignancies
	Age-related macular degeneration
	Cognitive assessment (using the MOCA-blind questionnaire)
	Dementia
	Comorbidities (using the Charlson Index)
	Obesity (using the Body Mass Index)
	Functional capacity (using the VES-13 questionnaire)
Breast	Breast cancer-specific survival
	Bone metastases-free survival
	Invasive disease-free survival-ductal carcinoma insitu (IDFS-DCIS)
Colorectal	Colorectal cancer-specific survival
Gastro-oesophageal	Disease-free survival
Prostate	Prostate cancer-specific survival
	Time to initiation of salvage treatment
	Bone metastases-free survival

dose. This analysis will be performed only in the cohorts that show a positive result for aspirin vs. placebo and will be stratified by cohort. By making these analyses conditional on a benefit of aspirin being observed in the primary analysis, the likelihood of a false-positive result is reduced. The rationale for combining the data across cohorts is to maximise power, as we anticipate that any difference between doses of aspirin will be smaller than the difference between aspirin and placebo. The trial has also established collaborative links with research groups running other aspirin cancer trials internationally with a view to future meta-analyses.

3.3. Sample size breast cohort

Based on data from recent trials, we expect that five-year invasive disease-free survival (IDFS) in the control group will be approximately 80% [29–32]. 717 IDFS events will be required to achieve 90% power to detect a 4% (HR = 0.78) improvement in this rate. Assuming that the cohort takes 3 1/2 years to recruit, with analysis six years later, we anticipate that 3100 participants will be required to observe this number of events.

3.4. Sample size colorectal cohort

Based on data from recent trials, we expect that five-year disease-free survival (DFS) in this cohort will be approximately 70% [33,34]. 899 DFS events will be required to achieve 90% power to detect a 5% (HR = 0.80) improvement in this rate. Assuming that the cohort takes 3 1/2 years to recruit, with analysis six years later, we anticipate that 2600 participants will be required to observe this number of events.

3.5. Sample size gastro-oesophageal cohort

Based on data from recent trials, we expect that five-year overall survival in this cohort will be approximately 45% [35–39]. 1120 deaths will be required to achieve 80% power to detect a 6% (HR = 0.84) improvement in this rate. Assuming that the cohort takes six years to recruit, with analysis five years later, we anticipate that 2100 participants will be required to observe this number of events.

3.6. Sample size prostate cohort

The radical prostatectomy and radical radiotherapy groups are powered to assess effects separately. In the radical prostatectomy group, we anticipate that biochemical recurrence-free survival (bRFS) at five years will be approximately 75% [40]. For the radical radiotherapy group, five year bRFS is estimated to be approximately 65% [41]. To achieve 90% power to detect an 8% improvement in these rates, 673 bRFS events will be required. Assuming that the cohort takes five years to recruit, with analysis five years later, we anticipate that 2120 participants will be required to observe this number of events.

Sample size calculations for all cohorts are based on a two-sided 5% significance level and account for a degree of loss to follow-up and slower recruitment in the early stages of the trial. Target registrations have been inflated to allow a 10% dropout after the run-in period.

3.7. Ethical considerations

The trial will be conducted in compliance with the approved protocol, the Declaration of Helsinki 2008, the principles of Good Clinical Practice (GCP), Commission Directive 2005/28/EC with the implementation in national legislation in the UK by Statutory Instrument 2004/1031 and subsequent amendments, the UK Data Protection Act (DPA number: Z5886415), and the National Health Service (NHS) Research Governance Framework for Health and Social Care (RGF). International centres will comply with the principles of GCP as laid down by the ICH topic E6 (Note for Guidance on GCP) and applicable national regulations. The Add-Aspirin trial is registered with the International Standard Randomised Controlled Trial Number ISRCTN74358648, and has also been submitted for registration with the Clinical trials Registry of India (REF/2016/06/011465). The Add-Aspirin trial was approved by the South Central – Oxford C research ethics committee and is part of the UK National Cancer Research Network (NCRN) portfolio. In India, the trial has been approved by the Directorate of the National Cancer Grid (NCG), and is part of the NCG trials portfolio. University College London (UCL) and the Tata Memorial Centre (TMC) are co-sponsors of the trial and have delegated responsibility for the overall management of the Add-Aspirin trial to the MRC CTU at UCL and Tata Memorial Centre CTU for India.

4. Discussion

The Clinical Trials Authorisation for the Add-Aspirin trial was granted by the Medicines and Healthcare products Regulatory Agency (MHRA) on 25th November 2014, and ethics approval was given on 4th June 2014. The first participant was recruited on 8th October 2015. At the time of writing on 1st May 2016, the Add-Aspirin trial is recruiting across 120 centres in the UK, and is recruiting ahead of its overall projected targets, with the breast cohort the fastest recruiter. The last of the four cohorts is now expected to complete recruitment in 2021. Current recruitment figures are available at www.addaspirintrial.org.

4.1. Challenges and methodological solutions

There are a number of practical and operational challenges presented by a large adjuvant trial of a generic and repurposed intervention. These include the need for cost efficiencies due to a lack of industry financial support for a trial of a generic pharmaceutical; ensuring sufficient long-term adherence in a largely asymptomatic population; and the potential for control arm contamination due to over the counter

(OTC) availability of aspirin. These have been addressed using a number of contemporary methodological approaches.

4.2. Overarching protocol

A platform trial design was chosen because there is evidence that aspirin is potentially effective in multiple tumours. Investigating the use of both 100 mg daily and 300 mg daily aspirin across cohorts addresses uncertainty surrounding the optimal aspirin dose required to achieve anti-cancer effects, potentially saving many years of research time. Keeping all four cohorts within a single protocol ensures that the management of each cohort is as comparable as possible (with the exception of some site specific procedures). This allows a combined analysis of overall survival as a co-primary outcome measure and cross cohort secondary analyses of toxicity, cardiovascular and other health benefits, thus increasing the overall potential impact of the trial. In addition, this design provides the capacity to add further tumour sites and also provides a potential platform for evaluation of other repurposed agents. An overarching protocol provides economies of scale both centrally and site level, including site set-up, regulatory approval, central staffing, coordination, oversight and data management. The resulting cost efficiencies improve the financial viability of the trial given the lack of industry support, and provide value for money for our charitable and governmental funders.

4.3. Antecedent aspirin use

A proportion of individuals who are otherwise eligible for the trial will already be taking aspirin regularly. It is conceivable that pre-existing aspirin use could alter tumour biology [42], and that those already taking aspirin might be randomised to placebo which would be unethical. Consequently a decision was made to exclude current or previous regular aspirin users.

4.4. Over the counter aspirin

Unlike most other cancer trials, the intervention in the Add-Aspirin can be purchased without a prescription. There is a risk that some potential participants might opt to not enter the trial and purchase aspirin independently. OTC availability of aspirin also leads to the potential for control arm contamination and an increased risk of toxicity if they are randomised to 300 mg aspirin. To combat OTC aspirin use, site staff are trained to re-enforce the key message that as yet, there is no clear evidence from a randomised trial that adjuvant aspirin use improves survival, and equipoise is emphasised in all information provided to potential participants. For those already registered, site staff are trained to regularly ask about and discourage OTC aspirin use. Randomisation to two doses of aspirin or placebo also leads to a 2:1 chance of receiving a potentially active agent.

4.5. Timing of entry considerations

The timing of trial registration has been aligned across all four cohorts to make the treatment of each as similar as possible, however a number of adjustments have been necessary to account for the variety of treatment modalities and pathways, and differences in patient characteristics between cohorts. The timing of entry around adjuvant chemotherapy requires particular consideration. The risk of developing dyspepsia during adjuvant chemotherapy varies according to the regimen used, the need for dexamethasone as a supportive therapy, and the incidence of risk factors in that group. Dyspepsia is a common occurrence during adjuvant chemotherapy for breast cancer and as such, patients can only register once chemotherapy is complete. In the colorectal and gastro-oesophageal cohorts dyspepsia developing as a consequence of adjuvant chemotherapy is less common, and registration is permitted when six weeks of adjuvant chemotherapy has been administered (without developing dyspepsia and subject to acceptable platelet counts).

4.6. Run-in period

The run-in period was implemented after funders' concern about the risk of poor adherence. Adherence will be assessed during the run-in period using three different methods to allow a more accurate assessment. These include a participant diary card, return of used blister packs and participant interview at an end of run-in assessment. The run-in period provides an early opportunity to assess feasibility, in terms of early toxicity, recruitment and patient acceptability, and provides a population for the randomised phase who are more likely to tolerate and adhere to the trial treatment for the duration of the trial [43]. This strategy has also been used successfully in other aspirin trials [44,45]. We do not believe that eight weeks of aspirin will reduce the effect between the aspirin and placebo comparison in the trial as data have consistently shown that long-term treatment (a minimum of 2 years [1], and up to 5–10 years [2,3]) with aspirin is required for the anti-cancer effects of aspirin to become identifiable [1–3]. The use of the run-in period is being monitored carefully and will be reviewed on completion of an initial feasibility phase. Other methods of encouraging adherence include the use of blister packs labelled with days of the week, provision of diary cards, participant newsletters and promotion of the trial website which includes updates and reaction to stories related to aspirin in the news media.

4.7. Drug supply

Aspirin is a generic drug, Bayer AG donated all doses of the blinded active intervention and matched placebo, but not the packaging, labelling, blinding or distribution, which therefore represented a major operational and funding challenge. These challenges have been met by outsourcing some of these processes and development of an in-house drug supply management system to track stock levels at sites and automatically trigger re-orders based on projected demand. The system also includes an unblinding capability.

4.8. Co-enrolment

Since aspirin is intended to be given following or alongside standard primary therapy, rather than replacing any element of current treatment, it will be appropriate to include participants who have already taken part in trials of primary treatments wherever possible. This will allow assessment of the efficacy of aspirin in participants who have received both current and potentially future standard of care treatments. Including participants from other treatment trials will help to ensure the future relevance of both trials, is important for recruitment feasibility, and maximises the opportunities for patients to participate in trials. We have found that the acceptability of co-enrolment to researchers varies by tumour group. This may be due to variation in the amount of trial activity or even differences in patient group demographics. Our approach has been to consider co-enrolment on a trial-by-trial basis, discussing this with the relevant trial teams, with a careful assessment of any conflicts in eligibility criteria, scheduling and potential impact on safety and the results of either trial. Where concerns exist, or reassurance is required, statistical modelling is conducted to assess the potential impact on trial results (there is often limited overlap leading to negligible impact). Co-enrolment has been agreed with 16 trials to-date and is planned with other trials currently in development.

4.9. Recruitment in India

Since aspirin is easily available worldwide, demonstrating its implementation in different resource settings will increase the global impact of the results. Recruitment in India is also important to ensure adequate

recruitment in the gastro-oesophageal cohort and also allows the development of new international collaborations. Academic multi-centre trials are rare in India and the set-up of Add-Aspirin has helped with development of a research infrastructure for this and future trials. Recent changes in clinical regulations in India have delayed opening and the trial is anticipated to open in India later in 2016.

4.10. Conclusions

Aspirin is a low-cost, generic drug that is easily available worldwide. Consequently, if aspirin is shown to be beneficial as an adjuvant treatment, even with a modest effect, it would change practice globally. Compared with many new agents or complex regimens, the intervention could be implemented quickly and on a broad scale, including in lower resource settings, with the potential to have a huge impact on the global cancer burden.

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Disclosures

Ruth E. Langley has received financial support through grants from Cancer Research UK and the National Institute for Health Research (NIHR) Health Technology Assessment (HTA) as Chief Investigator of the Add-Aspirin trial, has received compensation from Bayer and Aspirin Foundation for service on scientific advisory boards, and has received a supply of aspirin and placebo from Bayer Pharmaceuticals AG for the Add-Aspirin trial. Richard Wilson has received a supply of aspirin and placebo from Bayer Pharmaceuticals AG for the FOCUS4-B trial in metastatic colorectal cancer and for which he is the co-Chief Investigator.

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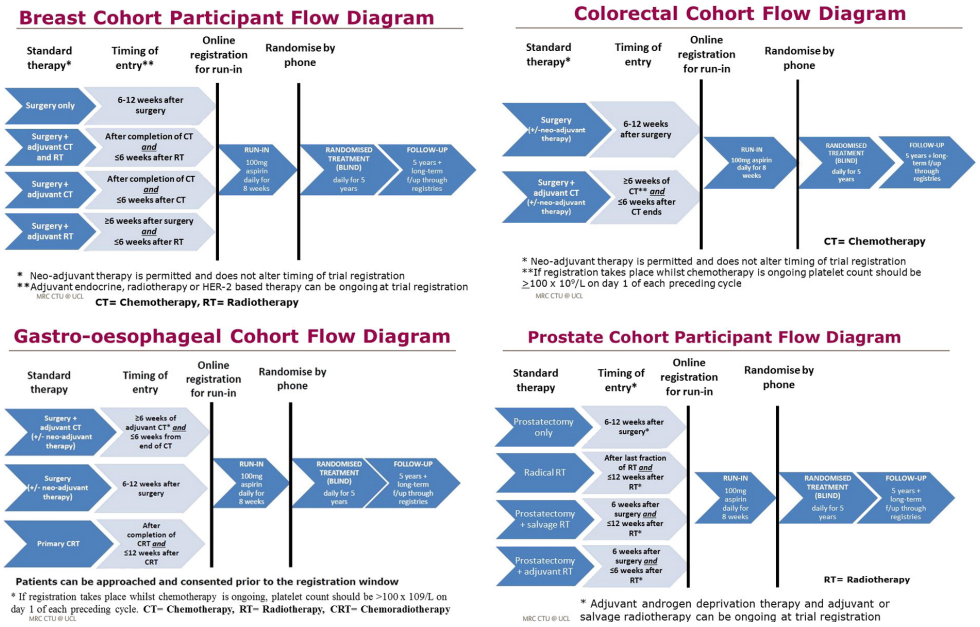
Appendix A. The Add-Aspirin trial investigators

Gastro-oesophageal Cohort Trial Management Group		
Professor Ruth Langley	Chief Investigator and Lead Investigator, Gastro-oesophageal Cohort (UK)	London, UK
Professor C S Pramesh	Lead Investigator – India, Lead Investigator, Gastro-oesophageal Cohort (India)	Mumbai, India
Dr Richard Hubner	Medical Oncologist – Gastro-oesophageal Cohort	Manchester, UK
Professor Janusz Jankowski	Gastroenterologist – Gastro-oesophageal Cohort	Warwick, UK
Mr Tim Underwood	Surgeon – Gastro-oesophageal Cohort	Southampton, UK
Professor Anne Thomas	Medical Oncologist – Gastro-oesophageal Cohort	Leicester, UK
Verity Henson	Clinical Trials Officer	Bristol, UK
Professor John Bridgewater	Medical Oncologist – Gastro-oesophageal Cohort	London, UK
Breast Cohort Trial Management Group		
Dr Alistair Ring	Lead Investigator – Breast Cohort (UK)	London, UK
Professor David Cameron	Medical Oncologist – Breast Cohort. Lead Investigator, Translational Research	Edinburgh, UK
Professor Sudeep Gupta	Lead Investigator – Breast Cohort (India)	Mumbai, India
Colorectal Cohort Trial Management Group		
Professor Richard Wilson	Lead Investigator – Colorectal Cohort	Belfast, UK
Dr Tim Iveson	Medical Oncologist – Colorectal Cohort	Southampton, UK
Professor Robert Steele	Surgeon – Colorectal Cohort	Dundee, UK
Dr Daniel Swinson	Medical Oncologist – Colorectal Cohort	Leeds, UK
Ms Farhat Din	Surgeon – Colorectal Cohort	Edinburgh, UK
Prostate Cohort Trial Management Group		
Professor Howard Kynaston	Lead Investigator – Prostate Cohort	Cardiff, UK
Dr Duncan Gilbert	Clinical Oncologist – Prostate Cohort	Brighton, UK
Mr Paul Cathcart	Surgeon – Prostate Cohort	London, UK
Cross-Study Collaborators		
Professor Mahesh Parmar	Director, MRC CTU	London, UK
Professor Peter Rothwell	Clinical Neurologist	Oxford, UK
Professor Carlo Patrono	Pharmacologist	Rome, Italy
Professor Sir John Burn	Clinical Geneticist	Newcastle, UK
Dr David Adlam	Cardiologist	Leicester, UK
Dr Michael Peake	Clinical Lead, National Cancer Intelligence Network	London, UK
Participant Representatives		
Lindy Berkman	Participant Representative, NCRI Consumer Forum	UK
Mairead MacKenzie	Participant Representative, Independent Cancer Patient Voices	UK
Vandana Gupta	Participant Representative, VCare	India
Arnold Goldman	Participant Representative	UK
MRC CTU at UCL		

Marta Campos	Trial Manager	MRC CTU, UK
Anna Thomason	Trial Manager	MRC CTU, UK
Ben Sydes	Data Manager	MRC CTU, UK
Shabinah Ali	Data Manager	MRC CTU, UK
Alex Robbins	Data Manager	MRC CTU, UK
Sam Rowley	Statistician	MRC CTU, UK
Dr Fay Cafferty	Project Leader/Senior Statistician	MRC CTU, UK
Dr Chris Coyle	Trial Physician and Trial Clinical Research Fellow	MRC CTU, UK

Appendix B. Figures describing cohort specific timing of entry criteria



Appendix C. Trial schedules

Assessments		Prior to registration	Prior to randomisation (end of run-in period)	Months since randomisation											
				3	6	9	12	18	24	30	36	42	48	54	60
All cohorts															
Main assessments	Registration assessments[a]	✓													
	End of run-in assessment[b]		✓												
	Follow-up assessments[c]			✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Intermittent assessments	VES-13 questionnaire (65 ≥ years at registration)	✓													✓
	Cognitive assessment	✓					✓								✓
Blood tests	FBC, LFT, U&E & eGFR	✓	✓		✓		✓		✓		✓		✓		✓
	C-Reactive Protein (CRP)	✓			✓		✓								
	Fasting lipid profile	✓													
Other tests	Tumour and blood sample	✓													
Breast cohort															
Imaging	Mammography	✓					✓		✓		✓		✓		✓
Colorectal cohort															
Imaging and procedures	CT (chest/abdomen/pelvis)	✓					✓		✓						✓
	Colonoscopy	✓						✓		✓					✓
Blood tests	CEA test	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

(continued on next page)

(*continued*)

Assessments	Prior to registration	Prior to randomisation (end of run-in period)	Months since randomisation											
			3	6	9	12	18	24	30	36	42	48	54	60
Prostate cohort														
Blood tests PSA	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Gastro-oesophageal cohort														
No cohort specific investigations														

[a] Registration assessments include: eligibility, co-enrolment (if applicable), blood pressure, height, weight, blood results, concomitant medication and comorbidities.
[b] End of run-in assessments include: symptoms and toxicity, adherence, blood pressure.
[c] Follow-up assessments include: symptoms and toxicity, adherence, blood pressure, weight, concomitant medication.

(*continued*)

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PERSONALIZED MEDICINE IN COLORECTAL CANCER (D CUNNINGHAM AND EC SMYTH, SECTION EDITORS)

Aspirin and Colorectal Cancer Prevention and Treatment: Is It for Everyone?

Christopher Coyle[1] · **Fay Helen Cafferty**[1] · **Ruth Elizabeth Langley**[1]

Abstract There is now a considerable body of data supporting the hypothesis that aspirin could be effective in the prevention and treatment of colorectal cancer, and a number of phase III randomised controlled trials designed to evaluate the role of aspirin in the treatment of colorectal cancer are ongoing. Although generally well tolerated, aspirin can have adverse effects, including dyspepsia and, infrequently, bleeding. To ensure a favourable balance of benefits and risks from aspirin, a more personalised assessment of the advantages and disadvantages is required. Emerging data suggest that tumour PIK3CA mutation status, expression of cyclo-oxygenase-2 and human leukocyte antigen class I, along with certain germline polymorphisms, might all help to identify individuals who stand to gain most. We review both the underpinning evidence and current data, on clinical, molecular and genetic biomarkers for aspirin use in the prevention and treatment of colorectal cancer, and discuss the opportunities for further biomarker research provided by ongoing trials.

Keywords Aspirin · Acetylsalicylic acid · NSAID · Colorectal cancer · Chemoprevention · Secondary prevention · Primary prevention · Adjuvant therapy · Bleeding · PIK3CA · BRAF · Single-nucleotide polymorphism · Human leukocyte antigen

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Introduction

Evidence for the anti-cancer effects of aspirin has emerged from in vitro and animal models, epidemiological studies and randomised data, with the most extensive evidence pertaining to colorectal cancer (CRC). Research suggests that aspirin is effective in primary prevention, reducing the risk of adenomas [1] and CRC [2•, 3]. There is also evidence for a possible role in the treatment of cancer, particularly in the adjuvant setting (preventing recurrence and decreasing the likelihood of metastases after potentially curative therapy) [4, 5•].

The potential benefits of aspirin have to be weighed against the risk of adverse effects, particularly in the primary prevention setting. Upper gastrointestinal symptoms (UGS) are a common concern associated with aspirin use, and can limit adherence, but are usually avoidable. The most undesirable effect of aspirin is an increased bleeding tendency, which can manifest as occult gastrointestinal bleeding, epistaxis or purpura. Serious extra-cranial bleeding is rare (an estimated 3.6 additional events per 10,000 people treated for a year with aspirin [6]), with the vast majority of bleeding episodes resolved without sequelae [7•], and intracranial haemorrhage is rarer still (an estimated 0.8 additional events per 10,000 people treated for a year with aspirin [6]).

Identifying biomarkers or clinical characteristics which predict benefit from aspirin use could lead to a more targeted intervention and protect some individuals from unnecessary treatment and possible side effects. A number of potential clinical, molecular and genetic biomarkers have been evaluated including the following: genes mutated in CRC (PIK3CA and BRAF), molecules proposed to have a role in the mechanism through which aspirin exerts its anti-cancer effects (cyclo-oxygenase (COX) enzymes and human leukocyte antigen (HLA) class I expression), and key genetic polymorphisms that may influence the actions of aspirin.

This review will summarise the current data supporting a personalised approach to aspirin use in relation to CRC and

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highlight the need to discover and validate biomarkers in ongoing trials. The article is structured into four sections: (i) primary prevention of CRC, (ii) treatment of CRC, (iii) safety biomarkers and (iv) other benefit-risk considerations.

Aspirin and Colorectal Cancer Prevention

The ability of aspirin to prevent colorectal carcinogenesis has been observed in animal models [8–10]. The first clinical evidence emerged in 1988 from a case-control study conducted in Melbourne, Australia, which showed that aspirin reduced the risk of developing CRC [11]. This finding was subsequently corroborated by several other epidemiological studies, with a meta-analysis in 2012 of 30 case-control and cohort studies ($n = 37{,}519$ CRC cases) showing that aspirin was associated with a lower risk of developing CRC (relative risk (RR) 0.73, 95 % confidence interval (CI), 0.67–0.79) [12]. Two large epidemiological studies have recently provided further supporting data. The Association of American Retired Persons Diet and Health study (AARP) included 301,240 adults aged between 50 and 71 years. An estimated 14 % reduction in CRC was observed with daily aspirin use (hazard ratio (HR) 0.86, CI 0.79–0.94) during 10 years of follow-up [13]. A Danish case-control study of 10,280 CRC cases and 102,800 controls showed a reduction in the risk of CRC (odds ratio (OR) 0.73, CI 0.54–0.99) for those continuously taking aspirin for at least 5 years [14•].

Randomised data substantiate these observations, with a meta-analysis of individual participant data on cancer incidence in randomised trials designed to investigate the effect of aspirin on vascular disease showing that aspirin reduced the 20-year risk of CRC by 24 % (HR 0.76, CI 0.63–0.94), improving to 32 % if taken for ≥5 years (HR 0.68, CI 0.54–0.87) [3]. Similarly, long-term follow-up from the Women's Health Study (WHS), a randomised placebo-controlled trial designed to assess the effects of aspirin (100 mg on alternate days) in the primary prevention of cardiovascular disease, showed that allocation to aspirin reduced the incidence of CRC by 20 % (HR 0.80, CI 0.67–0.97) [2•].

Recommending aspirin for all has been approached with caution due to the difficulties of predicting the chance of benefit, and the risk of toxicity on an individual level and therefore attention, has naturally turned towards individuals at highest risk of CRC who may benefit most.

Aspirin and Colorectal Cancer Prevention: Who Benefits?

Groups at the highest risk of developing CRC include those with a hereditary CRC syndrome, a history of colorectal adenomas or an inflammatory bowel disease. Aspirin can exacerbate inflammatory bowel disease and is therefore avoided, but there is evidence to support an effect of aspirin in these other high-risk groups.

The most robust evidence exists for Lynch syndrome (hereditary non-polyposis colorectal cancer), the most common inherited CRC syndrome. The CaPP2 (Cancer Prevention Project 2) trial randomly allocated patients with Lynch syndrome to 600 mg daily aspirin or placebo and found a reduction in CRC incidence in those that remained on aspirin for more than 2 years (HR 0.41, CI 0.19–0.86, $p = 0.02$) [15]. This is in the context of an overall incidence of CRC in the CaPP2 trial population of 5.6 % (48/861) over 4.5 years of follow-up, and is likely to represent the group with the highest absolute reduction in risk of CRC. Intriguingly, a sub-analysis of this trial showed that the increase in risk of CRC associated with obesity can be abrogated by aspirin in this population [16•]. Obesity is, thus, a potential predictive biomarker for aspirin benefit, and further research in populations other than Lynch syndrome is warranted. Less evidence is available to support an effect of aspirin in familial adenomatous polyposis (FAP), another inherited CRC syndrome. The CaPP1 trial randomised patients with FAP (prior to preventive surgery), in a 2×2 factorial design, to 600 mg daily aspirin, resistant starch or placebo. They found a trend towards reduced polyp load in aspirin users; however, this did not reach statistical significance (relative risk 0.77, CI 0.54–1.10) [17]. The median duration of aspirin use was only 17 months and it is plausible that a treatment effect may have emerged with longer exposure.

Adenomas are precursor lesions for most cases of CRC, and their prevention or regression has been proposed to represent a surrogate marker for CRC risk [18]. A meta-analysis of four randomised controlled trials, including 2967 individuals with previous adenomas or CRC (without an inherited CRC syndrome), found that those allocated to aspirin had a reduced risk of any subsequent adenoma (risk ratio 0.83, CI 0.72–0.96) or advanced adenoma (risk ratio 0.72, CI 0.57–0.90) [1]. This corresponded to a reduction in the absolute risk of any adenoma of 6.7 % (CI 3.2–10.2 %). Further research is needed to confirm whether individuals with a history of adenomas benefit more from aspirin than those without.

Whilst those at highest risk of CRC are most likely to gain from chemoprevention with aspirin, it may be possible to identify those that benefit in lower risk populations. Newly emerging data show that aspirin users with certain single-nucleotide polymorphisms (SNPs) have a reduced risk of developing CRC, and their absence may describe a group who will not benefit. A case-control study of 840 CRC patients and 1686 matched controls examined CRC risk according to expression of the T allele of rs6983267, which is associated with reduced WNT/β-catenin signalling, a major oncogenic pathway in CRC, proposed to be affected by aspirin. They observed that aspirin reduced CRC risk in the cohort as a whole (OR 0.71, CI 0.60–0.85), but the effect was most marked in individuals with a T allele of rs6983267 (OR 0.83, CI 0.74–

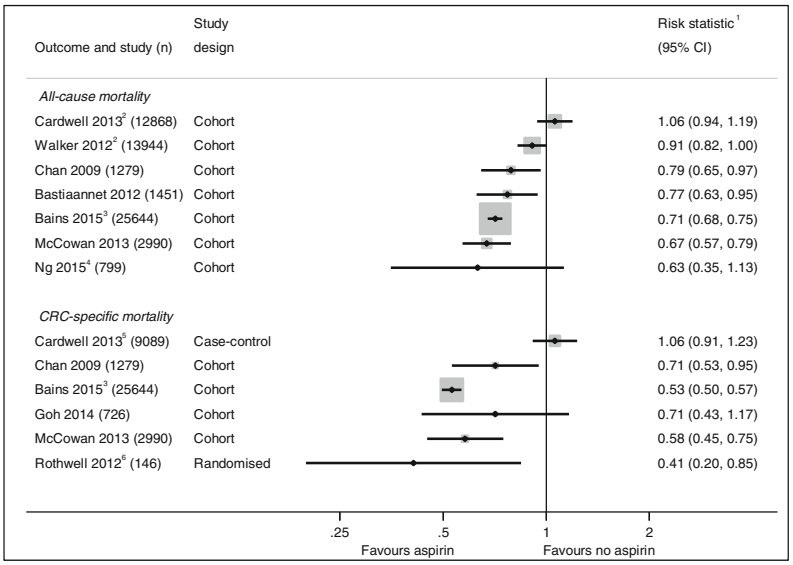

Fig. 1 Studies investigating CRC outcomes according to aspirin use following diagnosis. No summary statistic is presented given the high heterogeneity of studies. Multivariate (adjusted) statistics are presented in all cases. *1* All risk statistics are hazard ratios except the studies by Cardwell [23•] and Rothwell [30], which are odds ratios, and Bastiaannet [26] which is a rate ratio. *2* Studies have an overlapping population, both using UK General Practice Data. *3* Published in abstract form only at time of authorship. *4* Cohort taken from randomised chemotherapy trial and is limited to colon cancer. *5* Cohort only matched for CRC-specific mortality analysis. *6* Meta-analysis of cancer outcomes in randomised cardiovascular trials

0.94) [19••]. A larger case-control study (8634 cases, 8553 controls) examined the risk of CRC according to expression of two different SNPs, rs2965667 (located close to the microsomal glutathione S-transferase 1 gene, often upregulated in CRC) and rs16973225 (located close to the interleukin 16 gene, which has been implicated in CRC carcinogenesis) [20••]. It was observed that use of aspirin and/or non-steroidal anti-inflammatory drugs (NSAIDs) was associated with a reduced risk of CRC amongst individuals with TT genotype of SNP rs2965667 (OR 0.66, CI 0.61–0.70) and the AA genotype of rs16973225 (OR 0.66, CI 0.62–0.71), but not in those with other rarer genotypes. Germline genetic polymorphisms have the potential to identify individuals that benefit from aspirin, as well as those that do not, within populations at lower risk of CRC, and further investigation using existing datasets is required.

Aspirin and Colorectal Cancer Treatment

In vitro studies show that aspirin inhibits proliferation and induces apoptosis in CRC cell lines [21, 22] suggesting a possible role for aspirin in the treatment of CRC. Figure 1 summarises the results of the clinical studies investigating the effect of aspirin use after a CRC diagnosis. The first epidemiological data emerged from the Nurses' Health Study (NHS) and Health Professionals Follow-up Study (HPFS) showing that regular aspirin use after a diagnosis of CRC is associated with a reduction in CRC deaths (HR 0.71, CI 0.53–0.95) and overall mortality (HR 0.79, CI 0.65–0.97) [24]. This has recently been corroborated by a large cohort of CRC patients from the Cancer Registry of Norway where improvements in overall survival (HR 0.71, CI 0.68–0.75) and CRC-specific survival (HR 0.53, CI 0.50–0.57) were seen with aspirin use after CRC diagnosis [25]. Similar improvements in mortality were observed in data from the Eindhoven Cancer Registry (ECR) (overall survival RR 0.77, CI 0.63–0.95) [26] and in population data collected in Tayside, Scotland (overall mortality HR 0.67, CI 0.57–0.79, and CRC-specific mortality HR 0.58, CI 0.45–0.75) [27]. Observational data from the CALGB 89803 trial (which compared two different adjuvant chemotherapy regimens in patients with stage III colon cancer) has also shown a trend towards improved overall survival (HR 0.63, CI 0.35–1.12) and disease-free survival (HR 0.68, CI 0.42–1.11) in those patients using aspirin both during and after chemotherapy [28•]. A study of 13,994 CRC patients from the UK General Practice Research Database found a strong trend towards a reduction in overall mortality; however, this failed to reach significance (HR 0.91, CI 0.82–1.00) [29].

Data on cancer outcomes from randomised trials investigating the effects of aspirin in vascular disease corroborate the trends seen in epidemiological studies. A meta-analysis which included 13,833 individuals who developed CRC in four vascular trials has shown significant reductions in CRC deaths (HR 0.66, CI 0.51–0.85) [3]. Another meta-analysis of five vascular trials has shown that aspirin is associated with a reduction in the risk of having metastases when CRC is diagnosed (OR 0.36, CI 0.18–0.74) and of subsequently developing them during follow-up when not present at diagnosis (HR 0.26 CI 0.11–0.57) [30].

Table 1 Studies examining PIK3CA mutation, aspirin use and colorectal cancer outcomes

Study	PIK3CA mutation (%)	PIK3CA mutant					PIK3CA wild type				
		No aspirin	Aspirin	Outcome	HR	95 % CI *p* value	No aspirin	Aspirin	Outcome	HR	95 % CI *p* value
NHS and HPFS [36]	16.7	95	66	OS	0.54	0.31–0.94 $p=0.01$	466	337	OS	0.94	0.75–1.17 $p=0.96$
				CSS	0.18	0.06–0.61 $p<0.001$			CSS	0.96	0.69–1.32 $p=0.76$
VICTOR trial [37•]	11.6	90	14	OS	0.29	0.04–2.33 $p=0.19$	681	111	OS	0.95	0.56–1.61 $p=0.26$
				CSS	0.11	0.001–0.83 $p=0.027$			CSS	0.94	0.59–1.49 $p=0.79$
MCS and RMH [38•]	12.4	136	49	OS	0.96	0.58–1.57 $p=0.86$	Study of PIK3CA-mutated tumours only				
				CSS	0.60	0.34–1.16 $p=0.14$					
ECR[a] [33••]	15.8	73	27	OS	0.73[b]	0.33–1.63 $p=0.4$	348	147	OS	0.55	0.40–0.75 $p<0.001$

Multivariate (adjusted) statistics are presented in all cases

OS overall survival, *CSS* colorectal cancer-specific survival, *RFS* recurrence-free survival, *NHS* Nurses' Health Study, *HPFS* Health Professionals Follow-up Study, *MCS* Moffitt Cancer Centre, *RMH* Royal Melbourne Hospital, *ECR* Eindhoven Cancer Registry, *HR* hazard ratio

[a] Colon cancer only

[b] Rate ratio

Aspirin and Colorectal Cancer Treatment: Who Benefits?

Much of the work on potential biomarkers relating to aspirin use as a treatment for CRC has come from three large cohorts (NHS [24], HPFS [24] and ECR [26]). An initial analysis of the NHS/HPFS dataset reported that the beneficial effects of aspirin on CRC outcomes were restricted to individuals whose tumours overexpressed COX-2 (HR 0.39, CI 0.20–0.76)and not observed for those with weak or absent expression (HR 1.22, CI 0.36–4.18) [24]. Whilst inhibition of either COX-1 or COX-2 is sufficient to inhibit tumourigenesis in mouse models [31], uncertainty exists about the role of COX enzymes in relation to the anti-cancer effects of aspirin, particularly given that the daily doses of aspirin used in vascular prevention are not considered sufficient for sustained COX inhibition in systemic tissues [32]. The predictive utility of COX-2 has not been seen in other colorectal studies [33••] or comparative studies in breast cancer [34].

Although the mechanism by which aspirin exerts its anti-cancer effects remains unknown, one proposed hypothesis was that aspirin, through its anti-platelet effects, could expose circulating tumour cells to immune-mediated destruction by natural killer cells [35] and that this effect would be restricted to tumours with low or absent HLA class I expression. However, analyses of a random sample of colon tumour samples ($n=999$) from the ECR found that the benefit from aspirin therapy was largely restricted to tumours expressing HLA class I antigens (risk ratio 0.53, CI 0.38–0.74), and was not seen in those who had lost expression (risk ratio 1.03; CI 0.66–1.61) [33••]. This interesting observation, contrary to the original study hypothesis, requires validation in further datasets. HLA class I expression is seen in about a third of colorectal tumours and so could identify a sizeable group who might benefit from aspirin after a CRC diagnosis.

There has been significant interest in the phosphatidylinositol 3-kinase (PI3KCA) gene as a potential biomarker of aspirin response (Table 1). This follows the publication of data from the NHS/HPFS demonstrating that individuals with PIK3CA mutations taking regular aspirin after a diagnosis of CRC had markedly improved CRC-specific survival (HR 0.18, CI 0.06–0.61) compared to those with wild-type tumours (HR 0.96, CI 0.69–1.32). The same association was observed for overall survival (mutated PIK3CA HR 0.54, CI 0.31–0.94, wild-type PIK3CA HR 0.94, CI 0.75–1.17) [36]. This finding was supported by a small ad hoc analysis of the randomised VICTOR trial, where rofecoxib (a Cox-2 inhibitor) was being evaluated after CRC resection but the trial was closed early when rofecoxib was withdrawn from the market. Individuals with PIK3CA mutations taking regular aspirin after diagnosis had improved recurrence rates (HR 0.11, CI 0.001–0.83), whereas those lacking PIK3CA mutation did not (HR 0.92, CI 0.60–1.42), although the number of participants taking aspirin with the mutation was small ($n=14$) [37•]. However, data from two recent studies has not confirmed the association. In the ECR dataset, the survival benefit associated with aspirin use after a colon cancer diagnosis was seen in those with wild-type PIK3CA tumours (rate ratio 0.55, CI 0.40–0.75), as well as those with PIK3CA-mutated tumours where there was a trend towards a survival benefit but this did not reach statistical significance (rate ratio 0.73, CI 0.33–1.63) [33••]. In addition, in a cohort of patients with PIK3CA-mutated CRCs from the Moffitt Cancer Centre and Royal Melbourne Hospital ($n=1487$), no overall survival benefit was observed (HR 0.96, CI 0.58–1.57) and, whilst there was a trend towards a CRC-specific survival benefit, this was not significant (HR 0.60, CI 0.34–1.16) [38•]. PIK3CA mutations only occur in 10–15 % of patients with CRC, whereas the epidemiological data suggest that a greater

proportion of CRC patients benefit from aspirin use after a CRC diagnosis; therefore, this biomarker needs further investigation, ideally in randomised trials.

A cohort of 1226 patients with a diagnosis of CRC from the NHS and HPFS have also been analysed for BRAF mutation status. The effect of aspirin, after a cancer diagnosis, on cancer-specific and overall survival did not differ according to BRAF mutation status, but may have lacked statistical power. Interestingly, in terms of cancer prevention, aspirin was associated with a lower risk of developing a BRAF wild-type CRC (HR 0.73, CI 0.64–0.83), but not with BRAF-mutated CRC (HR 1.03, CI 0.76–1.38) [39•].

The risk of CRC recurrence after potentially curative treatment depends on a number of prognostic factors, which include stage, mode of presentation, microsatellite instability status and whether adjuvant chemotherapy was administered. Any relative improvement in CRC outcomes with aspirin will need to be considered in the context of an individual's absolute risk of recurrence.

Identifying Those at Risk of Toxicity

Aspirin has been used for over 100 years [40] and has a well-documented toxicity profile. Standard contraindications to aspirin use include the following: a history of active or recurrent peptic ulceration, active gastrointestinal bleeding, previous intracranial haemorrhage, a haemorrhagic diathesis or a coagulation disorder. Avoiding co-administration of other NSAIDs, anti-coagulants or corticosteroids also reduces the risk of adverse effects [41].

UGS are common side effects associated with aspirin, with one survey reporting 15.4 % ($n = 152/986$) of long-term low-dose aspirin users experiencing UGS [42]. A history of gastro-oesophageal reflux disease or dyspepsia prior to starting aspirin has been shown to be strongly predictive of UGS on aspirin (OR 17.6, CI 11.52–26.88) [42]. *Helicobacter pylori* infection has been proposed to be a marker of increased risk of developing dyspepsia and a bleeding gastrointestinal ulcer with aspirin [7•]; however, most data supporting this association relates to non-aspirin NSAID use; and therefore, further data is needed to confirm a relationship with aspirin [43]. The HEAT trial (ISRCTN10134725), examining *H. pylori* eradication to prevent ulcer-related bleeding and dyspepsia in aspirin users, is ongoing.

The most common cause for concern in relation to aspirin use is the risk of bleeding. Data from six cardiovascular primary prevention RCTs ($n = 95{,}000$) estimated that aspirin increased the risk of serious bleeding (excluding intracranial haemorrhage) by 0.04 % per year (from 6.6 events per year in 10,000 individuals to 10.2 events [6]). Age has been shown to be a key predictor of bleeding risk with a recent systematic review estimating that the risk of major bleeding increases between three- and fourfold between the ages of 50–54 and 70–74 years [7•]. Intracranial haemorrhage is even rarer with aspirin estimated to increase the risk by less than 0.01 % per year (from 2.7 events in 10,000 individuals treated for a year in the control groups to 3.5 events in the aspirin groups, HR 1.39, CI 1.08–1.78) in the aforementioned analysis of six cardiovascular RCTs of aspirin. This study also revealed that mean blood pressure is associated with an increased risk of intracranial haemorrhage (rate ratio 2.18, CI 1.65–2.87) [6].

Certain genetic polymorphisms have been proposed as potential biomarkers for NSAID-induced gastrointestinal ulceration and bleeding. A study in a Japanese population ($n = 480$) found that a functional SNP of the COX-1 gene (rs1330344) has been shown to be significantly associated with gastric ulceration (OR 5.80, CI 1.59–21.1) [44]. Additionally, two polymorphisms of CYP2C9 (an enzyme responsible for the metabolism of aspirin) have been found to be significantly associated with bleeding risk in NSAID users [45, 46].

Biomarkers including increasing age, previous dyspepsia and certain SNPs have the potential to identify those at greatest risk of aspirin toxicity. Furthermore, diagnosing and treating conditions like hypertension and *H. pylori* infection could also reduce the change of adverse effects.

Other Benefit-Risk Considerations

Aspirin is an established treatment for the secondary prevention of cardiovascular disease but is not generally recommended for its primary prevention, however a recommendation for its use in both the prevention of cardiovascular disease and cancer is currently under consideration by the United States Preventive Services Task Force [47]. Little is known about the cardiovascular benefits of aspirin in those with cancer as this is an exclusion criterion in most large cardiovascular trials. CRC and cardiovascular disease have a number of common risk factors, including obesity, high cholesterol and diabetes, and any cardiovascular benefits might add to the rationale for aspirin use in the context of CRC prevention or treatment. Data from cardiovascular trials has additionally suggested that some individuals are resistant to the biological effects of aspirin [48] which also has the potential to limit anti-cancer activity. The existence of aspirin resistance is challenged by the finding that serum thromboxane B2 (a serum marker of platelet activation) is suppressed by aspirin in 99 % of healthy subjects [49]. Other explanations to account for the phenomena include variability in some functional assays of platelet function or undetected poor adherence to aspirin [50]. Fast recovery of platelet function in some individuals might have previously been categorised as resistance, and this might be addressed with twice daily doses [51]. Both the cardiovascular effects of aspirin and the possibility of aspirin resistance could alter the overall risk-benefit profile, and thus require further investigation in existing datasets and ongoing trials.

Conclusions

The current data on the benefits and risks of prophylactic aspirin in the general population has recently been reviewed by Cuzick et al. who concluded that aspirin use for greater than 5 years (75–325 mg/day), starting between the ages of 55 and 65, has a favourable benefit-harm profile [52••]. Phase III trials investigating the role of aspirin for cancer prevention in the general population are likely to be challenging due to the length of follow-up and number of participants required. However, in higher risk groups, trials are more feasible. The CaPP3 trial (Cancer Prevention Project 3), examining different doses of aspirin for the prevention of Lynch syndrome cancer (ISRCTN16261285), and the seAFOod (Systematic Evaluation of Aspirin and Fish Oil Bowel Polyp Prevention Trial), examining the effect of aspirin and fish oil in patients at high risk of colorectal adenomas (ISRCTN05926847), are ongoing and the results are awaited.

The role of aspirin in the treatment of CRC is also being evaluated in a number of recruiting phase III trials. Add-Aspirin (ISRCTN74358648) is a double-blind placebo-controlled trial for patients with colorectal, breast, gastro-oesophageal and prostate cancer, investigating the effects of aspirin in the adjuvant setting. Participants are randomised to aspirin 300 mg, aspirin 100 mg or placebo for at least 5 years, and it is separately powered for each tumour type to assess the effects of tumour-specific outcomes. Other ongoing trials in this setting include ASCOLT (Aspirin for Dukes C and High Risk Dukes B CRCs, NCT00565708) and ASPIRIN (A Trial of Aspirin on Recurrence and Survival in Elderly Colon Cancer Patients, NCT02301286). There are also three upcoming trials investigating the effects of aspirin in molecularly stratified groups. In the adjuvant setting, ALASCCA is a randomised, double-blind, placebo-controlled phase III trial of aspirin in colorectal patients with mutations in the PI3K signalling pathway (PIK3CA, PIK3R1 or PTEN mutations), and SAKK 41/13 (Swiss Group for Clinical Cancer Research), a double-blind, placebo-controlled randomised trial of adjuvant aspirin treatment in PIK3CA-mutated colon cancer patients (NCT02467582). In the advanced setting, FOCUS4-B (ISRCTN90061546) plans to investigate the role of aspirin in individuals with PIK3CA mutant advanced CRC.

Prior to being considered as an intervention in randomised trials, aspirin has not followed the modern target-driven drug development pathway taken by other anti-cancer agents. Consequently, biomarker discovery and validation is at an early stage. Ideally, predictive biomarkers are co-developed with a drug and validated in phase I and II trials prior to defining the population for phase III trials [53]. However, potential biomarkers for aspirin have mostly emerged from observational studies where there is a risk of confounding and, therefore, it is highly important that they are investigated further in ongoing trials. Research into the mechanisms underlying the anti-cancer effects of aspirin may also reveal new biomarkers. The likelihood of a single biomarker that can identify individuals that will or will not benefit from aspirin is low, and thus, it will be important to investigate patterns of multiple biomarkers to select individuals who will gain from aspirin therapy.

Aspirin is a low-cost, generic agent, available in both resource-poor and resource-rich countries. If it can be shown to be effective, there is the potential for a major impact on the global burden of CRC. Identifying those who are most likely to benefit will be essential to maximising this potential.

Compliance with Ethical Standards

Conflict of Interest Ruth E. Langley has received financial support through grants from Cancer Research UK and the National Institute for Health Research (NIHR) Health Technology Assessment (HTA) as Chief Investigator of the Add-Aspirin Trial, has received compensation from Bayer and Aspirin Foundation for service on scientific advisory boards, and has received a supply of aspirin and placebo from Bayer Pharmaceuticals AG for the Add-Aspirin Trial.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- • Of importance
- •• Of major importance

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review

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Metformin as an adjuvant treatment for cancer: a systematic review and meta-analysis

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Background: Metformin use has been associated with a reduced risk of developing cancer and an improvement in overall cancer survival rates in meta-analyses, but, to date, evidence to support the use of metformin as an adjuvant therapy in individual cancer types has not been presented.

Patients and methods: We systematically searched research databases, conference abstracts and trial registries for any studies reporting cancer outcomes for individual tumour types in metformin users compared with non-users, and extracted data on patients with early-stage cancer. Studies were assessed for design and quality, and a meta-analysis was conducted to quantify the adjuvant effect of metformin on recurrence-free survival (RFS), overall survival (OS) and cancer-specific survival (CSS), to inform future trial design.

Results: Of 7670 articles screened, 27 eligible studies were identified comprising 24 178 participants, all enrolled in observational studies. In those with early-stage colorectal cancer, metformin use was associated with a significant benefit in all outcomes [RFS hazard ratio (HR) 0.63, 95% confidence interval (CI) 0.47–0.85; OS HR 0.69, CI 0.58–0.83; CSS HR 0.58, CI 0.39–0.86]. For men with early-stage prostate cancer, metformin was associated with significant, or borderline significant, benefits in all outcomes (RFS HR 0.83, CI 0.69–1.00; OS HR 0.82, CI 0.73–0.93; CSS HR 0.58, CI 0.37–0.93); however, there was significant heterogeneity between studies. The data suggest that prostate cancer patients treated with radical radiotherapy may benefit more from metformin (RFS HR 0.45, CI 0.29–0.70). In breast and urothelial cancer, no significant benefits were identified. Sufficient data were not available to conduct analyses on the impact of metformin dose and duration.

Conclusions: Our findings suggest that metformin could be a useful adjuvant agent, with the greatest benefits seen in colorectal and prostate cancer, particularly in those receiving radical radiotherapy, and randomised, controlled trials which investigate dose and duration, alongside efficacy, are advocated.

Key words: metformin, repurposing, adjuvant, prostate cancer, colorectal cancer, breast cancer

introduction

Although cancer survival rates in the UK have doubled in the last 40 years, half of those diagnosed with cancer still die from their disease within 10 years [1, 2]. Adjuvant treatment after potentially curative cancer therapy improves survival rates, but relapse rates remain high in some tumour types, and for others, there are no proven adjuvant treatments. In the quest to improve cancer outcomes, a number of established medications with known anti-cancer properties have been considered as adjuvant anti-cancer therapies. Examples include aspirin [3], vitamin D [4], bisphosphonates [5], statins [6] and metformin.

Metformin exhibits a number of attributes that make it appealing for repurposing as an anti-cancer therapy. It has been in use for over half a century and is the most widely prescribed anti-diabetic medication in the world [7]. Consequently, it has been administered alongside most cancer treatments without the emergence of any important interactions. Additionally, data on the toxicity profile of metformin in those without type II diabetes mellitus (DM) are already available from clinical trials investigating its role as a treatment for polycystic ovarian syndrome [8]. Metformin is also generically available worldwide at low cost.

Metformin has been shown to have anti-cancer activity both *in vivo* and *in vitro* [9], with the underlying mechanism subject to ongoing investigation. It has been proposed that the anti-cancer properties of metformin result from both direct effects on cancer cells, particularly through inhibition of the AMPK/mTOR pathway [10], and indirect effects on the host, by virtue of its blood glucose-lowering properties and anti-inflammatory effects [11, 12]. Both mechanisms are anticipated to be important, although their relative contribution may differ according to

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cancer stage. *In vivo* evidence has emerged from window studies showing an anti-proliferative effect in breast cancer [13, 14] and a reduction in precancerous changes in the colorectum [15]. Meta-analyses have examined the role of metformin in the primary prevention of cancer, where it was found to significantly reduce overall cancer incidence; however, findings were inconsistent when individual tumour types were considered [16–20]. Meta-analyses have also investigated the effect of metformin use across all stages of disease and have found that it reduces overall cancer mortality rates, but, again findings are conflicting for individual tumour types [21–28], suggesting analyses are best conduced for individual tumour types separately. Most recently, a randomised phase III trial of non-DM patients showed that low-dose metformin was effective in the chemoprevention of metachronous colorectal adenomas or polyps when compared with placebo [29].

Benefits in the primary prevention, or advanced setting, do not necessarily translate to utility in the adjuvant setting as the mechanism of action may be different. Our objective was to conduct a systematic review and meta-analysis of randomised and non-randomised studies to investigate the effect of metformin use compared with non-use on recurrence-free survival (RFS), overall survival (OS) and cancer-specific survival (CSS) in adults who have potentially curable solid tumours. There have been a number of calls for systematic reviews and meta-analyses to be conducted as part of the scientific justification, and to inform the design, of new clinical trials [30, 31]. This is particularly relevant in the field of drug repurposing. The aim of this analysis was to advise further clinical investigation of metformin in the adjuvant setting.

methods

All methods for this systematic review and meta-analysis are outlined in a prospectively registered protocol available online [32] (PROSPERO identifier CRD42015020519), and reporting follows PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines.

eligibility criteria

Eligible studies include randomised, controlled trials and non-randomised studies (observational, cohort and case–control) that have investigated the use of metformin, with a comparator of no metformin, in participants over 16 years old with potentially curable solid tumours (defined as those either undergoing radical therapy with curative intent or those with an early-stage cancer where cure is normally the objective of standard treatment). Studies must have reported data on at least one of RFS, CSS or OS for individual tumour types.

search strategy

Electronic searches of databases (Medline, EMBASE, Cochrane Central Register of Controlled Trials), clinical trial registries (clinicaltrials.gov, ISRCTN and EU Clinical Trials Register) and conference proceedings (American Society of Clinical Oncology, and European Society of Medical Oncology) were conducted. All sources were searched from inception until 31 May 2015 (conference abstracts 2005–2015). Bibliographies of the reports of all identified studies and review articles were hand-searched for further potentially eligible studies. Further details of the search strategy are available in supplementary data S1, available at *Annals of Oncology* online.

study selection

All retrieved studies were assessed for eligibility and, when sufficient information was not available from the title and/or abstract, the full-text publication or (for conference abstracts) the associated poster or presentation was acquired and where this was not available, we contacted the study author. For studies with multiple publications, or where there was overlap in the patients studied, the most recent publication was chosen. Any queries were checked by a second reviewer and resolved by consensus. No study was excluded for weakness of study design or quality. For the purpose of this analysis, studies presenting data separately by tumour type were treated as separate studies. Articles were grouped by cancer type according to the site of origin and histology.

data items and collection

Data on patient characteristics, interventions and outcomes were extracted for all studies into a predesigned table. These were cross-checked by a second independent reviewer and any disagreements were resolved by consensus. A list of data extracted is available in supplementary data S2, available at *Annals of Oncology* online. Studies were evaluated to determine whether they accounted for potential confounding factors [body mass index (BMI), age, gender, cancer-specific prognostic factors and the use of other anti-DM medications], either by demonstrating that there was no significant difference in their distribution between treatment groups or by inclusion in multivariable analyses. In order to minimise the potential for confounding by DM status, where the comparator included both non-DM patients and DM non-metformin users, we extracted data based on a DM non-metformin comparator in preference. Where a time-varying covariate was used to model treatment effect, the most conservative HR was selected. Where reported, the HR after adjustment for potential confounding factors was extracted in preference to an unadjusted value. Since all eligible studies were of cohort design, the Newcastle–Ottawa quality assessment scale for cohort studies (NOS) [33] was used to evaluate methodological quality.

statistical analysis

HRs and associated statistics were either extracted directly from the study reports or estimated from the Kaplan–Meier curves or summary statistics using published methods [34–36]. Where sufficient data were available on outcomes for individual cancer types, a meta-analysis was conducted with a primary outcome of RFS and secondary outcomes of OS and CSS. HRs were combined across trials using a fixed-effect model. Heterogeneity was assessed using the χ^2 test and the I^2 statistic. A random-effects model (DerSimonian and Laird) [37] was used to assess whether the results were robust to the choice of model. Probability values were two-sided, with $P < 0.05$ considered of statistical significance.

We also preplanned analyses to explore whether the size or the direction of the effect of metformin therapy varied according to specific study or patient characteristics, including: DM status of the comparator group (with and without non-DM patients in the comparator group), prostate cancer primary treatment type (prostatectomy or radical radiotherapy) and study design. The resulting HR estimates from study group analyses were compared using the χ^2 test for interaction. We also planned to explore the impact of metformin dose/exposure on the outcomes described above where available. We also conducted unplanned sensitivity analyses for the primary outcome of RFS where at least two studies were available after restrictions. This was carried out according to study quality (restricted to studies with an NOS score ≥ the median); publication type (restricted to studies where a full publication was available); setting (restricted to hospital-based studies); follow-up (restriction of follow-up <3 years); and by the potential confounding factors accounted for (restricted to studies that adjusted for BMI, age, gender, cancer-specific prognostic factors and other DM medications). An additional unplanned exploratory analysis was also conducted according to whether the study was from a Western (North America or Europe) or non-Western population after a wide geographical distribution of studies was noted. Study group and sensitivity analyses were only conducted where study numbers were sufficient to be meaningful. Statistical analyses were carried out using STATA version 14.

results

After screening 7670 reports and conference abstracts, we identified 23 full publications and 4 conference abstracts that met our eligibility criteria, comprising 24 178 participants [38–64]. All were retrospective cohort studies except for one prospective cohort study embedded in a clinical trial [41]. The PRISMA study selection diagram is shown in Figure 1. The majority of identified studies examined the effect of metformin in one of four tumour types: prostate, colorectal, breast and urothelial cancer, which, therefore, represent the main focus of this analysis. A summary of the main characteristics for studies of breast, colorectal and prostate cancer is presented in Table 1, and a table of study characteristics for other cancer types is presented in Table 2.

colorectal cancer

RFS was assessed in two studies (623 patients), OS in five studies (1936 patients) and CSS in two studies (535 patients). Overall, metformin use appeared to demonstrate significant improvements in RFS (HR 0.63, CI 0.47–0.85), OS (HR 0.69, CI 0.58–0.83) and CSS (HR 0.58, CI 0.39–0.86) (Figure 2), although there was variation between the results of the individual studies for RFS ($I^2 = 83.1\%$, $P = 0.015$) and OS ($I^2 = 82.3$ $P < 0.001$). When the random-effects model was applied, the benefits seen for both OS (HR 0.62, CI 0.40–0.97) and CSS (HR 0.58, CI 0.39–0.86) remained, but there was no longer a significant

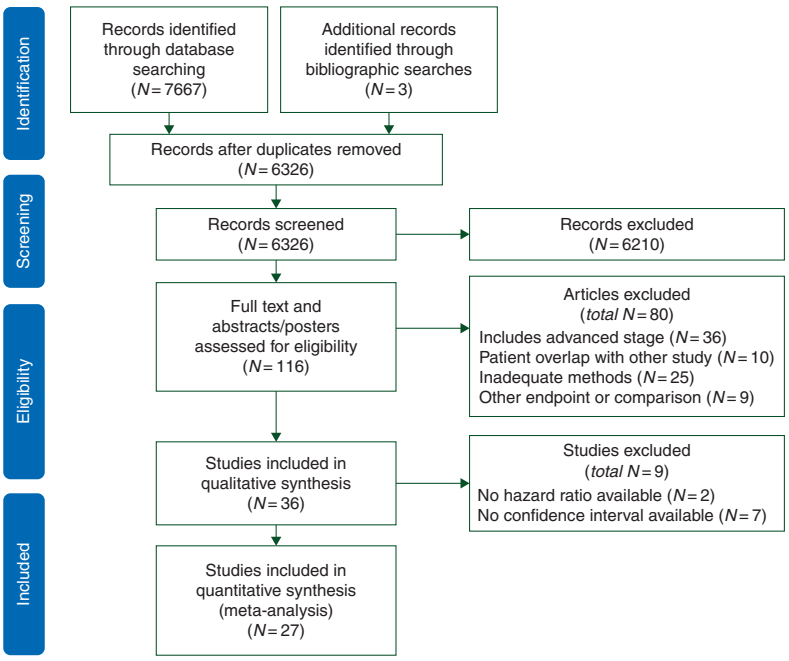

Figure 1. PRISMA study selection diagram.

Table 1. Main study characteristics: colorectal, prostate and breast cancer

Tumour group	Study author	Patient characteristics		Study characteristics				Comparator DM status		Outcomes			Definition of metformin exposure	Median follow-up (months)	Potential confounders (R = reported and not significant, M = included in multivariate model, x = not assessed, or significant but not adjusted for)					NOS score
		Treatment	Stage/other restrictions	Sample size (*met/total*)	Article type	Study location	Setting (H = Hospital, P = Population)	DM	Non-DM	RFS	OS	CSS			BMI	Age	Sex	Cancer-specific variables	Other DM meds	
Colorectal adenocarcinoma	Spillane [38]	Not specified	I–III	*207/315*	Full	Ireland	P	✓	X	X	✓	✓	In year before diagnosis	46	X	M	M	M	M	7
	Lee, GE [39]	Not specified	II–III	*223/356*	Abstract	Singapore	H	✓	X	✓	✓	X	At diagnosis	78	X	M	X	M	X	5
	Lee, JH [40]	Not specified	III[a]	*96/220*	Full	Korea	H	✓	X	X	✓	✓	>6 m exposure	41	M[b]	M[b]	M[b]	M[b]	M[b]	8
	Singh [41]	Not specified	III /colon only	*115/267*	Abstract	USA and Canada	H	✓	X	✓	✓	X	Before randomisation	Not given	X	M	M	M	X	5
	Zanders [42]	Not specified	I–III	*512/778*	Full	The Netherlands	P	✓	X	X	✓	X	Cumulative exposure	41	X	M	M	M	M	7
Prostate adenocarcinoma	Allott [43]	Prostatectomy	Localised	*155/369*	Full	USA	H	✓	X	✓	X	✓	At surgery	59/73[c]	M	M	n/a	M	X	8
	Kaushik [44]	Prostatectomy	Localised	*323/885*	Full	USA	H	✓	X	✓	✓	X	In 3 months before surgery	61	M	M	n/a	M	R	7
	Rieken WJU [45]	Prostatectomy	Localised	*287/6486*	Full	USA and Europe	H	X	✓	✓	X	X	At surgery	25	X	M	n/a	M	n/a	6
	Spratt [46]	Radical radiotherapy	Localised	*157/319*	Full	USA	H	✓	X	✓	✓	✓	At diagnosis or after radiotherapy	104	R	M	n/a	M	R	8
	Margel [47]	Prostatectomy or radical radiotherapy	Localised[a]/ ≥66 years old	*Total 955*	Full	Canada	P	✓	X	X	✓	✓	Cumulative exposure	56	X	M	n/a	M	M	8
	Zannella [48]	Radical radiotherapy	Localised	*114/504*	Full	Canada	H	✓	✓	✓	X	X	At the time of radiotherapy	82	X	R	n/a	M	X	5
	Danzig [49]	Prostatectomy	Localised	*98/767*	Full	USA	H	✓	X	✓	X	X	At surgery	27	X	M	n/a	M	X	6
	Taira [50]	Brachytherapy	Localised	*126/2298*	Full	USA	H	✓	✓	X	✓	X	Diagnosis to 3 months after brachytherapy	100	M	M	n/a	M	X	7
Breast adenocarcinoma	Oppong [51]	Adjuvant chemo	I–III	*76/141*	Full	USA	H	✓	X	✓	✓	X	Diagnosis to 6 months after	87	R	M	n/a	M	M	8
	Bayraktar [52]	Adjuvant chemo	I–III/triple negative	*63/130*	Full	USA	H	✓	X	✓	✓	X	During adjuvant chemo	62	M[d]	M	n/a	M	R	8
	Lega [53]	Breast cancer surgery	Infer I–III/ ≥66 years	*868/1774*	Full	Canada	P	✓	X	X	✓	✓	Cumulative exposure	54	X	M	n/a	M	M	6

NOS, Newcastle–Ottawa Quality Assessment Scale for Cohort Studies; BMI, body mass index; met, metformin; N/A, not applicable; RFS, recurrence-free survival; OS, overall survival; CSS, cancer-specific survival.

[a]Data from subanalysis.

[b]Main analysis only.

[c]Metformin/non-metformin.

[d]Adjustment for body weight.

Table 2. Main study characteristics: other cancer types

Tumour group	Study author	Patient characteristics		Study characteristics				Comparator DM status		Outcomes			Definition of metformin exposure	Median follow-up (months)	Potential confounders (R = reported & not significant, M = included in multivariate model X = not assessed, or significant but not adjusted for)					NOS Score
		Treatment	Stage/other restriction	Sample size (*met/total*)	Article type	Study location	Setting (H = hospital, P = population)	DM	Non-DM	RFS	OS	CSS			BMI	Age	Sex	Cancer specific	Other DM meds	
Urothelial carcinoma	Rieken BJU [54]	TURBT	pTa–pT1 N0 M0 /urothelial carcinoma of bladder (NMI)	*43/1035*	Full	USA and Europe	H	X	✓	✓	✓	X	At surgery	64	X	M	R	M	n/a	8
	Rieken UO [55]	Radical surgery	M0 /invasive urothelial carcinoma of bladder	*80/1382*	Full	USA and Europe	H	X	✓	✓	✓	✓	At diagnosis	34	M	M	M	M	n/a	8
	Rieken EJS [56]	Radical surgery	M0/upper tract urothelial carcinoma	*194/2330*	Full	USA, Europe and Japan	H	X	✓	✓	✓	✓	At surgery	36	X	M	M	M	n/a	6
Head and neck (squamous cell carcinoma)	Kwon [57]	Curative surgery or radiotherapy	No distant metastases	*99/1072*	Full	Korea	H	X	✓	✓	✓	✓	Ever exposure	65	M	M	R	M	n/a	8
	Thompson [58]	Not specified	Disease-free at 3 months/oral-oropharynx	*33/78*	Full	USA	H	✓	X	✓	X	X	Diagnosis to relapse	44	X	R	R	R	X	5
Renal cell carcinoma	Hakimi [59]	Partial/radical nephrectomy	T2–T3 N0 M0	*55/784*	Full	USA	H	✓	✓	✓	X	✓	At surgery	41	M	M	R	M	X	6
	Psutka [60]	Partial/radical nephrectomy	Localised	*83/200*	Full	USA	H	✓	X	✓	✓	✓	In 90 days before surgery	97	R	M	R	M	X	8
Pancreatic adenocarcinoma	Ambe [61]	Radical surgery	Resectable	*19/44*	Abstract	USA	H	✓	X	X	✓	X	At surgery	Not given	R	R	R	R	X	7
Non-small-cell lung carcinoma	Fortune-Greeley [62]	Not specified	data on stage I–II	*Not given*	Abstract	USA	H	✓	X	X	✓	X	Not given	Not given	M	M	X	M	X	6
Endometrial cancer	Ko [63]	Not specified	I–IV (RFS data extracted)	*200/363*	Full	USA	H	✓	X	✓	X	X	At diagnosis	33	R	M	n/a	M	R	8
Gastric cancer	Lee, CK [64]	Gastrectomy	I–III	*132/326*	Full	Korea	H	✓	X	✓	✓	✓	Cumulative exposure	74	M	M	M	M	M	9

NOS, Newcastle–Ottawa Quality Assessment Scale for Cohort Studies; BMI, body mass index; met, metformin; N/A, not applicable; NMI, non-muscle invasive; TURBT, transurethral resection of bladder tumour; RFS, recurrence-free survival; OS, overall survival; CSS, cancer-specific survival.

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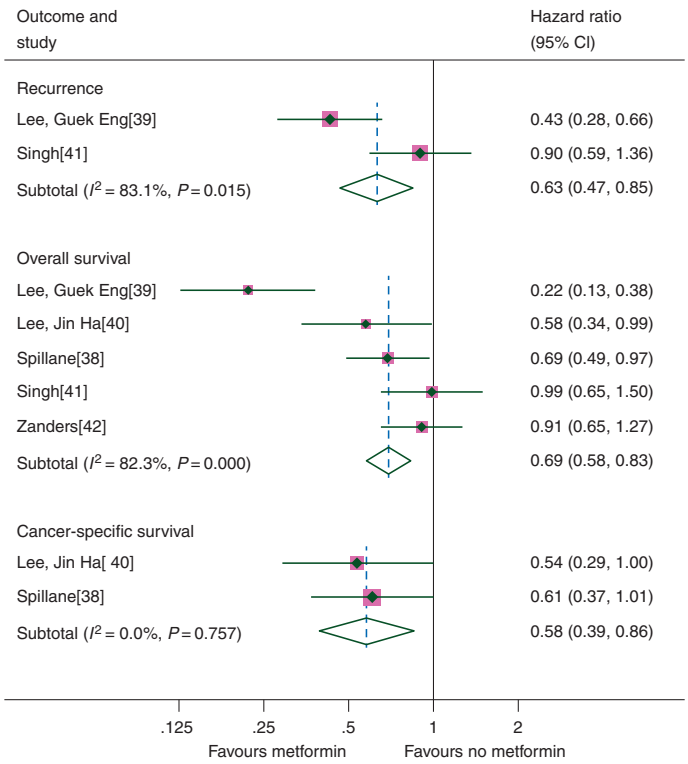


Figure 2. Colorectal cancer outcomes according to metformin use.

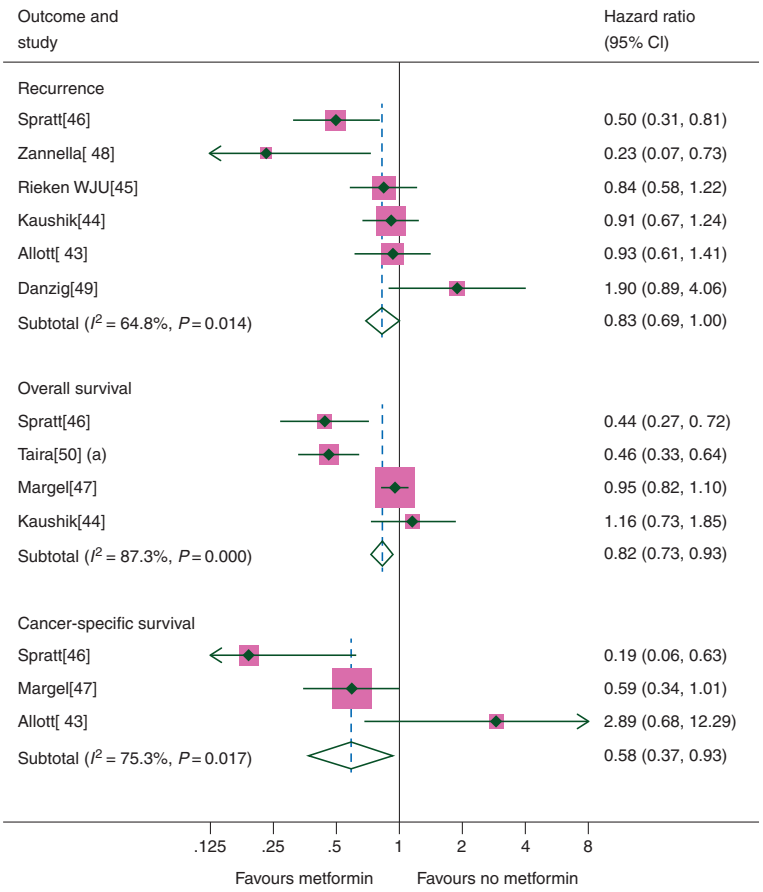
benefit of metformin on RFS (HR 0.62, CI 0.30–1.29). In an unplanned exploratory analysis that grouped studies with Western and non-Western populations separately, we found there was a significant interaction between the effect of metformin on OS and the population studied ($\chi^2 = 14.31$, $P < 0.001$). In studies in non-Western populations, there was a highly significant benefit of metformin on OS (HR 0.36, CI 0.25–0.53); however, there was evidence of heterogeneity ($I^2 = 85.8\%$, $P = 0.013$). In studies with Western populations, only a trend towards a significant effect was identified (OS HR 0.84, CI 0.68–1.03) with no clear evidence of heterogeneity ($I^2 = 4.6\%$, $P = 0.350$). In unplanned sensitivity analyses, there appeared to be a larger relative benefit of metformin on OS when analyses were restricted to studies that had follow-up of >3 years (HR 0.64, CI 0.52–0.78). Further details of study group and sensitivity analyses for all tumour types are available in supplementary Table S1, available at *Annals of Oncology* online.

prostate cancer

RFS was assessed in six studies (9330 patients), OS in four studies (4457 patients) and CSS in three studies (1643 patients). Metformin use demonstrated a borderline significant improvement in RFS (HR 0.83, CI 0.69–1.00), and significant improvements in OS (HR 0.82, CI 0.73–0.93) and CSS (HR 0.58, CI 0.37–0.93) (Figure 3); however, the relationship was inconsistent across studies (RFS $I^2 = 64.8\%$, $P = 0.014$; OS $I^2 = 87.3\%$, $P < 0.001$; CSS $I^2 = 75.3\%$, $P = 0.017$), which was reflected when the random-effects model was applied (RFS HR 0.80, CI 0.57–1.13; OS 0.69, CI 0.44–1.10; CSS 0.64, CI 0.19–2.12).

In a pre-specified analysis, there was significant interaction between the effect of metformin and the primary treatment type on RFS (χ^2 test for interaction 9.03, $P = 0.003$). For patients receiving radical radiotherapy [46, 48], there was a significant benefit from metformin (HR 0.45, CI 0.29–0.70), whereas no significant benefit was seen for patients who underwent radical prostatectomy (HR 0.94, CI 0.77–1.15) (Figure 4). Only a single study was able to provide data on OS and CSS in those having radical radiotherapy; however, significant improvements were seen in both (OS 0.44, CI 0.27–0.72; CSS 0.19, CI 0.06–0.63) [46]. We found no evidence of an interaction between the effect of metformin on RFS and the presence or absence of non-DM patients in the comparator group (χ^2 0.49, $P = 0.48$).

In unplanned sensitivity analyses, there appeared to be a larger relative benefit of metformin on RFS when analyses were restricted to studies that had a follow-up of >3 years (HR 0.77, CI 0.62–0.96) or considered other DM medications in their analysis (HR 0.79, CI 0.64–0.98).



(a) Hazard rations for Taira et al.[50] were estimated from Kaplan Meier curves and summary statistics using published methods. [29-31]

Figure 3. Prostate cancer outcomes according to metformin use.

breast cancer

RFS was assessed in 2 studies containing 271 patients and OS in 3 studies including 2045 patients. Metformin demonstrated a trend towards improvement in RFS (HR 0.77, CI 0.49–1.22) (Figure 5); however, no effect was seen in OS (HR 0.99, CI 0.92–1.05). There was no evidence of variation between the results of the studies either for RFS (I^2 = 0.0%, P = 0.74) or OS (I^2 = 0.0%, P = *0.75*). As CSS was only available for one study containing 1774 patients, no meta-analysis was possible for this outcome; however in this study, metformin did not appear to have an impact on CSS (HR 1.01, CI 0.86–1.19). There were insufficient study numbers for any meaningful study group or sensitivity analyses.

urothelial cancer

Studies included patients with upper tract urothelial carcinoma and urothelial carcinoma of the bladder. RFS was assessed in 3 studies including 4747 patients, and OS in 3 studies including 4747 patients, of which 2 also assessed CSS including 3712 patients. There was no clear evidence that metformin improved either RFS (HR 0.91, CI 0.73–1.14), OS (HR 0.94, CI 0.76–1.16) or CSS (HR 0.88, CI 0.66–1.17) (Figure 6). Although there was some evidence of inconsistency between the results of studies for both RFS (I^2 = 59.0%, P = 0.087) and OS (I^2–51.5%, P = 0.127), the results did not change significantly when the random-effects model was applied (RFS HR 0.84, CI 0.57–1.24; OS HR 1.00, CI 0.72–1.39; CSS HR 0.88, CI 0.66–1.17). There were insufficient study numbers for any meaningful study group or sensitivity analyses.

other cancer types

There were insufficient studies identified to warrant meta-analyses for other cancer types, the findings of which are presented in Table 3. In head and neck cancer, a positive trend towards improved RFS and CSS was seen in one study [57], but there

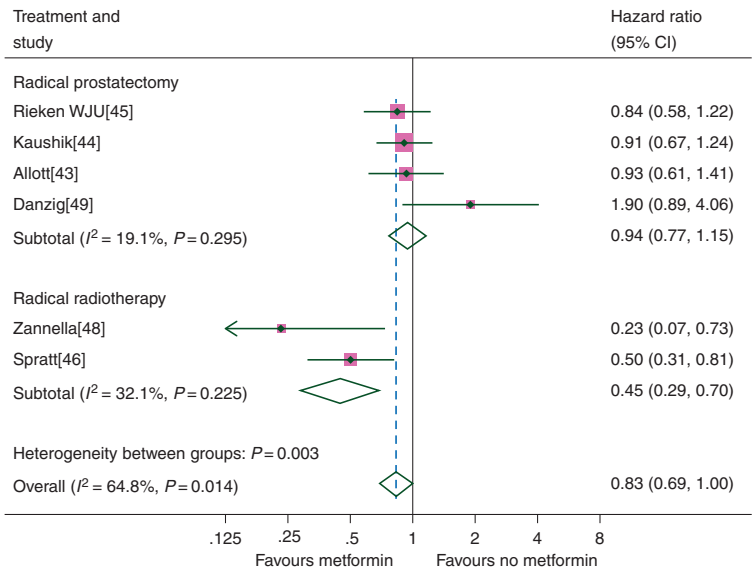


Figure 4. Prostate cancer recurrence-free survival according to metformin use for different treatment groups.

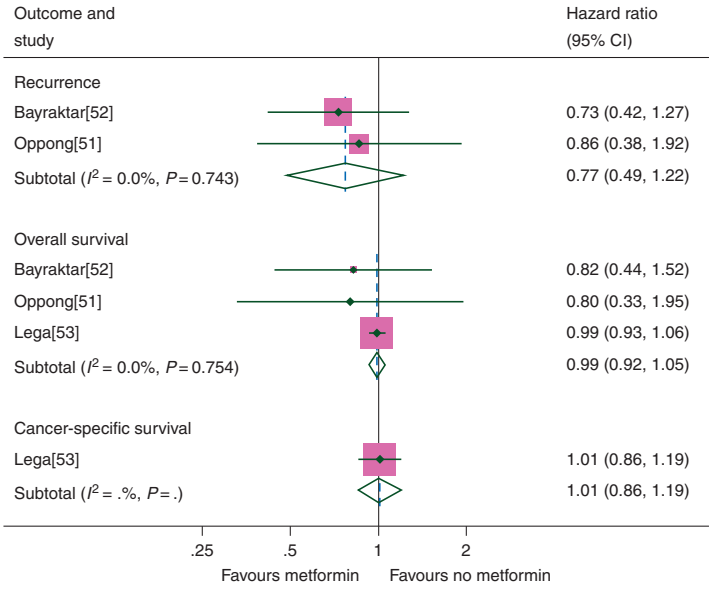


Figure 5. Breast cancer outcomes according to metformin use.

was no effect on OS. However, the second study identified showed a potential detriment of metformin use on RFS [58]. In renal cell carcinoma, two studies were identified, both showing a non-significant inverse relationship with metformin use and RFS, and no significant benefit in OS or CSS. Single studies were identified showing a significant improvement in OS in lung cancer, RFS and OS in endometrial cancer and RFS, OS and CSS in gastric cancer. A small single study in pancreatic cancer did not suggest any effect of metformin; however, this study had a very small sample size.

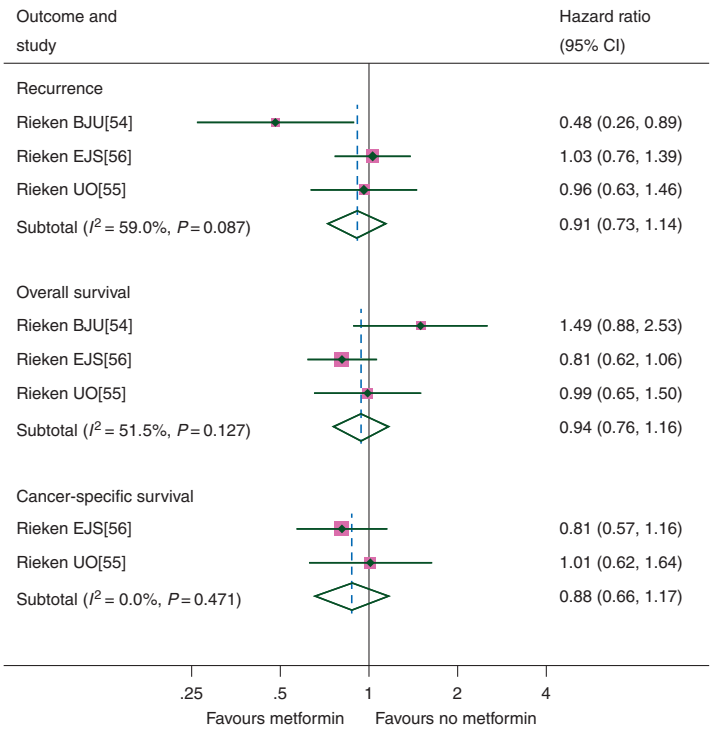


Figure 6. Urothelial cancer outcomes according to metformin use.

Table 3. Cancer outcomes by metformin use for tumour types with limited numbers of studies

Tumour group	Study author	Sample size	Recurrence-free survival HR (95% CI)	Overall survival HR (95% CI)	Cancer-specific survival HR (95% CI)
Head and neck	Kwon [57]	1072	0.76 (0.49–1.21)	0.95 (0.59–1.50)	0.79 (0.42–1.50)
	Thompson [58]	78	1.26 (0.62–2.56)	—	—
Renal cell carcinoma	Hakimi [59]	784	1.22 (0.66–2.27)	—	0.76 (0.21–2.70)
	Psutka [60]	200	1.07 (0.61–1.88)	0.74 (0.48–1.15)	0.83 (0.41–1.67)
Pancreas	Ambe [61]	44	—	0.54 (0.16–1.68)	—
Lung	Fortune [62]	Not given by stage	—	0.85 (0.77–0.93)	—
Endometrial	Ko [63]	363	0.56 (0.34–0.91)	0.43 (0.24–0..77)	—
Gastric	Lee, CK [64][a]	326	0.86 (0.80–0.94)	0.87 (0.80–0.95)	0.87 (0.78–0.96)

[a]HR for each 6 months of metformin use.

duration and dose

The impact of different exposures to metformin on early-stage cancer outcomes is examined in some of the identified studies; however, limited data and differences in the methods used to investigate exposure preclude any study-group analyses. In colorectal cancer, Spillane et al. [38] conducted additional analyses on dose intensity and found survival benefits for high-intensity metformin users not using other diabetic therapies (CSS HR 0.44, CI 0.20–0.95; OS HR 0.41, CI 0.24–0.70), but no significant benefits were identified in other subgroups. In gastric cancer, Lee et al. [64] found that increased cumulative duration of metformin use improved cancer-specific and all-cause mortality. Single studies in colorectal [42] and prostate cancer [43] also investigated the impact of different exposures to metformin but found no significant associations.

discussion

Our analysis suggests that metformin could be a useful adjuvant agent, particularly in colorectal and prostate cancer. The number of studies identified for each tumour type is likely to reflect the incidence and demographics of the disease, particularly the likelihood of presentation with early-stage disease and a diagnosis of DM.

The variation in the adjuvant effects of metformin according to tumour type could be explained by differences in both patient characteristics and tumour biology. The effect of metformin on AMPK signalling has been hypothesised to be a major pathway through which metformin exerts its anti-cancer effects [10]. AMPK signalling dysregulation is also associated with metabolic syndrome [65], a cluster of conditions which include raised fasting glucose, dyslipidaemia, high blood pressure and central obesity [66]. Metabolic syndrome is also known to increase the risk of developing some cancers, particularly colorectal cancer [67], where it is also associated with poorer recurrence and survival outcomes [68]. In addition, it is known to develop as a consequence of androgen deprivation therapy in men with prostate cancer [69]. Metformin may improve OS by reducing the number of cardiovascular deaths associated with metabolic syndrome; however, the improvements in RFS and CSS identified suggest a direct anti-cancer effect. In prostate cancer, our study group analysis suggests that the beneficial effects of metformin use could be limited to those undergoing radical radiotherapy. The AMPK pathway is known to play a role in regulating cellular responses to radiotherapy, [70] and studies in xenograft mice models suggest that metformin can improve tumour oxygenation and therefore radiation response [71].

The limitations of our meta-analysis include the inherent weaknesses of observational data, particularly potential measurement errors in the exposure to metformin, and variation in the definition of metformin use, and the risk of time-related biases [72]. A high degree of variation between the results of studies was observed for a number of the outcomes investigated in most of the cancer types. Our sensitivity analyses were designed to explore possible reasons for this to inform future observational and clinical trial design; however, only a small number of analyses were possible due to insufficient study numbers. For both prostate and colorectal cancer, the relative effect size appeared to increase for studies with follow-up of 3 years or greater, highlighting the importance of ensuring adequate duration of follow-up in future studies. Similarities have been seen in studies of aspirin, where greater benefits have been seen with longer follow-up [73–75]. A limited number of studies investigated the relation with frequency, dose and duration of metformin in early-stage cancer; however, findings are inconsistent and further research is required to better understand this relationship.

Previous studies have suggested that a diagnosis of DM has a negative impact on cancer outcomes [76, 77]; therefore, inclusion of non-DM patients in comparator groups could underestimate the beneficial effect of metformin. Owing to insufficient study numbers, it was only possible to analyse the effect of the presence or absence of non-DM patients in the comparator group for RFS in prostate cancer, where no evidence for an effect was found.

Other meta-analyses have investigated the effect of metformin on survival outcomes, across all stages of cancer, in individual tumour types, the findings of which are presented in supplementary Table S2, available at *Annals of Oncology* online. In colorectal cancer, four meta-analyses have examined the effect on OS [21–24], two of which also investigated colorectal CSS [23, 24]. All meta-analyses identified significant improvements in these end points, which is consistent with the findings of this study. For prostate cancer, findings are less consistent. Five meta-analyses have examined the effect of metformin on OS [22, 23, 25–27], two of which also investigated prostate CSS [25, 26]. Only two meta-analyses identified a significant benefit in OS [23, 25], with no benefit identified in prostate CSS. This differs from the findings of this study where significant benefits in OS and prostate CSS were identified, which could suggest that metformin is better suited to the adjuvant setting for prostate cancer. In breast cancer, four meta-analyses examined OS [21–23, 28], two of which investigated breast CSS. Two meta-analyses identified a significant benefit in OS [21, 23, 28], the other approached significance (HR 0.81, CI 0..64–1.04) [22] and the two meta-analyses investigating breast CSS also showed significant improvements [23, 28]. This differs from the findings of this study where no significant benefit in OS and breast CSS was identified. This could suggest that metformin may be effective in those with established breast cancer, which is consistent with the findings of breast cancer window studies where direct anti-tumour effects have been identified [13, 14].

Investigation of metformin in the primary prevention setting presents a number of challenges, where the balance between adverse effects and benefits is likely to be less favourable and difficult to detect in a clinical trial because of the low event rate. While the advanced setting can provide a sufficient event rate, there is evidence to suggest that metformin requires long-term use to exert its anti-cancer effect [78], and therefore, patients with established cancer with more limited prognoses may not be able to receive metformin long enough for a therapeutic benefit to emerge. Therefore, the adjuvant setting could be most suitable for investigating the anti-cancer effects of metformin.

current trial activity

In colorectal cancer, a phase III trial of metformin versus standard care assessing recurrence and survival in stage III disease is now in set-up phase in South Korea (NCT02614339). In prostate cancer, the Metformin Active Surveillance Trial (NCT01864096), an ongoing randomised phase III trial of metformin versus placebo given before primary therapy is assessing time to progression in men with low-risk prostate cancer. The STAMPEDE trial (NCT00268476), a multi-arm multi-stage randomised, controlled trial investigating a number of agents in the treatment of hormone-naïve, high-risk, localised and metastatic prostate cancer, aims to evaluate whether the addition of metformin improves survival in this group. Recruitment to this comparison is due to open in autumn 2016. In breast cancer, our results did not identify any meaningful benefit of metformin use in the adjuvant setting; however, this could be due to the limited number of studies identified. Additional supporting data are available in the primary prevention and treatment setting (across all stages), where meta-analyses have shown a beneficial effect [21, 23, 28, 79]. A randomised phase

III trial of metformin versus placebo assessing recurrence and survival in early-stage breast cancer has recently completed recruitment (MA-32, NCT01101438) and the results are awaited.

conclusions

The findings of this meta-analysis support the concept of randomised clinical trials using metformin in the adjuvant setting, with the strongest supporting evidence in colorectal and prostate cancer, particularly those treated with radical radiotherapy. Such trials could also further our understanding of the relationships between cancer outcomes and the dose and duration of metformin. The authors are not aware of any ongoing adjuvant phase III trials of metformin in prostate cancer, or colorectal cancer in Western populations. In other tumour types, where there is currently less evidence, further observational studies are needed to advise suitability for investigation in any future randomised, controlled trials.

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disclosure

The authors have declared no conflicts of interest.

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Overview

Co-enrolment of Participants into Multiple Cancer Trials: Benefits and Challenges

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Abstract

Opportunities to enter patients into more than one clinical trial are not routinely considered in cancer research and experiences with co-enrolment are rarely reported. Potential benefits of allowing appropriate co-enrolment have been identified in other settings but there is a lack of evidence base or guidance to inform these decisions in oncology. Here, we discuss the benefits and challenges associated with co-enrolment based on experiences in the Add-Aspirin trial – a large, multicentre trial recruiting across a number of tumour types, where opportunities to co-enrol patients have been proactively explored and managed. The potential benefits of co-enrolment include: improving recruitment feasibility; increased opportunities for patients to participate in trials; and collection of robust data on combinations of interventions, which will ensure the ongoing relevance of individual trials and provide more cohesive evidence to guide the management of future patients. There are a number of perceived barriers to co-enrolment in terms of scientific, safety and ethical issues, which warrant consideration on a trial-by-trial basis. In many cases, any potential effect on the results of the trials will be negligible – limited by a number of factors, including the overlap in trial cohorts. Participant representatives stress the importance of autonomy to decide about trial enrolment, providing a compelling argument for offering co-enrolment where there are multiple trials that are relevant to a patient and no concerns regarding safety or the integrity of the trials. A number of measures are proposed for managing and monitoring co-enrolment. Ensuring acceptability to (potential) participants is paramount. Opportunities to enter patients into more than one cancer trial should be considered more routinely. Where planned and managed appropriately, co-enrolment can offer a number of benefits in terms of both scientific value and efficiency of study conduct, and will increase the opportunities for patients to participate in, and benefit from, clinical research.

Key words: Adjuvant; aspirin; cancer; co-enrolment; randomised controlled trial

Statement of Search Strategies Used and Sources of Information

The paper largely reflects expert opinions and experiences of the authors, and their knowledge of the literature. The Pubmed database was searched for relevant articles, but a formal search strategy was not defined.

Introduction

Co-enrolment – entering patients into more than one clinical trial either concurrently or sequentially – is rarely reported or discussed in oncology literature. As such, co-enrolment policies may be specified in the trial protocol or decisions made by an institute or recruiting investigator, without a clear rationale or evidence base. With a lack of guidance or consensus on when co-enrolment is appropriate, it is unsurprising that the decision not to co-enrol may be seen as the safe option.

Recent trends in oncology research – such as the use of longer term, maintenance therapies and evaluation of repurposed agents (whose use alongside other treatments may already be well documented) – as well as the ever-increasing number of trials competing for the same patients, mean that co-enrolment is becoming more relevant. More routine consideration of opportunities to enter patients into multiple trials is warranted.

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Co-enrolment has been explored in other (non-cancer) settings – particularly those where trial recruitment is challenging and/or there are many (large) competing trials – including resuscitation [1], critical care [2–4] (including neonatal [5] and paediatric [6] settings) and peri-natal research [7]. Here, co-enrolment offers the opportunity to maximise use of the patient population and increase the speed and efficiency of research delivery. In settings such as HIV [8] and anaesthesia [9], where large, pragmatic trials are common and/or participants might be receiving several other medications, co-enrolment may also provide important data on drug interactions.

Across different settings, researchers report barriers to co-enrolment and, frequently, a lack of (universal) support from the research community or ethics committees [2,6,9]. Common barriers range from ethical and scientific considerations to safety concerns [1–3,6,7,9]. The need for further reporting of co-enrolment and more research on this topic, is noted [4,7,10].

The potential benefits of co-enrolment, as well as possible barriers, are relevant in oncology trials and warrant further exploration. Here, we report our experiences with exploring and managing co-enrolment opportunities within a large, multicentre oncology trial.

The Add-Aspirin Trial

The Add-Aspirin trial is a randomised controlled trial (RCT) assessing whether regular aspirin use after curative treatment for an early stage tumour can prevent recurrence and prolong survival (Figure 1) [11–13]. The intervention is being tested in four tumour types (breast, colorectal, gastro-oesophageal and prostate) by means of parallel cohorts. Patients enrol following potentially curative therapy – this incorporates a range of treatment pathways for each tumour site, including surgery with any appropriate (neo-) adjuvant therapies, radical chemoradiation (oesophageal) and radical radiotherapy (prostate). Participants are randomised to daily aspirin 100 mg, 300 mg or placebo. The trial is recruiting across the UK, and will also open in India, with a target of approximately 10 000 participants.

Co-enrolment may be relevant to patients entering Add-Aspirin subsequent to enrolling in a primary therapy trial and may also arise at the time of recurrence during participation in Add-Aspirin. A proactive approach to exploring co-enrolment opportunities with other trial teams has been adopted to agree when this might be appropriate and how it can be facilitated and managed within the ongoing trials.

Benefits of Co-enrolment

Co-enrolment is particularly relevant in Add-Aspirin as the intervention is being given after initial treatment, so participants from trials of primary therapies represent a significant proportion of the eligible population. However, the potential advantages of co-enrolment apply more widely to multicentre oncology RCTs – particularly pragmatic trials – as a number of different interventions will be relevant to a patient over the course of their disease and treatment. Allowing appropriate co-enrolment improves the efficiency of recruitment, helping to ensure the feasibility of trials running concurrently, and maximises opportunities for patients to participate in, and benefit from, clinical research.

A further advantage is the opportunity to assess trial interventions alongside one another, helping to ensure the

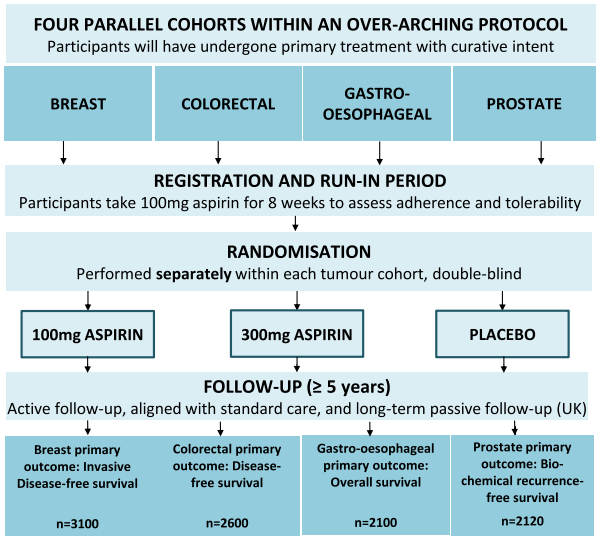

Fig 1. The Add-Aspirin trial.

ongoing relevance of the studies. If two interventions might potentially both be given to a patient in future practice, collection of information on their combined use will be valuable for establishing the importance of each one, providing more cohesive evidence to inform the management of future patients.

There are, of course, potential concerns in allowing patients to enrol in multiple RCTs. In what follows, we consider the scientific, safety and ethical issues.

Impact on Trial Results

A principle concern with co-enrolment is the potential effect on the results of the trials, particularly when they are evaluating a common outcome measure. We would argue that, although this issue deserves careful consideration, in many cases any effect will probably be negligible, and should not generally be a prohibitive factor.

In Add-Aspirin, due to the timing of the intervention, we are commonly considering the case of sequential co-enrolment – patients entering Add-Aspirin having previously enrolled in another trial. This would also be the case if considering trials of second- or third-line treatment after relapse in patients who had participated in a primary therapy trial. Here, assuming there is no interaction between the interventions, there is no concern about an effect on the results of the second trial (Add-Aspirin). However, there is the potential for an effect on the results of the first trial if participants from the different arms enter Add-Aspirin at different rates. This may occur because patients from one arm of the trial are either more likely to be eligible (for example, patients need to be disease-free, which may be more likely in the experimental arm of the first trial) or they are more likely to be willing to participate (for example, if one arm of the first trial has a shorter or less toxic treatment). In these scenarios, if aspirin is effective, it will have a differential effect in the trial arms of the first trial with the potential to affect the power. Although stratification within Add-Aspirin for the trial arm in the first trial will help to ensure balance in terms of those individuals entering Add-Aspirin, there may still be an overall imbalance in terms of aspirin allocation between the arms of the first trial when those who did not join Add-Aspirin are also considered.

We have estimated the magnitude of any potential effect in different scenarios and found that it is generally limited by a number of factors (Figure 2). Statistical modelling, using ranges of assumptions, suggests that any effect on the power of the first trial will probably be small. Table A1 (web appendix) provides an example showing selected models, including some felt to illustrate the largest plausible effect. Significant effects were only anticipated with relatively large (improbable) differences in participation rates and were further increased when there were unexpectedly large effects of aspirin. Co-enrolling trials could be monitored for this unlikely set of circumstances, with the potential to stop co-enrolment if there were concerns. Our models do not consider the potential effect of aspirin use outside the Add-Aspirin trial (participants from the first trial already taking aspirin), which may further limit any effect. Similar limiting factors are noted in other settings [9]. However, this should be carefully considered on a trial-by-trial basis, before any co-enrolment, and subsequently monitored.

Interaction Effects

Our modelling has generally assumed that there are no interaction effects between trial interventions – this is

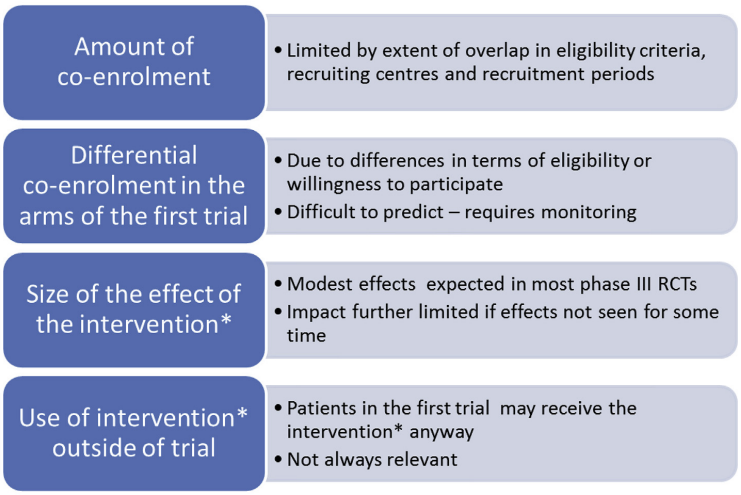

Fig 2. Factors affecting the potential effect of co-enrolment on power. *Intervention being evaluated in the second trial.

reasonable in most scenarios considered in relation to Add-Aspirin. However, others argue that the potential for an interaction between two trial interventions should not necessarily prohibit co-enrolment and, in fact, can facilitate evaluation of the interaction (particularly relevant where interventions are already in use outside of the trials) [7–9]. The information gained from co-enrolled participants may be insufficient to formally establish if there is an interaction but will be more robust data than would otherwise be available [7–9]. Modelling based on pragmatic anaesthesia trials suggested that a large detrimental effect on the power of the first trial would only be seen with a large antagonistic interaction, substantial co-enrolment and limited use of the second trial intervention outside of the trial [9].

A factorial randomisation can be viewed as preferable to co-enrolment between separate trials, but may not always be practical or sensible – for example, if the combination of the two interventions is only relevant to a small subgroup or, as with Add-Aspirin, one intervention is given at a later time, dictating the most appropriate timing for randomisation. Furthermore, the (statistical) advantages of a factorial design, compared with co-enrolment, may not be great [4,8,9]. The potential for loss of power for assessing one intervention, in the presence of the other, may still exist, and factorial trials are not normally powered to detect interactions.

Safety Considerations

There may, of course, be safety concerns with patients receiving interventions from two different trials. This will be highly dependent on the interventions. If there are concerns or a high degree of uncertainty about toxicity risks then co-enrolment will probably be avoided. However, in a trial of a marketed product or intervention that is already in use in normal practice, co-enrolment will be more acceptable [9]. In Add-Aspirin, participants receive low-dose aspirin or placebo. In most of the primary treatment trials where co-enrolment may be relevant, there will already be patients taking aspirin alongside the trial intervention. Allowing participants to subsequently enrol in Add-Aspirin may facilitate the collection of more robust data on the use of aspirin alongside (or following) the intervention to guide future practice.

Concerns regarding liability, in the event of a personal injury claim being made by a trial participant who is enrolled in multiple trials, have been raised as a potential barrier to co-enrolment, but we do not believe this is justified. Existing indemnity arrangements for each trial should suffice.

The Participant's Perspective

In addition to potential scientific benefits, allowing trial co-enrolment, where appropriate, will maximise opportunities for patients to participate in research. However, the approach must be both ethically sound and acceptable to (potential) participants – these are perhaps the most complex issues surrounding co-enrolment and there is currently a lack of guidance or evidence in the literature to inform this.

In our discussions with other trial teams regarding co-enrolment, some researchers have expressed concerns that asking patients to join more than one trial may over-burden them, a view that has proved to be a barrier in other settings [6]. The participant representatives on the Add-Aspirin Trial Management Group (co-authors on this paper), have been strong advocates of co-enrolment from the outset, and would argue that there is an opposing ethical obligation to provide all of the information required to allow an individual to decide for themselves about joining any trial that is relevant to them. Not approaching a patient to participate in a trial that they could be eligible for because they are already enrolled in another study would be denying them an opportunity. Similar conclusions were reached in a review of co-enrolment considerations in the anaesthesia setting, with the authors feeling that preventing patients from autonomously co-enrolling is difficult to justify ethically [9].

A survey of patients in a research-active breast cancer unit provides evidence to support these views [14]: three-quarters of respondents (37/50, 74%) would have considered entering more than one study if adequate written information was provided. Most (32/50, 64%) did not believe that participation in clinical research should be restricted to a maximum number of studies – and, of those who did, only two indicated that it should be limited to a single study. Furthermore, two-thirds of respondent (34/50, 68%) did not think that involvement in more than one study was a significant burden.

A similar survey of 50 families approached about multiple (up to six) clinical trials in a neonatal intensive care unit suggested similar attitudes (despite the setting, where the potential to over-burden families may be even more of a concern) [5]: three-quarters (74%) of parents indicated that they would enrol their baby into two or more studies; almost all (98%) felt they wanted to make the decisions about study enrolment themselves, rather than a clinician deciding.

Although the data from these studies are reassuring, they are limited and may reflect the views of select groups of individuals. Further exploration with patients and with groups representing patient and public involvement (PPI) is warranted.

Measures to Increase Acceptability

Comments from respondents in the breast unit study emphasised the importance of individual choice as well as concerns around extra hospital visits interfering with normal life [14]. These are areas that need to be addressed in trials where co-enrolment is deemed appropriate.

Researchers have an obligation to carefully consider the timing of approaching potential participants about each trial, ensuring that the information provided (not only about the individual trials but also about the implications of joining more than one) is clear and there is sufficient opportunity for questions. In the paediatric intensive care setting, Harron *et al.* [6] advocated careful development and piloting of a strategy for the whole consent process when multiple studies may be available to an individual.

Wherever possible, there should be compatibility between follow-up schedules for two trials where co-enrolment is possible in order to minimise the number of additional hospital visits and assessments/tests compared with standard care. Ideally, this would be planned at the design stage. Where co-enrolment decisions may be made during the trial, allowing some flexibility in schedules will work towards this aim – enabling research nurses to plan clinic visits that will meet the requirements of both trial schedules. In Add-Aspirin, follow-up schedules have been planned to largely align with standard care – and this will be the case with many pragmatic trials. Additionally, there is some flexibility regarding the timing of assessments.

In the above considerations, engagement with and input from participant representatives and PPI groups is vital to ensure that the approach is acceptable to participants and will not lead to unnecessary additional burden.

Ethical Approvals

Some researchers report resistance from ethics committees as an obstacle to allowing individuals to enter multiple trials [2,14]. This has not been the experience in Add-Aspirin – the potential for participants from multiple primary treatment trials to enrol in Add-Aspirin has been written into the trial protocol from the outset, and was not raised as an issue by the ethics committee who approved the study, nor by the regulators nor funders of the trial. Thus, there is perhaps a need for a more consistent approach to trial co-enrolment by research ethics committees. We would suggest that co-enrolment, where appropriate, should generally be supported in order to allow potential participants the autonomy to decide about enrolling in any trial that is relevant to them. However, this should be on the provisos that: the informed consent process and trial follow-up schedules have been carefully considered; the safety of receiving both trial interventions has been deemed acceptable; and any other appropriate measures are in place to minimise any extra burden on participants as far as possible.

The potential scientific advantages of allowing co-enrolment (where appropriate), in terms of increasing both the value and efficiency of the research, provide further ethical justification for the approach. Myles *et al.* [9] argue that an important ethical consideration in research planning is the efficient conduct of studies and fairer allocation of resources for research, and that allowing co-enrolment can contribute to this aim. Furthermore, if two interventions being evaluated in trials might potentially both be given to a patient in future practice, there is

Table 1
Proposed measures for trial teams managing co-enrolment within a randomised controlled trial

	Proposed measure	Purpose
Design	Identify trials where co-enrolment may be considered	Assess potential impact and agree where co-enrolment is appropriate in advance
	Develop appropriate consent process*	Ensure that being approached about multiple studies will be acceptable to patients
	Ensure compatibility of follow-up schedules, allowing flexibility where possible/appropriate*	Minimise extra visits/assessments, ensuring that participation in multiple studies will be acceptable to patients
	Provide guidance on co-enrolment in the protocol (and trial website/other documents as appropriate)	Ensure only appropriate co-enrolment takes place and follows the strategy developed for consent and follow-up
	Consider stratifying by treatment arm in the first trial in the randomisation algorithm for the second trial (where significant overlap is expected)	Ensure treatment allocation in the second trial is balanced (in terms of those individuals who *enter* the second trial)†
Conduct	Implement eligibility checks around co-enrolment at entry	Ensure only appropriate co-enrolment takes place
	Consider implementation of screening logs	Identify any recruitment issues as a result of co-enrolment decisions or any barriers to co-enrolment
Monitoring	Collect and regularly review co-enrolment information, including treatment allocation in the other trial, on case report forms	Active monitoring with the potential to take action – by capping recruitment from one arm, for example – if a large imbalance occurs (although this is unlikely)
	Establish agreements to share information between data monitoring committees (blinded trials)	It may be appropriate for monitoring to be carried out by data monitoring committees in the case of blinded trials

* In discussion with participant representatives and/or patient and public involvement groups.
† This will not ensure balance overall if participants from the different treatment arms of the first trial enter the second trial at different rates. Thus, careful monitoring is still required.

arguably an ethical obligation for researchers to collect information on the combined use of the therapies in order to establish the importance and safety of each one in the context of the other, and provide more cohesive evidence to inform the management of future patients [7].

Managing Co-enrolment

Where co-enrolment to multiple oncology RCTs is permitted, given the issues outlined here, it requires careful management and monitoring. We propose a number of measures (Table 1).

A precedent for designing and conducting RCTs to facilitate co-enrolment has been set in the HIV field (Terry Beirn Community Programs for Clinical Research on AIDS; CPCRA) [8]. Measures include shared data collection forms; standardised definitions and criteria for assessing and reporting outcomes and adverse events; a single, common follow-up schedule; and an analysis approach that explores drug interactions.

Discussion

For the vast majority of trials where co-enrolment with Add-Aspirin has been considered, we have found that it is likely to be acceptable both in terms of the safety of participants and maintaining the integrity of trial results. As such, the importance of giving individuals the autonomy to make their own decisions about trial participation provides a compelling ethical argument for allowing co-enrolment, wherever appropriate, providing that it is done in a way that will be acceptable to participants. We have encountered a number of perceived barriers that may not be well-founded and there is a need for further evidence to promote greater understanding about the potential impact of co-enrolment.

The benefits of co-enrolment align with the original aims in establishing the National Cancer Research Network, which include improving the co-ordination and quality of research, widening participation, increasing the numbers of patients involved and speeding up the delivery of research for the ultimate benefit to patients [15]. As such, we suggest it should be routinely considered by the associated clinical studies groups in reviewing trial portfolios, with the aim of maximising co-enrolment opportunities.

The implications of individuals participating in more than one trial are multifactorial and should be carefully considered on a trial-by-trial basis. Evidence relating to acceptability is limited, and more research is needed. However, as it is probably highly dependent on the patient group and the specific trials under consideration, engagement with relevant PPI groups and representatives, from the planning stage and throughout the trial, is crucial to ensure that the appropriate measures are in place. There is an onus on the trial teams to evaluate acceptability and any potential (scientific) consequences in advance, and to monitor co-enrolment closely throughout the trial, managing any issues appropriately with a pre-defined strategy. Trial protocols should not enforce a complete ban on co-enrolment without sound justification.

Much could be learnt from the CPCRA programme, where efforts to facilitate co-enrolment led to a quarter (22.5%) of patients from six RCTs entering more than one trial [8]. The programme was developed by a single research group – a high degree of co-operation and strong lines of communication would be required to achieve similar where trials are being conducted by different groups. In other settings, the establishment of co-enrolment policies or consensus guidelines has been advocated [4,8,10]. This could be a way forward in oncology research.

A more considered and co-operative approach to co-enrolment will not only benefit individual trials, but may contribute to an evidence base showing the extent of co-enrolment and any observed impact or issues. Limited reports from other settings have not indicated any negative impact [3,6]. Based on the experiences in Add-Aspirin, we hope that such data might ultimately reassure researchers of the benefits of allowing participants to co-enrol where there are multiple oncology trials that are relevant to them. Add-Aspirin opened in October 2015 and, to date, the possibility of co-enrolling relevant patients has been agreed for 40 other trials, across the four tumour types. The number of participants who have been co-enrolled remains small at this early stage of recruitment.

Conclusions

Opportunities for co-enrolment of participants into multiple cancer trials should be more routinely considered. Where planned and managed appropriately, co-enrolment can offer a number of benefits in terms of both the scientific value and efficiency of study conduct, and will increase the opportunities for patients to participate in, and benefit from, clinical research.

Acknowledgments

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Appendix

Table A1
Example power calculations to assess the potential impact of co-enrolment

Modelling assumptions			Estimated impact on trial X				
Effect of aspirin on 5 year survival*	Participation rates in Add-Aspirin†		5 year survival in trial X with co-enrolment			Power (loss/gain in power)	Extra patients (OR follow-up) needed for 80% power
	Control	Intervention	Control	Intervention	Difference		
Trial X result is positive (5 year survival 55% control versus 45% intervention) in the absence of co-enrolment							
6%	10%	10%	45.4%	55.4%	10.0%	79.9%	2 (1 month)
	20%	20%	45.8%	55.8%	10.0%	79.9%	3 (1 month)
	30%	30%	46.2%	56.2%	10.0%	79.8%	5 (1 month)
	10%	15%	45.4%	55.6%	10.2%	81.5%	–
	10%	20%	45.4%	55.8%	10.4%	82.9%	–
	15%	30%	45.6%	56.2%	10.6%	84.3%	–
	15%	10%	45.6%	55.4%	9.8%	78.3%	44 (3 months)
	20%	10%	45.8%	55.4%	9.6%	76.6%	89 (5 months)
	30%	15%	46.2%	55.6%	9.4%	74.8%	138 (8 months)
10%	10%	10%	45.7%	55.7%	10.0%	79.9%	3 (1 month)
	20%	20%	46.3%	56.3%	10.0%	79.8%	5 (1 month)
	30%	30%	47.0%	57.0%	10.0%	79.8%	7 (1 month)
	10%	15%	45.7%	56.0%	10.3%	82.4%	–
	10%	20%	45.7%	56.3%	10.7%	84.7%	–
	15%	30%	46.0%	57.0%	11.0%	86.8%	–
	15%	10%	46.0%	55.7%	9.7%	77.2%	75 (5 months)
	20%	10%	46.3%	55.7%	9.3%	74.2%	154 (9 months)
	30%	15%	47.0%	56.0%	9.0%	71.1%	244 (15 months)
Trial X result is negative (5 year survival 45% in both arms) in the absence of co-enrolment							
6%	10%	10%	45.4%	45.4%	0.0%		
	20%	20%	45.8%	45.8%	0.0%		
	30%	30%	46.2%	46.2%	0.0%		
	10%	15%	45.4%	45.6%	0.2%		
	10%	20%	45.4%	45.8%	0.4%		
	15%	30%	45.6%	46.2%	0.6%		
	15%	10%	45.6%	45.4%	−0.2%		
	20%	10%	45.8%	45.4%	−0.4%		
	30%	15%	46.2%	45.6%	−0.6%		
Trial X result is negative (5 year survival 45% in both arms) in the absence of co-enrolment							
10%	10%	10%	45.7%	45.7%	0.0%		
	20%	20%	46.3%	46.3%	0.0%		
	30%	30%	47.0%	47.0%	0.0%		
	10%	15%	45.7%	46.0%	0.3%		
	10%	20%	45.7%	46.3%	0.7%		
	15%	30%	46.0%	47.0%	1.0%		
	15%	10%	46.0%	45.7%	−0.3%		
	20%	10%	46.3%	45.7%	−0.7%		
	30%	15%	47.0%	46.0%	−1.0%		

The table illustrates the potential impact of co-enrolment into Add-Aspirin on the power of a hypothetical study, trial X. Selected results are shown from models performed under a range of assumptions about the factors listed in Figure 2, including scenarios felt to illustrate the largest plausible impact on power.
Trial X: A hypothetical two-arm superiority randomised controlled trial of a new peri-operative chemotherapy regimen versus standard in gastro-oesophageal patients. Designed with 80% power to detect a 10% improvement (from 45% to 55%) in survival at 5 years, requiring 500 patients per arm. Patients who are disease free at the end of treatment may become eligible for Add-Aspirin.

* A 6% survival benefit at 5 years is hypothesised in Add-Aspirin (gastro-oesophageal). Models are repeated for larger benefits to illustrate potential effects on power.

† A range of participation rates are used to assess potential impact – actual rates are unlikely to reach 30% (limited by overlap in recruiting centres and recruitment periods, as well as trial X participants being ineligible or unwilling to participate in Add-Aspirin). Differences in rates between arms are also unlikely to be as large as illustrated here.

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Appendix H:

Posters