

Early Phase Disease Modification Trials with Selisistat and Optical Coherence Tomography as a Biomarker in Huntington's Disease

1 Declaration

I, Salman Haider, confirm that the work presented in this Thesis is my own. Where contributions of others are involved, I confirm that this has been clearly indicated in the Thesis. The copyright of this Thesis rests with the author and no quotation or information derived from it may be published without the prior written consent of the author.

2 Acknowledgements

I would like to thank Professor Sarah Tabrizi for giving me the opportunity to work in Huntington's disease and for her invaluable support, humour and patience. I would like to thank Professor Tom Warner, my secondary supervisor, for his guidance and support. I am also deeply grateful to all the staff, patients and their families with whom I worked so closely. The Huntington's community remain a continuing source of inspiration to me. Finally I would like to acknowledge my family, in particular my parents including my late father, who gave me a gift that keeps on giving, and one that can never be repaid.

3 Abstract

Huntington's disease (HD) is a monogenic neurodegenerative disorder with no known cure.

Selisistat is a novel, highly potent Sirt1 inhibitor with supportive pre-clinical data. Facilitation of autophagy and amelioration of transcriptional dysregulation are proposed as mechanisms of action. A first in disease Phase 1B study showed safety and tolerability. The peripheral immune dysfunction in HD could be modulated by Sirtuins which have both pro and anti-inflammatory activities. However selisistat did not alter the cytokine profile in this study. Phase 2 data over a longer duration was also well tolerated however potential hepatotoxicity is a concern. Sub-analysis of clinical assessments did not reveal any significant effect. Confirmation of proposed mechanisms of action is lacking and no Phase III studies are planned.

Significant clinical heterogeneity exists in HD phenotypes which must reflect differing neuronal susceptibilities. A novel total motor score based sub-division of HD phenotypes failed to demonstrate any changes in a whole brain voxel-based morphometry (VBM) analysis.

Clinical assessment alone lacks sensitivity over shorter time-spans in HD, and thus reliable, tolerable and sensitive biomarkers are required. Optical coherence tomography is a potential novel biomarker. The hypothesis that neuroretinal structures may be surrogate marker of intracranial disease was tested in a pilot biomarker study, the first of its kind in HD. Evidence of a statistically significant ($p < 0.01$) reduction in macular volume in HD subjects versus age and sex-matched controls is seen. No change in RNFL measures was seen. A correlation with increasing disease severity on ordinal regression was also noted. No correlation of macular volume and RNFL thickness with change in whole brain and caudate volumes. Furthermore, OCT was well tolerated by the majority of participants. Retinal abnormalities in HD have been confirmed in three subsequent independent OCT studies.

(Word Count: 289)

4 Impact Statement

Huntington's disease is fatal neurodegenerative for which no disease modifying treatment exists. The body of work presented below has contributed to the field of Huntington's disease research through demonstrating safety and tolerability of selisistat. It has raised concerns about the potential hepatotoxicity of the medication. It has further re-emphasised the importance of ensuring the presence of sound pre-clinical work demonstrating firstly, target engagement and the secondly that the target is a key modulator of the disease process. This underpins the successful transition of therapeutic candidates into clinical trials.

Selisistat did not demonstrate modulation of the innate immune response, which is altered in Huntington's disease. The role of optical coherence tomography as potential safe, quick and well tolerated biomarker has been suggested by work carried out here. The importance of demonstrating target engagement in therapeutic development prior to clinical trials is a well-established in principle in clinical trials here and its absence here, underscores its importance.

There is no obvious impact of this work outside the wider scientific or non-scientific community.

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5 Introduction

5.1 Huntington's Disease

Huntington's disease is a monogenic, autosomal dominant, neurodegenerative condition. The onset of disease is currently defined as the point at which characteristic motor signs develop, where a patient moves from being a *premanifest gene carrier* to having *manifest* disease. This distinction is somewhat arbitrary because most patients develop cognitive or psychiatric symptoms (or both) during the premanifest period, often many years before any motor signs are seen.

Due to relatively early loss of function and productivity and the chronic, slowly progressive nature of the disease with its increasing requirements for medication, social and multidisciplinary care, HD carries a substantial resource burden (Aubeeluck & Wilson, 2008). Furthermore, as the disease is fully penetrant, a positive predictive HD gene test results in development of disease at some point. The *premanifest* phase therefore creates an invaluable window of opportunity, both to study the disease in its earliest stages, and a unique point at which to intervene therapeutically.

Although no cure for the disease exists, in the last few years there has been an exponential rise in the number of publications, delineating its basic and clinical science, and therapeutic trials. Promising avenues exist, to broaden our understanding of disease processes from which suitable targets can be pinpointed, in the availability of suitably target-directed therapies, and finally in our ability to adequately and reliably establish disease modification.

To date however no disease modifying therapy for a neurodegenerative disease exists.

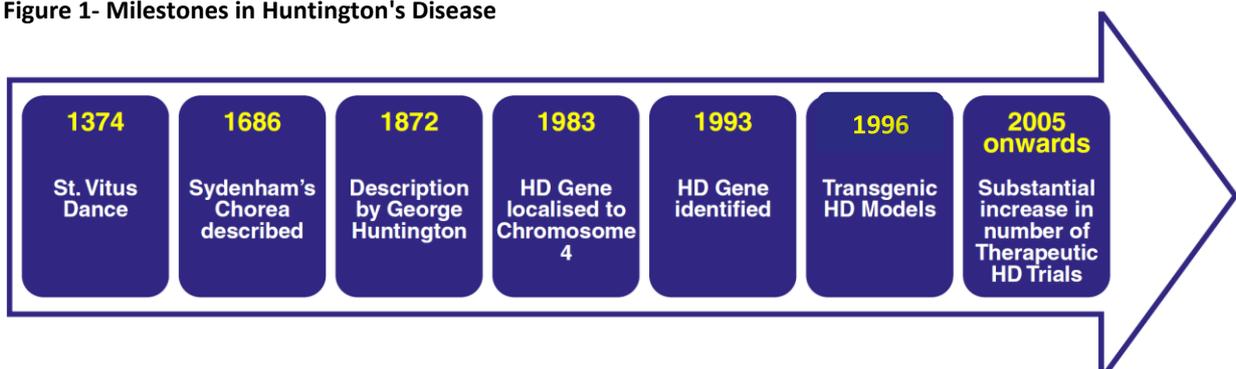
5.2 History

Originally termed Huntington's chorea, the nomenclature changed to its current form relatively recently in order to better represent the plethora of clinical features beyond the movement disorder. The first definite description of Huntington's disease (HD) was given by Charles Oscar Waters in 1841, who gave a clear account of the natural history of the disease. On the 15th February 1872, George Huntington, aged 21 and a recent graduate from Columbia University, presented the earliest most complete description of HD before the Meigs and Mason Academy of Medicine at Middleport, Ohio. It was published two months later in the Medical and Surgical Reporter of Philadelphia (Huntington, 1872).

In an address to the New York Medical Society in 1909, Huntington summarised some important features we recognise today as being part of the disease, alongside the classical description of chorea, “. . . two women, mother and daughter, both tall, thin, almost cadaverous, both bowing, twisting, grimacing.”

By far the most important milestone of modern times was the herculean collaborative effort, driven notably by affected families, galvanising doctors and scientists and culminating in the identification of a mutation in the *Huntingtin (HTT)* gene in 1993 (The Huntington's Disease Collaborative Research Group, 1993) (**Figure 1**). The gene is located on the short arm of chromosome 4 (4p 16. 3) and in HD, it contains a CAG repeat expansion in exon 1, which results in an abnormal polyglutamine tract in the protein, huntingtin (HTT). The advent of gene silencing trials may herald another key milestone in the history of Huntington's disease and full results are anxiously awaited.

Figure 1- Milestones in Huntington's Disease



2010). HD is one of the most frequently occurring hereditary neurological disorders, after Neurofibromatosis Type 1, Charcot Marie Tooth disease and Duchenne muscular dystrophy (Harper, 1992).

5.2.2 Late Onset Huntington's Disease

This has been variously termed senile chorea or late onset HD, describing a subset of HD patients, who present above the age of 75. Of all known HD patients, approximately 25 per cent present above the age of 50. Although other causes of chorea should be looked for in this age group, the most likely cause remains HD. Chorea is almost universal but the course is milder and slower with mild cognitive and psychiatric disease. Gait disturbance and dysphagia are also seen but are not severe. It is usually associated with CAG repeat sizes of around 40, though lengths of up to 48 have been recorded. Often a family history can be lacking, which may reflect expansion of an intermediate range allele (James *et al.*, 1994; Kremer *et al.*, 1993).

5.2.3 Juvenile Onset Huntington's Disease

Although Lyon is credited with the first description of Juvenile HD in 1863, closer examination reveals this cohort as more likely benign hereditary chorea, given the absence of significant disease progression (Bates *et al.*, 2002; Lyon, 1863). Juvenile HD is defined as an age of onset prior to the age of 20. It accounts for 5-10% of all HD presentations and is paternally transmitted in 90% of cases. CAG repeat expansions are almost always greater than 55.

In the largest case series of juvenile subjects, over half experienced symptom onset under the age of 14, while 1 in 10 cases occurred under the age of 10. Under the age of 10, developmental delay specifically in speech and language may be prominent which may manifest as failure to progress at school. Behavioural changes, learning difficulties, rapid cognitive decline, psychiatric disease and parkinsonian motor features, with predominating rigidity and bradykinesia seen in 50% of cases, as

well as dystonia and ataxia. The range of psychiatric disease can include drug and alcohol abuse as well as eating disorders (Ribai *et al.*, 2007).

In a multi-sourced survey of prescribing practices in juvenile HD, the most commonly prescribed agents were anti-psychotics, anti-depressants, anti-parkinsonian medications and anti-epileptics, of which Valproic Acid was the most commonly used. Polypharmacy was an issue in minority of cases. The most common symptoms reported by the families were speech difficulties, dysphagia, stiffness, spasticity, sleeping difficulty, pain and behavioural problems (Robertson *et al.*, 2012).

In contrast to the adult form, chorea itself is rare and early oropharyngeal dysfunction appears more commonly in juvenile cases. Similarly, seizures occur in around 25-50% of juvenile HD cases. They are usually generalised or myoclonic in nature though absence seizures have also been noted and occasionally seizures prove intractable (Gonzalez-Alegre & Afifi, 2006).

5.2.4 Akinetic-Rigid or Westphal Variant

Westphal described an 18-year-old patient with prominent rigidity, which later became known as the akinetic-rigid Westphal variant (Westphal, 1883) and is most typically associated with juvenile HD. However, this variant has also been noted rarely in young adult populations, with much lower CAG repeat sizes. In one 3 patient case series no significant eye movement or cognitive abnormalities were seen though mild cardiovascular dysautonomia was present (Racette & Perlmutter, 1998) (Reuter *et al.*, 2000). Pathologically, the Westphal variant may be different, with studies demonstrating loss of both direct and indirect striatopallidal pathways (Albin *et al.*, 1990) (Bugiani *et al.*, 1984).

5.2.5 Adult Onset Disease

In the classical adult onset disease, symptoms usually manifest between the ages of 30-50 and progress over 15-20 years. Here we find the classical triad of motor, cognitive, and psychiatric

features. However, even within the same family, there can be a striking difference in the relative manifestation and severity of these features.

5.2.6 Clinical Symptoms

5.2.6.1 Motor

Although there are a legion of motor signs in HD, they can be reasonably divided into those representative of impairment of voluntary motor control e.g. limb inco-ordination or loss of fine motor control of fingers, those in keeping with loss of involuntary motor control e.g. loss of balance and postural reflexes and finally, those that indicate the presence of involuntary movements e.g. chorea, tics and dystonia. There can evidently be overlap of features, for example where chorea or dystonia of the trunk are superimposed upon impairment of postural reflexes, impacting additively on balance and mobility.

Intriguingly, even quite marked chorea often remains unnoticed by the patient. It can also be passed over initially by relatives as nervousness but it is usually their persistence that prompts the first medical consultation. Alternatively, in those with a clear family history, partners or relative can comment on similarities of behaviour or movement between generations. Descriptions such as 'twitchy', 'fidgety' or 'restless' can be applied, and subjects themselves can report symptoms of akathisia. Typically, there are minor movements of the hands or toes, though movements can be much larger amplitude of a whole body part where they are termed ballistic. Subtle deterioration in fine motor control can be suggested by a change in handwriting or loss of dexterity in the use of a computer, mobile phone or cutlery.

This can be accompanied by walking difficulty, which is related to composite loss of voluntary motor control, excess voluntary movements, as well as impaired co-ordination and balance. It can produce a gait that is often difficult to classify, with

varying amounts of appendicular and limb ataxia, chorea and dystonia. Early mild swallowing impairment or dysarthria can also be seen. However, it is clear that the motor features can vary significantly in their relative predominance and location- chorea itself can be seen extra-axially in the face, mouth and tongue.

Generally, mild amounts of dystonia, bradykinesia and rigidity of limbs co-exist with relatively more prominent chorea, which itself reaches a peak in mid-stage disease, after which this balance reverses as disease progresses. In moderate and advanced stages, loss of both involuntary and voluntary movement occurs with abnormal fixed postures especially of the trunk or neck, which are usually dystonic in origin. Flexion contractures can ensue reflecting overriding spasticity and rigidity and representing more florid degeneration of corticospinal tracts.

The exception is the Westphal, akinetic-rigid variant, where rigidity and bradykinesia prevail and tremor and chorea are rarely seen. This is the characteristic presentation of juvenile HD though does occur in around 10% of adult cases. Axial rigidity appears to be more significantly associated with disability and loss of ambulation than chorea (Nance, 1998).

Motor impersistence is also seen in HD, characterised by an inability to maintain muscle contraction at a fixed level which has given rise to the term "milkmaid's grip". The patient is unable to apply a constant force during the handshake (Gordon *et al.*, 2000) and it can also be elicited by asking the patient to maintain a fully protruded tongue for ten seconds.

5.2.6.2 Balance and Posture

Balance impairment can occur early on in concert with the above motor features, but may only be noticed when the patient is in an environment that presents a greater motor challenge- at night or on getting onto or off an escalator or whilst walking on uneven ground. Formal quantitative analysis using an obstacle test, sit-

to-stand test and a step and turn test using kinematic and kinetic variables has shown that HD patients perform slower in all three tests, produce less rising force and have greater sway velocity of the centre of gravity. Patients display significant abnormalities in postural control deficits when replicating motor skills employed in activities of daily living, emphasising the functional impairment these symptoms confer (Panzera *et al.*, 2011).

5.2.6.3 Eye movements

The broken pursuit movements and slowed saccades (vertical worse than horizontal) seen early on the disease worsen, requiring head movements or eye blinking to initiate and at the advanced stages, a complete supranuclear gaze palsy is possible (Quinn & Schrag, 1998). A characteristic sign is gaze impersistence with difficulty fixating on an object. These oculomotor abnormalities are likely to represent disruption to the fronto-striatal control of eye movements (Lasker & Zee, 1997).

5.2.6.4 Dysphagia

The swallowing difficulties seen in HD likely reflect several processes: lack of coordination between the oral and pharyngeal stage, tachyphagia, or rapid uncontrolled swallowing secondary to impaired sensory and corticobulbar function, buccolingual chorea resulting in food being transferred inadvertently, failure of the normal respiratory-deglutition cycle as well as oesophageal dysmotility (Manor *et al.*, 2018; Shoulson & Fahn, 1979). Patients report difficulty swallowing solid and liquids and can choke occasionally also, emphasising the importance of speech and language therapists, dieticians and judicious investigation in the form of video fluoroscopy. In the later stages, progressive swallowing and speaking impairment gives way to anarthria and mutism.

5.2.6.5 Continence

Urge incontinence with associated frequency and urgency is probably the most commonly reported complaint, usually occurring in mid stage disease. In advanced stages patients become doubly incontinent. There is minimal literature specifically on this subject though a study of 6 patients revealed four with detrusor instability

while two had a normal study suggesting a non-organic disturbance. A characteristic urodynamic pattern, consisting of choreiform movements of the pelvic floor musculature during filling with selective suppression of choreiform contractions in the perineum during detrusor contraction was observed though to best of my knowledge, this has never been followed up (Wheeler *et al.*, 1985).

In a large study of autonomic symptomatology in HD, using a clinical questionnaire rather than formal urological investigation, urgency, urinary incontinence, frequency, nocturia, incomplete emptying and straining on defecation were all found in both manifest and premanifest subjects (Aziz *et al.*, 2010).

5.2.6.6 Sleep disturbance

The aetiology of sleeping difficulties in HD is likely to be multi-factorial as patients have a profound circadian rhythm disturbance, which mirrors that seen in transgenic mice, where diminished gene expression in the suprachiasmatic nucleus is noted (Maywood *et al.*, 2010) (Morton *et al.*, 2005). Hypothalamic-pituitary dysfunction does undoubtedly play a role leading to sleep fragmentation and sleep-wake cycle reversal. Depression and apathy may contribute also through a loss of daytime stimulation and activity. Involuntary movements can hinder getting off to sleep as well as contribute to middle insomnia. Clinical research has shown that sleep disturbance in HD includes insomnia, advanced sleep phase, periodic leg movements, REM sleep behaviour disorders and reduced REM sleep (Arnulf *et al.*, 2008) as well as reduced melatonin levels (Kalliolia *et al.*, 2014).

5.2.6.7 Dysautonomia

This is a relatively new clinical entity in the HD phenotype though research has shown patients with HD commonly have impairment of the autonomic nervous system (Bar *et al.*, 2008;Kobal *et al.*, 2004). In a recent study, the most prevalent symptoms in 63 patients with HD and 21 pre-manifest mutation carriers, were dysphagia, erectile and ejaculatory dysfunction, sialorrhoea though xerostomia can

also be seen, early abdominal fullness and light-headedness whilst standing (Aziz *et al.*, 2010).

5.2.6.8 Cognitive

A global dementia is not seen in HD until the later stages, rather an affectation of specific domains and their connections- a focal frontal subcortical dementia, manifesting typically as a dysexecutive syndrome, where patients complain of mental slowing, difficulty multi-tasking, failure to initiate, plan and organise tasks, maintain concentration and rigid thinking. Occasionally this may manifest or indeed be supplemented with a prominent behavioural change such as aggression or alternatively withdrawal and change of personality.

Symptoms may be present from the premanifest stage, with one study showing that 38% of subjects in the premanifest stage had mild cognitive impairment (Duff *et al.*, 2010). Deficits in processing speed, visuospatial processing, time estimation and timekeeping, learning and working memory are also seen in this group. Correspondingly, they report difficulty in picking up new tasks though retrieval of task specific memories is affected also. There is also reliable evidence of olfactory impairment in both premanifest and manifest subjects (Paulsen, 2011; Stout *et al.*, 2011). It is clear also that cognitive and behavioural symptoms place the greatest burden on carers and correlate with functional decline and institutionalisation (Hamilton *et al.*, 2003).

Language is markedly spared until the advanced stages where there is undoubtedly a mixed component of both dysphasia and dysarthria. However, comprehension is believed to be well preserved even in late stages of the disease. Communication however can be impacted upon by diminished processing speed and loss of coordinated oro-motor function with respiration (Paulsen, 2011).

Characterisation of the cognitive impairment seen in HD has been driven largely by clinical research. Although there is substantial evidence of cognitive impairment in HD, there is no accepted cognitive battery for testing in premanifest HD clinically. Current practice may include the Verbal Fluency, Symbol Digit, Stroop, Category fluency as well as Trails A and B and the Hopkins Verbal Learning Test, as derived from several years of observational data (Paulsen, 2011;Tabrizi *et al.*, 2011;Tabrizi *et al.*, 2012).

In summary there are a large number of clinical symptoms that occur in the course of Huntington's disease (**Table 1**).

Table 1- Symptoms & Signs of Huntington's Disease (adapted from (Novak & Tabrizi, 2010))

Neurological
<p>Motor:</p> <p>Chorea, dystonia, bradykinesia, rigidity, spasticity, myoclonus, tics, motor impersistence.</p> <p>Loss of postural reflexes and balance, walking difficulty and impairment of voluntary motor control.</p> <p>Dysphagia and dysarthria.</p>
<p>Non-motor:</p> <p>Urge incontinence</p> <p>Sleep disturbance including sleep wake cycle reversal, insomnia, periodic leg movements, REM sleep behaviour disorders.</p>
Cognitive
<p>Perseveration, impulsivity, perceptual distortions, lack of insight, distractibility and difficulty in learning new information. Difficulties with planning, initiating and organising thoughts, activities and communication.</p>
Psychiatric
<p>Depression, obsessive-compulsive disorders, anxiety, bipolar disorder, irritability, apathy, hypersexuality, psychosis.</p> <p>Verbal and/or physical aggression.</p>
Other
<p>Weight loss, muscle wasting.</p>

5.2.7 Stages of Huntington's Disease

A working classification of HD denotes early, middle and late stages while the most commonly used clinical scale rates the person's functional abilities over five domains- the Shoulson and Fahn Total Functional Capacity Rating Scale (Shoulson & Fahn, 1979) (**Table 2**).

The early stage (I-II) represents the point at which patients have minimal limitation, are usually able to maintain employment without compromise and continue to live independently. Minor involuntary movements, psychiatric disease and some difficulty when multitasking may be reported.

The major difference at the moderate stage (III) is the loss of employment while still maintaining activities of daily living. Chorea is usually at its peak at this stage, with loss of fine motor control, increasing falls and abnormal mobility and gait. Mental inflexibility will manifest more prominently.

At advanced disease stage (IV-V), complete immobility is present as individuals are bed bound and require assistance in all activities of daily living. Profound dysarthria also occurs to point of severe communication difficulties. Chorea gives way to rigidity, dystonia, and bradykinesia. There is a high risk of medical complications.

Table 2- Clinical Staging of Huntington's Disease (Huntington Study Group, 1996; Shoulson et al., 1989a)

Total Functional Capacity Rating Scale		
Domain	Ability	Score
Occupation	Unable	0
	Marginal work only	1
	Reduced capacity for usual job	2
	Normal	3
Finances	Unable	0
	Major assistance	1
	Slight assistance	2
	Normal	3
Domestic Chores	Unable	0
	Impaired	1
	Normal	2
Activities of Daily Living	Total care	0
	Gross tasks only	1
	Minimal impairment	2
	Normal	3
Care level	Full-time nursing care	0
	Home of chronic care	1
	Home	2
TOTAL	Range 0 - 13	

TFC Total Score	Stage
11 - 13	I
7 - 10	II
3 - 6	III
1 - 2	IV
0	V

5.3 Huntington's Disease Pathophysiology

5.3.1 Introduction

In the two decades that have passed since the discovery of the gene, much progress has been made in elucidating the pathological processes of HD. Huntingtin (HTT) is ubiquitously expressed throughout the body but is found in particularly high concentration in the brain, in neurones more so than glia, and testes and to a moderate extent in the lungs, liver and heart. It is predominantly a cytoplasmic protein, which is amenable to cleavage by proteases.

5.3.2 Neuropathology

It is well established that in HD there is selective neurodegeneration of vulnerable neuronal populations. In specific terms, neuronal loss and atrophy occur particularly in the neostriatum- the caudate and putamen, though the former is affected to a greater extent.

As the disease progresses, generalised brain atrophy ensues such that at post-mortem the brain is between 300-400 grams lighter than the average adult brain weight for that age (Mann *et al.*, 1993) and the striatum only accounts for 20g of this. Interneurones are generally spared while in contrast, the striatal medium spiny neurons, which comprise 90% of the striatal neuronal population, are selectively lost. These are predominantly the enkephalin containing projections to the external globus pallidum rather than the substance P neurones that connect to the internal globus pallidum. From a functional neuroanatomical perspective, the occurrence of chorea early in disease, may reflect preferential damage to the indirect pathway of basal ganglia-thalamocortical circuitry (Paulsen *et al.*, 2005). In later disease the direct pathway is affected, as well as cortical neurons, which may contribute to the loss of motor control, abnormalities of eye movements and neuropsychiatric symptomatology.

The substantia nigra, distinct cortical layers, the hippocampus, angular gyrus in the parietal lobe, Purkinje cells of the cerebellum, hypothalamus and the thalamus are all also affected in HD (Heinsen

et al., 1999; Jeste *et al.*, 1984; Kremer *et al.*, 1991; Macdonald *et al.*, 1997; Macdonald & Halliday, 2002; Spargo *et al.*, 1993). At a microscopic level, cortical neurons show decreased staining of nerve fibres, neurofilaments, tubulin, and microtubule-associated protein 2 and diminished complexin 2 concentrations, suggesting impairment of synaptic function, cytoskeleton and axonal transport (DiProspero *et al.*, 2004; Modregger *et al.*, 2002). Nuclear, cytoplasmic and neuritic HTT inclusions are seen even in the premanifest stage of disease and are the pathological hallmark of HD. Ubiquitinated HTT aggregates are found throughout vulnerable and seemingly more preserved regions (DiFiglia *et al.*, 1997). Accordingly, a direct toxic effect is not supported by current research, instead it may represent the increasingly self-injurious attempt by the neurons to sequester toxic protein fragments and render them biologically inert that leads eventually to their downfall (Saudou *et al.*, 1998).

At 15 years prior to calculated disease onset, there is demonstrable striatal, thalamic and cortical white matter loss and whole brain atrophy on brain MRI (Paulsen *et al.*, 2010; Rosas *et al.*, 2005; Tabrizi *et al.*, 2011). In pathological studies, 80% of the human HD brains show atrophy of the frontal lobes, bilateral atrophy of the striatum in 95%, while non-striatal regions show atrophy of variable severity or have normal appearance (Halliday *et al.*, 1998; Vonsattel & DiFiglia, 1998).

The most well recognized neuropathological classification is the Vonsattel grade. There are five grades: grade 0, in which HD brains show no gross or generalised microscopic abnormalities consistent with HD, despite premortem symptomatology and positive family history, progressing to grade 4, which shows extreme atrophy (Vonsattel *et al.*, 1985).

Importantly, compensatory and pathological changes co-exist simultaneously in many different areas in the brain, indicating that there is process of dysfunction prior to degeneration, and raising the possibility that these tissues could be salvaged with an effective intervention.

5.3.3 Role of Huntingtin Protein

Huntingtin is a 348kDA protein, expressed at different levels depending on the tissue type and is present within the nucleus and cytoplasm. The normal functions of huntingtin are not yet fully known however, neural development, modification of neurotransmitter synthesis and transport as well as a role in the integrity and function of the cytoskeleton are proposed (Bates *et al.*, 2015). This latter role in organelle trafficking as well as in ciliogenesis and endocytosis, suggest a key role for Huntingtin in multiple cellular processes (Saudou & Humbert, 2016). However, while diminished huntingtin function may play a part in pathogenesis, Huntington's disease is primarily mediated by a toxic gain-of-function, through alteration of the conformational state of huntingtin and thus its subsequent interactions.

The human HD gene comprises 67 exons and encodes 3,144 amino acids, making up the wild type HTT protein and is expressed throughout the brain, in cytoplasm, nuclei, dendrites and axon terminals. The expanded polyglutamine (>36 repeats) mutant HTT is similarly distributed (Vonsattel *et al.*, 2008).

Proteolysis of the N-terminal fragment of mutant huntingtin may be important as a pathogenic mechanism as they are a major component of the hallmark nucleolar and cytoplasmic HTT aggregates found in pathological studies from animal and cellular models. However aggregates in themselves are not thought to be toxic and may in fact reflect a compensatory cellular mechanism to cordon off toxic fragments. Mutant HTT itself and these N-terminal fragments however have an ability to sequester other proteins including transcription factors, which may be a more valid pathological pathway.

Dysfunctional axonal trafficking is evidenced in transgenic mice and in mammalian neurones, where mutant HTT appears to restrict trafficking of vesicles and mitochondria (Ross & Tabrizi, 2011).

Homozygous HD patients, who by definition only produce mutant HTT, have similar phenotypes to their heterozygous counterparts, suggesting that gain of toxic function may be the predominating effect (Durr *et al.*, 1999).

5.3.4 Mitochondrial pathology in Huntington's disease

Mutant htt appears to be toxic to mitochondria in R6/2 mice (Beal & Ferrante, 2004). This is complemented by in vivo work using Magnetic Resonance spectroscopy in HD subjects, including pre-symptomatic, under maximal exercise, which reveals defective mitochondrial oxidative metabolism (Lodi et al., 2000). Multiple mitochondrial DNA deletions were found by polymerase chain reaction (PCR) analysis as well as variable deficits in complex I of the mitochondrial respiratory chain, in muscle of patients with HD (Arenas et al., 1998). Reflecting their early involvement in disease, myoblast cell cultures from presymptomatic and symptomatic HD subjects showed mitochondrial abnormalities, impaired cell differentiation and htt inclusions (Ciammola et al., 2006).

The transcription co-activator PGC-1 α plays a role in mitochondrial biogenesis and cellular metabolism. Mutant htt is proposed to inhibit expression of PGC-1 α through a promoter interaction and disruption of its transcriptional pathway. PGC-1 α is reduced exclusively in the medium spiny neurons which are preferentially affected in HD striatum (Cui et al., 2006), as well as muscle and adipose tissue of HD subjects (Chaturvedi et al., 2009;Phan et al., 2009). Its involvement across several tissues marks it out as a key target for further study.

In post-mortem studies, severe deficiencies in mitochondrial complex II and III and sometimes complex IV have been reported in the striatum (Bowling & Beal, 1995;Gu *et al.*, 1996). Mutant huntingtin may also impact on mitochondrial function through direct physical interaction.

5.3.5 Transcriptional dysfunction

The R6/2 mouse model has provided evidence of transcriptional dysregulation, early on in disease. Transcriptional down-regulation of mRNA levels of different genes of influential neuronal structures and proteins has been noted in humans and animal models. Indeed there exists a significant degree of consistency in profiles across these models, underscoring its importance pathologically. Mechanistically, nuclear translocation of toxic N-terminal fragments can affect various parts of the transcriptional process such as DNA, chromatin modification and interact negatively with

transcription factors, to cause transcriptional dysregulation (Kumar *et al.*, 2014;Luthi-Carter *et al.*, 2002;Luthi-Carter *et al.*, 2000).

A micro-array analysis of human HD brains versus unaffected controls reveal transcriptional changes in the caudate, motor cortex and cerebellum, including in genes governing cannabinoid CB1 receptor, dopamine D1 receptor and enkephalin (Hodges *et al.*, 2006).

5.3.6 Proteasomal and autophagic dysfunction

HD mouse model studies have shown that truncated N-terminal htt fragments with an expanded polyQ tract are susceptible to misfolding and aggregation and are more harmful. The degradation of these proteins therefore has received some focus from the research perspective. Nuclear HTT inclusions are also ubiquitin positive across cellular, animal models and human HD brains, suggesting involvement of the ubiquitin proteasome system (UPS) (DiFiglia *et al.*, 1997)

In various cellular and animal models of HD, as well as in the post-mortem brains of HD patients, nuclear polyQ inclusions are positively labelled by antibodies against ubiquitin (DiFiglia *et al.*, 1997;Gutekunst *et al.*, 1999). This colocalization led to subsequent studies of whether there is the sequestration of UPS components by polyQ inclusions. Indirect assays of UPS function through pharmacological inhibition or labelling, have found aggregate accumulation in cellular models, however using whole cell homogenates of HD mouse brains, reduced UPS activity was not detected (Bett *et al.*, 2006;Diaz-Hernandez *et al.*, 2003;Wang *et al.*, 2008).

Removal of misfolded proteins and cell organelles is a slower process, termed autophagy, and is solely cytoplasmic. Ubiquitin-proteasome system (UPS) and autophagic inhibitors directly injected into the striatum of *Hdh* (CAG)¹⁴⁰ knockin mice demonstrated that UPS is more important to clearance of soluble mutant HTT than autophagy (Li *et al.*, 2010).

Although there is not as compelling a role for UPS and autophagic dysfunction in HD pathogenesis as some of the other mechanisms described, it is known that both UPS & mitochondrial function

declines with age. Thus in combination with ageing, and other unrelated cellular stressors induced by the presence of mutant HTT, a gradual slowing and then failure of removal of misfolded HTT may occur in the latter stages of the HD pathogenic process. Indeed work in neuronal and mouse models has suggested that there is a preliminary stage of UPS derangement in the presence of HTT inclusions, followed by adaptation and restoration of function (Ortega *et al.*, 2010; Mitra *et al.*, 2009). A separate disturbance in the autophagy-lysosome pathway has been inferred from experimental work showing deficiency in axonal transport as well of the autophagic apparatus in the sequestration of substrates (Wong & Holzbaur, 2014; Ochaba *et al.*, 2014; Jimenez-Sanchez *et al.*, 2017).

5.3.7 Immunity and inflammation

There is clear evidence from a number of sources for a role for the immune system and inflammatory pathways in the kindling, maintenance and progression of HD.

Mitochondrial dysfunction which is supported itself by several lines of evidence in HD, may activate “*inflammasomes*” with lymphoblasts from homozygous HD patients show mitochondrial abnormalities at an ultrastructural level (Squitieri *et al.*, 2006). Chemotaxis or migration of immune cells may be impaired in HD leading to unchecked, chronically heightened levels. In a BAC HD mouse model, deficient migration of macrophages to an inflammatory stimulus that was mutant HTT dependent was demonstrated. Cell migration of monocytes from HD patients was also shown to be impaired (Kwan *et al.*, 2012b).

Microglia are primary mediators of neuroinflammation in the CNS and in HD, microglia have been shown to be stimulated many years before onset of symptoms and has also been shown in an R6/2 mouse model (Simmons *et al.*, 2007; Tai *et al.*, 2006).

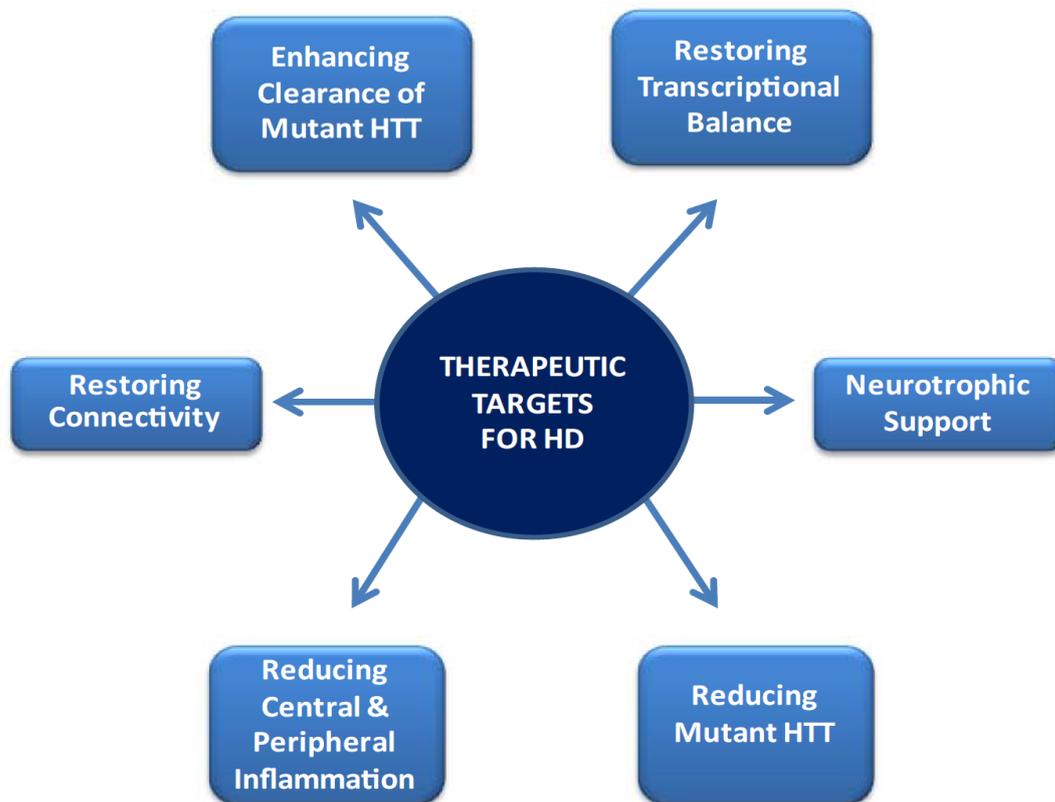
There is evidence of symmetry between the central and peripheral immune system in HD, with the finding of increased IL-6 and IL- 8 levels in plasma and striatum, mediated possibly by mutant HTT acting distinctly but similarly between compartments {Simmons, 2007 4837 /id; Bjorkqvist, 2008 102 /id}. R6/2 mice given an IL- 6 neutralizing antibody showed diminution in weight loss and improvement on rotarod (Bouchard *et al.*, 2012).

The role of the adaptive immune system, including dendritic cells, has only recently been explored. A study examining human HD T lymphocytes characteristics including subpopulations and cytokine output showed no functional differences in the presence of mutant HTT. This would suggest that alteration of *innate* immune system is the major contributor to the immune dysregulation seen in HD (Miller *et al.*, 2015).

5.4 Therapeutic Approaches in Huntington's Disease

5.4.1 Pathology to Therapy

Figure 2- Outline of potential therapeutic approaches in Huntington's disease



Based on the known pathogenesis of HD, several potential therapeutic approaches are possible (Figure 2).

5.4.2 Enhancing Clearance of Mutant Huntingtin

Conditional HD mouse models have supported the idea the continuous presence of mutant HTT is required to maintain toxicity and pathology. Consequently, HTT lowering strategies are an attractive therapeutic option (Regulier *et al.*, 2003; Yamamoto *et al.*, 2000). Huntingtin-lowering strategies include antisense oligonucleotides and RNA interference targeting mRNA, and zinc finger transcriptional repressors and CRISPR-Cas9 methods aiming to reduce transcription by targeting DNA.

5.4.2.1 Gene Silencing

Gene silencing or HTT lowering is arguably the most promising of all experimental therapies in Huntington's disease, given its monogenic and dominant inheritance. It offers the tantalising possibility to target the pathogenic gene directly and reduce HTT levels. There are two main candidates- RNA interference (RNAi), in which the silencing molecules are small interfering RNA (siRNA), and antisense oligonucleotides (ASOs).

In other genetic neurodegenerative disorders such as superoxide-dismutase 1 (SOD1) mutation-associated amyotrophic lateral sclerosis, ASOs therapy has been employed with no safety or tolerability issues reported (Miller *et al.*, 2013). In spinal muscular atrophy, nusinersen another ASO, is demonstrating slowing of disease progression in a previously untreatable neurodegenerative condition (Finkel *et al.*, 2016).

In rodent models, both approaches have demonstrated efficacy in lowering HTT protein, reducing pathology and improving phenotype via intracranial delivery (Carroll *et al.*, 2011; Franich *et al.*, 2008; Harper *et al.*, 2005). In primates, proof of concept studies have taken place with an RNAi based approach. ASO therapy infused via CSF into primate brain regions resulted in reasonable distribution including into the cortex without complications. HTT suppression continued for 3 months after the

therapy finished, which together suggests that intermittent HTT lowering may be an effective strategy (Kordasiewicz *et al.*, 2012).

Exosomes have emerged as another potentially exciting delivery method. They are endogenously occurring vesicles that transport RNAs and proteins and deliver to their intracellular targets. They have also been used to reduce expression of BACE1 gene in an Alzheimer's mouse model (Alvarez-Erviti *et al.*, 2011).

Other approaches to gene silencing include using mismatch-containing duplex RNA that targets mutant HTT. In vitro, this has been shown to alter mutant HTT production, and reduce generation of toxic HTT fragments (Neuroperspective, 2012).

Genomic editing via zinc-finger nuclease is a novel, potentially promising approach for Huntington's disease that has been successfully applied to treat liver cells from mice with haemophilia B (Li *et al.*, 2011).

Finally and most promisingly, in 2015-17 the IONIS-HTTRx trial was conducted with early HD patients receiving four lumbar intrathecal bolus doses of ASO or placebo, in a multiple escalating dose design with mutant CSF HTT levels as a potential therapeutic readout. An open-label extension study is planned and preliminary analysis of data would suggest that the drug is safe and well tolerated and does lead to reduction of mutant HTT levels in CSF (Ionis Pharmaceutical Press Release December 2017).

Trials for other ASOs are foreseen in the near future while viral vector based RNA interference therapies and zinc-finger transcriptional repressors are currently being investigated in animal experiments.

5.4.2.2 Other Approaches to Enhancing Clearance of Mutant Huntingtin

Studies have shown that acetylation of HTT has been shown to target the mutant protein for degradation. Acetylation at a single lysine (K444) facilitates trafficking of mutant HTT into autophagosomes and improves clearance of the mutant protein by macroautophagy (Jeong *et al.*, 2009).

Pharmacological activation of mTOR (mammalian target of rapamycin)-dependent autophagy with rapamycin, attenuated the toxic effects of mutant HTT in fly and mouse models of HD. Small-molecule enhancers of autophagy including mTOR-independent pathways such as autophagy inducers, are in preliminary development and being tested in cell models (Pan *et al.* 2009a). Inhibition of farnesyl transferase, a protein responsible for the farnesylation, or lipid modification, of a number of proteins has been touted as a possible means of influencing autophagy (Pan *et al.* 2009b). This is complicated by the fact that a knock-in mouse HD model suggests that HD-specific alterations in autophagy block the trafficking or degradation of HTT, necessitating a precise understanding and targeting of the autophagic process (Martinez-Vicente *et al.* 2010).

HD-derived lymphoblasts may provide a good cell model in which to study potential therapeutic compounds as autophagic deficits are established in this cell type. The important issues to address remain whether peripheral change can have central effect. Secondly, current approaches utilising peripheral inhibition of mTOR signalling have significant side-effects including ulcerative mucositis, anaemia, and neutropenia, which will need to be avoided, as new compounds develop (Mesa, 2006).

A screen of 253 approved drugs identified several candidates that appear to down-regulate huntingtin through autophagy, though this has not been definitely confirmed. They included L-type calcium channel blockers like nimodipine, the alpha-2 agonist clonidine, and the antihypertensive minoxidil (Williams *et al.*, 2008).

5.4.2.3 Genetic Modifiers and New therapeutic targets

About 50-70% of the variance in age of onset of HD symptoms is determined by HTT CAG repeat length with the remainder potentially also genetically based. A member of the DNA mismatch repair pathway, MSH3 has recently been identified via a genome wide association study as a novel genetic modifier of disease progression (Hensman Moss *et al.*, 2017a). Targeting this modifier directly or its downstream pathways therefore has the potential to significantly affect disease trajectory.

5.4.3 Restoring Connectivity

In HD mouse models, researchers have found that the levels of 3'-5'-cyclic adenosine mono phosphate (cAMP) in the striatum are lower than in normal mice. Mutant huntingtin disables cAMP signalling and transcription mediated by the cAMP response element-binding protein (CREB) (Gines *et al.*, 2003; Sugars *et al.*, 2004; Sugars & Rubinsztein, 2003). Enhancing synaptic function through preventing breakdown of cAMP and cyclic guanosine monophosphate (cGMP) is a potential mechanism being explored currently. However, Amaryliss, a phosphodiesterase (PDE) 10a inhibitor which improves striatal and cortical pathology in R6/2 HD mouse, did not meet its targets in the human trial (Pfizer, 2017).

A mGluR5 antagonist, had initially positive results in Fragile X but the final outcome was negative in HD (Neuroperspective, 2012). Targeting glutamate mediated excitotoxicity was the basis for a month long Novartis funded trial of a mGluR5 antagonist called AFQ056. The outcome measure was improvement of the chorea seen in HD, but it was negative and no further studies have been conducted (Reilmann *et al.*, 2015).

5.4.4 Restoring Healthy Gene Regulation

Mutant HTT mediated transcriptional dysregulation and reduced chromatin deacetylation has been well established as a pathogenic mechanism in HD (Cha, 2000; Benn *et al.*, 2008). Rolipram, a

PDE4 inhibitor, may be able to exert influence on transcriptional processes via modulation of cAMP response element binding protein (CREB) signalling with efficacy demonstrated in the R6/2 mouse model (DeMarch *et al.*, 2008;Giampa *et al.*, 2009).

Genetic and pharmacological reduction of Sir2, the orthologue of human SirT1, in drosophila, rescued neurodegeneration and promoted survival (Pallos *et al.*, 2008). The success of non-selective HDAC inhibitors such as suberoylanilide hydroxyamic acid (SAHA) in ameliorating HD phenotypes in the R6/2 mouse (Hockly *et al.*, 2003) prompted investigation of other individual HDACs, with class II HDAC-selective inhibitors in preclinical development. Trials of HDAC 4 inhibitors are also planned in future, after promising knockdown results in HD mice although the mechanism of action is not yet clear (Cronk *et al.*, 2012). Preclinical development and early phase results of a class III HDAC SirT1 inhibitor, Selisistat, are described in **Chapter 7**.

Other approaches include upregulation of SirT1. Genetic overexpression confers survival and neuropathological benefits as well as increased expression of brain-derived neurotrophic factor (BDNF) (Jeong *et al.*, 2012). In another separate study, genetic SirT1 overexpression improves motor function, reduces brain atrophy and attenuates metabolic abnormalities through maintaining BDNF concentrations and the signalling of its receptor, TrkB (Jiang *et al.*, 2012). However no highly selective, small molecule activators of SirT1 currently exist.

Transglutaminase inhibitors were suggested to have a role in preventing HTT aggregation. A transglutaminase inhibitor cystamine prolonged survival and improved motor features in an animal model of HD, though aggregation load was not altered (Bailey & Johnson, 2006;Dedeoglu *et al.*, 2002). Its effect may be explained by upregulation of neuroprotective genes, including perhaps BDNF, which has been seen in drosophila models (Borrell-Pages *et al.*, 2006). An aspartyl protease has a key role in the cleavage of HTT, raising the potential for the development of specifically targeted inhibitors (Neuroperspective, 2012).

5.4.5 Reducing Peripheral & Central Inflammation

Intrastriatal injection of quinolinic acid (QUIN), an NMDA receptor agonist, in rats was shown to recapitulate many features of HD (Schwarcz *et al.*, 1983). Glutamate receptor-mediated excitotoxicity and free radical formation have been correlated with decreased levels of the neuroprotective metabolite kynurenic acid in HD mouse models. Genetic deletion of kynurenine 3-monooxygenase (KMO) was found to suppress toxicity in a HD model (Giorgini *et al.*, 2005). KMO is largely expressed in microglial cells and not found in neurones (Giorgini *et al.*, 2008;Guillemin *et al.*, 2003), suggesting a pathological mechanism beyond that occurring within the neurone itself.

In the transgenic HD mouse model, a peripherally administered KMO3 inhibitor, prevents formation of the neurotoxic QUIN and ameliorates neurodegeneration (Zwilling *et al.*, 2011). The implied promise of this approach, which is yet to be validated in HD, is that by applying a compound peripherally, a meaningful central effect can be achieved.

Mutant HTT mediated innate immune dysregulation has been suggested as a modifier of disease (5.3.7). Transplantation of healthy wild-type bone marrow into HD mice restored cytokine reactivity to normal levels and led to synaptic plasticity and amelioration of phenotypic and neuropathological measures (Kwan *et al.*, 2012a).

Cannabinoid system is known to regulate inflammatory cascades. Cannabinoid (CB)2 receptor knockout HD mice have accelerated clinical pathology; treatment with peripherally administered CB2 agonists, dampens microglial activation and levels of circulating pro-inflammatory cytokines (Bouchard *et al.*, 2012;Palazuelos *et al.*, 2009;Sagredo *et al.*, 2009;Bouchard *et al.*, 2012).

Laquinimod reduces proinflammatory cytokine release in monocytes and acts to modulate astrocytic and microglial activity via NFκB signalling. Recent work in monocytes from premanifest and manifest HD patients and controls showed abrogation of the hyper-reactive cytokine release from all three groups with the manifest population impacted most strongly, and that this effect was NFκB independent (Dobson *et al.*, 2016).

5.4.6 Neurotrophic Support

The neurotrophic factor BDNF mediates neural development and neuroprotection, and is upregulated by normal huntingtin. A few studies have used adeno associated virus (AAV) to introduce BDNF expression into the subventricular zone, increasing striatal neuron survival in mouse models (Bemelmans *et al.*, 1999;Kells *et al.*, 2004).

Ampakines have been shown to upregulate the production of BDNF, which in turn leads to a proliferation of neural stem cells in the striatum. Reduced degeneration and improvements in cognitive function were seen in a HD transgenic mouse model (Simmons *et al.*, 2009). Research is being directed toward identifying TrkB receptor agonists to increase BDNF levels.

5.4.7 Cell Transplantation

Most cell-implant concepts for HD have focused on the striatum, and while this could be relevant to motor symptoms, the cognitive deterioration is unlikely to be affected. Surgically delivered striatal cell replacement therapy for HD has been attempted in small studies for over two decades, with the majority using human foetal tissue for cell harvesting. Although modest motor improvements have been seen, no sustained improvement has been achieved thus far. Importantly, deterioration of grafted cells was seen at autopsy in 3 subjects (Bachoud-Levi *et al.*, 2000;Bachoud-Levi *et al.*, 2006;Cicchetti *et al.*, 2009). Lack of integration with host neural architecture and early degeneration due to microglial activation are arguably the most significant difficulties to overcome. This approach appears to offer a temporary symptomatic benefit only. Autologous cell sourcing may be a viable alternative but needs to be developed.

5.4.8 Post-translational Modification

Post-translational modifications, such as phosphorylation, contribute significantly to the toxicity of mutant HTT. This is largely thought to be protective from the results of cell culture experiments (at

serine 13, 16 and 421) and thus therapeutic development would require targeting of the specific kinase to augment its function and repress the corresponding phosphatase activity (Zala *et al.*, 2008;Gu *et al.*, 2009;Warby *et al.*, 2005). By way of example, phosphorylation of serine 116 heightens mutant HTT toxicity (Watkin *et al.*, 2014), and thus targeting the responsible kinase would be a potentially promising strategy. HTT can undergo palmitoylation, specifically at cysteine 214, which enhances membrane association. Acetylation at lysine 444, augments its clearance (Jeong *et al.*, 2009). The N-terminus located 17 amino acids are highly disposed to modification, including phosphorylation, ubiquitination, and sumoylation (Ross *et al.*, 2017).

In murine models, intraventricular infusions of ganglioside GM-1, a natural component of 'lipid rafts' which serve a myriad of cell-signalling support roles in the brain, results in phosphorylation of mutant HTT, neutralising its toxic effects and improving motor function (Di *et al.*, 2012).

5.5 Clinical Trials Landscape in HD

5.5.1 Introduction

As an orphan disease, HD may not initially be perceived as financially attractive when it comes to outlay for drug development and clinical trials. However as a prototypical, neurodegenerative disease with a predictive, fully penetrant genetic test it does carry certain key advantages. Currently however, other than symptomatic treatment of chorea, there has been no disease modifying therapy established in human studies. While a lack of therapeutic compounds could be in part one explanation, the complexity and long-time course of the disease, necessitates development of valid biomarkers tested in well-designed and suitably powered clinical trials.

The traditional determinant of disease onset has been the development of motor features. Paradoxically the absence of a clearly successful intervention that leads to a change in the TMS *and* confers a functional benefit to the patient has also hamstrung research. The UHDRS assessments were designed to quantify functional deficits in multiple domains such as motor, cognitive, behavioural and psychiatric. They were not however designed with clinical trials in mind. Indeed early motor features can be underrepresented by the TMS and legitimate concerns over inter and

intra-rater reliability exist. Imaging, novel quantitative motor and neuropsychiatric and cognitive assessments at the perimanifest/prodromal phase, have the potential to supplant clinical assessments. Similarly however, these biomarkers must be held to same standard namely that improvement in their metrics mirrors a functional improvement or as a minimum, functional stability.

Broadly, clinical therapeutic approaches can be divided into 'Advanced therapies', with a regulatory definition of "new medical products based on genes (gene therapy), cells (cell therapy), and tissues (tissue engineering)—RNA-based therapies- interference RNA (RNAi), or antisense oligonucleotides (ASOs)"; new compounds; surgical strategies e.g. deep brain stimulation and finally 'Reinforcement or stimulation' therapies- repetition of a procedure e.g. aerobic exercise or cognitive training (Sampaio *et al.*, 2014).

5.5.2 Current Trials in HD

At the present moment, no drug has been shown to delay disease progression in HD. The vast majority of clinical trials to date in HD use drugs that were not developed specifically for the treatment of HD (**Table 3**).

Table 3- Currently active trials in Huntington's Disease (McColgan & Tabrizi, 2018)

Clinical trial
Open-label extension study of pridopidine (ACR16) in the symptomatic treatment of Huntington disease
Safety, tolerability, pharmacokinetics, and pharmacodynamics of IONIS-HTTRx in patients with early manifest Huntington's disease
Deep brain stimulation (DBS) of the globus pallidus (GP) in Huntington's disease (HD)
Resveratrol and Huntington disease
Exploring computerized cognitive training for people with Huntington's disease
Exercise effects in Huntington's disease
Beta in Huntington's disease (HD)
Feasibility of a video-oculography in patients with Huntington's disease VOG-HD study
A pilot evaluation of mindfulness-based cognitive therapy for people with Huntington's disease
A comparative phase 2 study assessing the efficacy of triheptanoin, an anaplerotic therapy in Huntington's disease
Follow-up measurement of brain PDE10A enzyme levels in Huntington's disease gene expansion carriers
A clinical study in subjects with Huntington's disease to assess the efficacy and safety of three oral doses of laquinimod
A study to evaluate sigma-1 and dopamine-2 receptor occupancy by pridopidine in the human brain of healthy volunteers and in patients with Huntington's disease
Tolerability, safety and activity of SRX246 in irritable subjects with Huntington's disease
Effect of tetrabenazine on Stroop interference in HD
Social cognition in Huntington's disease: cognitive study and functional and morphological imaging
Assessment of the psychological, cognitive and social resources of applicants for Huntington's disease and presymptomatic genetic testing
Alternatives for reducing chorea in HD
Brain stimulation in movement disorders
Validation and standardization of a battery evaluation of the socio-emotional functions in various neurological pathologies (GREFEX II)
Electronic-health application to measure outcomes remotely clinical trial
Laughter therapy effects on mood, stress and self-efficacy in people with neurological diseases
ExAblate transcranial MRgFUS for the management of treatment-refractory movement disorders

5.5.2.1 Cysteamine

Cysteamine increases brain-derived neurotrophic factor, a growth factor depleted in the brains of HD patients. The effect of cysteamine on motor progression in HD has been evaluated in a 3-year phase 2/3 trial. This revealed that it was safe and well tolerated but no statically significant change in motor progression was evidenced (Ross & Tabrizi, 2011).

5.5.2.2 PDE10A Inhibitors

Phosphodiesterase 10A (PDE10A) is found in the striatum and is reduced in HD patients many years before the onset of manifest disease (Niccolini *et al.*, 2015). In the Pfizer Amaryllis phase 2 trial, no change was seen in motor, cognitive or behavioural measures (Pfizer, 2017).

5.5.2.3 Pridopidine

Pridopidine, a dopamine modulator, has been studied in three separate large phase 3 trials, MermaiHD, HART and Pride-HD failed to meet their primary end-points (McColgan & Tabrizi, 2018). Pridopidine associates particularly with active D2

receptors and is thus viewed as a dopamine modulator, rather than a pure antagonist of the D2 dopamine receptor. In two large, phase III trials, although Pridopidine was safe and well tolerated it failed to meet the primary end-point. However, an improvement in the UHDRS total motor score (TMS) in two randomised controlled clinical trials at 90mg dosing led to a new phase II/III trial (PRIDEHD) to explore a new dose range but unfortunately this did not prove effective (de Yebenes *et al.*, 2011;The Huntington Study Group).

5.5.2.4 Deep Brain Stimulation

DBS implantation has been employed in Parkinson's disease, essential tremor, and dystonia. However, information regarding the potential use of DBS in HD is limited and its utility in managing chorea questionable, given that chorea usually declines with disease progression. Moreover, concerns over the potential negative cognitive impact of DBS must also be addressed. A small 12 week phase II trial is planned (Wojtecki *et al.*, 2016).

5.5.2.5 PBT2

PBT2 is a second-generation metal protein attenuating compound (MPAC) designed to restore copper and zinc ion homeostasis that can be disrupted in neurodegenerative diseases. The Reach2HD phase II trial was carried out in 2012/13, comparing a 6-month course of 100mg or 250 mg of PBT2 once-daily to placebo in 109 patients with mild to mid-stage HD. Compared with placebo, neither PBT2 100 mg nor PBT2 250 mg significantly improved cognitive scores, aside from the Trail Making Test Part B score in the PBT2 250 mg group. The authors concluded that PBT2 was generally safe and well tolerated but that a larger study is required to determine whether the cognitive improvement is a robust finding (Huntington Study Group, 2015).

5.6 Biomarkers in Huntington's Disease

Although primary clinical endpoints are readily apparent in some diseases, e.g. mortality and tumour size in cancer, for neurodegenerative diseases, especially Huntington's disease, such measures are not yet defined.

Indeed noting the chronic and degenerative nature of HD and related conditions, it is unrealistic for a given therapeutic intervention, to completely reverse the phenotype in manifest subjects; rather a slowing of disease progression may be a more reasonable outcome. Intervention in premanifest subjects may reasonably aspire to delay motor onset of the disease, the traditional determinant of the diagnosis of clinical HD.

Moreover given a mean life expectancy of 20 years from disease onset in HD, it is unlikely that outcomes used to determine the diagnosis of HD e.g. UHDRS motor or those that look at physical performance qualitatively, are sensitive enough to determine progression over the sort of time frames required for clinical trials.

Therefore in order to establish disease modification a biomarker or set of biomarkers is required that reliably marks disease progression over 6 months to 2 years. At present there are no biomarkers sensitive enough to detect disease progression over the sort of time frames that would be required for cost-effective clinical trials in premanifest HD. In early HD there are emerging measures over a 24 month period. It is likely that no single modality will be sufficient to assess the effect of a treatment and that a range of functional measures will be needed.

5.6.1 Observational Studies

Several large, multi-national, longitudinal, observational studies such as PREDICT-HD, TRACK-HD, COHORT, REGISTRY and ENROL-HD are examining clinical markers (Euro-HD Network REGISTRY Steering Committee, 2003;Paulsen *et al.*, 2008;Tabrizi *et al.*, 2009;, 2012).

5.6.1.1 REGISTRY & ENROLL-HD

The European HD Network's (EHDN) REGISTRY study is the largest study of HD to date and is an observational, prospective, multi-centre cohort study of HD which aims to establish a well-characterised European-based HD population to facilitate high-quality research. It collects phenotypical data and biospecimens from individuals who are at risk of, premanifest or manifest for HD, and includes age- and gender-matched control participants (Orth *et al.*, 2010). The Enroll-HD study will combine patients from COHORT and REGISTRY studies and include additional patients from Central and South America in the largest natural history study of HD (Enroll-HD, 2012).

5.6.1.2 PREDICT-HD

This study aims to identify biological predictors of disease onset and progression, in premanifest subjects. Psychomotor processing, emotion recognition, and working memory are found to be the most sensitive in distinguishing individuals according to time to predicted disease onset (Stout *et al.*, 2011).

5.6.1.3 TRACK-HD

TRACK-HD is a multi-site international study aimed at determining what assessments are suitable as outcome measures in clinical trials. Using a unique, novel objective quantitative motor assessment battery for finger tapping, tongue protrusion and postural control, one is able to differentiate patients according to disease stage (Tabrizi *et al.*, 2009). However, only the indirect circle tracing task changed significantly over 1 year in premanifest individuals. In the early HD group, the largest effect size was seen in the symbol digit modality test, a test of cognitive and motor processing (Tabrizi *et al.*, 2011).

Longitudinal imaging data has also been acquired and analysed as part of TRACK-HD where significantly greater progressive grey-matter, white-matter, whole-brain, and regional atrophy was seen in the premanifest and early HD groups, with the caudate

and white matter most affected. However, no significant functional changes over 24 months were seen in the premanifest group (Tabrizi *et al.*, 2012). Thus a series of validated assessments can be recommended as sensitive outcome measures, for use in disease modifying trials in early HD subjects.

5.6.1.4 PADDINGTON Imaging Biomarker Study

For the purposes of clinical trials, a crucial question is whether MRI brain and other assessments are sensitive enough to detect change over a 6 month interval. The PADDINGTON Imaging Biomarker Study seeks to answer this question. All subjects had a baseline visit and then at 6 months and 15 months. Subjects underwent clinical, neuropsychiatric, cognitive, quantitative motor and MRI assessments as well as donating fasting and non-fasting blood samples at each visit, for cholesterol metabolites, amino acid and immunity marker measurements. Longitudinal changes in macrostructural neuroimaging measures such as caudate atrophy and ventricular expansion were significantly larger in HD than controls, over the 6-month, 9-month and 15-month intervals, suggesting a potentially viable time-frame for clinical trials (Hobbs *et al.*, 2015).

5.6.2 Unified Huntington's Disease Rating Scale

The UHDRS is a comprehensive tool for the clinical assessment of HD patients developed first in 1996 and refined in 1999, comprising motor, behavioural, cognitive, functional and historical domains (Huntington Study Group, 1996;Huntington Study Group, 1999). It remains the most utilised assessment in observational and clinical trials. The Total Functional Capacity (TFC) subscale of the UHDRS represents the rate of functional decline in Huntington's disease patients but limited reliability and sensitivity of this informal 13-point scale may diminish its usefulness in clinical trials.

Progression of UHDRS item scores was assessed in a longitudinal study of 379 patients suffering from early HD, with linear progression found for all three functional measures and specific motor features including chorea, finger tapping and pronation/supination, gait, tongue protrusion and tandem

walking, suggesting a potential role for an abbreviated version (Meyer *et al.*, 2012). However, the time frames necessary in which to see a reliable change are not feasible for clinical trials which are usually designed with six month to one year efficacy intervals. Item scores may also be subject to bias and intra-rater and inter-rater variability (Henley *et al.*, 2005).

Moreover, no matter how rigorously clinical assessment is carried out, it represents the end-point of a series of pathological events. It can be influenced by other factors, some unrelated to the underlying pathology of the disease, such as medication, training and practice effects, as well as the general physical and psychological state of the patient.

5.6.3 Other Imaging Biomarkers

5.6.3.1 Functional MRI and Diffusion Tensor Imaging

In premanifest subjects with normal cognitive scores, fMRI revealed enhanced activation in selective cortical regions, which may be explained by a compensatory response to on-going primary striatal or localised cortical dysfunction (Paulsen, 2009). Alternatively, these unexpected activation patterns might be markers of neuronal dysfunction (Georgiou-Karistianis, 2009). However results have been inconsistent using this modality and though its potential to interrogate specific neural networks is clear, further technical advancements need to be made.

A longitudinal study of white matter microstructural change using Diffusion Tensor Imaging (DTI) demonstrated advancing but diffuse leukoencephalopathy, with some regions unchanged in the early HD stage (Gregory *et al.*, 2015a).

In another DTI study of premanifest and early HD subjects, significant correlations between depression scores and reduced fractional anisotropy (FA) in the splenium of the corpus callosum were noted. Those with prominent irritability showed more widespread loss of FA and were further from disease onset. This adds to the natural

history of the evolution of symptoms in HD, suggesting that white matter changes relating to both depression and irritability in HD occur at different times (Gregory *et al.*, 2015b).

5.6.3.2 Magnetic Resonance Spectroscopy (MRS)

Lower concentrations of putaminal N-acetyl aspartate were seen in HD subjects following an MRS study, suggestive of striatal pathology and confirming earlier studies (Sturrock *et al.*, 2010). MRS can also provide a means of assessing striatal glutamate and glutamine and therefore a way of modelling excitotoxicity in HD.

5.6.3.3 White Matter in Huntington's Disease

The white matter loss seen in HD occurs first within the corpus callosum, and in the posterior white matter tracts. The order in which these changes occur and why these white matter connections are specifically vulnerable is unclear. A diffusion tractography study in premanifest HD subjects, suggests that the length of white matter connections between a brain area and its neighbours predicts the rate of atrophy over 24 months (McColgan *et al.*, 2017).

Corticostriatal, interhemispheric, and intrahemispheric white matter connections at baseline and over 24 months in premanifest Huntington's disease are associated with gene expression profiles enriched for synaptic genes and metabolic genes, with the former carrying more genes associated with abnormal transcription in HD. This suggests that transcription dysregulation in synaptic genes rather than metabolic may be responsible for white matter loss seen in HD (McColgan *et al.*, 2018).

5.6.3.4 Positron Emission Tomography

PET has been employed to assess regional patterns of glucose uptake and dopaminergic signalling as potential disease markers in HD. Accordingly, striatal and cortical glucose reductions have been recorded. In patients with HD and in

premanifest gene carriers, longitudinal PET imaging revealed a rate of deteriorating striatal metabolism that seemed more aggressive in manifest than in premanifest individuals. Reduced D1 and D2 receptors, which are highly expressed in vulnerable medium spiny neurons, have been observed correlating with disease duration, cognitive impairment and motor deficits (Berent *et al.*, 1988; Ciarmiello *et al.*, 2006; Kuwert *et al.*, 1990; Young *et al.*, 1986).

The toxic effect of mutant huntingtin primarily in striatal medium spiny neurons, which highly express phosphodiesterase 10A (PDE10A) is well established. PDE10A could be a key therapeutic target in Huntington's disease. Combined positron emission tomography (PET) and multimodal magnetic resonance imaging was employed to determine PDE10A expression in vivo in a unique cohort of 12 early premanifest Huntington's disease gene carriers with a mean estimated 90% probability of 25 years before disease onset. PDE10A expression in early premanifest Huntington's disease was decreased in striatum and pallidum and increased in motor thalamic nuclei, compared to a group of matched healthy controls (Niccolini *et al.*, 2015).

5.6.4 24S-hydroxycholesterol

24S-hydroxy cholesterol (24OHC) is a product of brain oxidative cholesterol metabolism and is thought to be important for CNS development and function. Lowered concentrations of plasma 24OHC have been reported in patients and premanifest individuals, and they correlated with caudate atrophy and probability of onset of motor symptoms (Leoni *et al.*, 2008).

5.6.5 Novel Biomarkers

5.6.5.1 Genomic profiling

This approach has significant potential but a limited number of studies have been carried out, one of which found transcriptional alterations in HD participants, sensitive enough to distinguish between controls and premanifest individuals (Borovecki *et al.*, 2005).

5.6.5.2 Huntingtin Quantification

A novel soluble mutant *huntingtin* assay, using a technique known as Förster resonance energy transfer (FRET) has shown that mean mutant HTT levels in monocytes, T cells, and B cells differ significantly between patients, controls, pre-manifest mutation carriers and those at clinical onset. An association with disease burden scores and caudate atrophy rates in patients with HD was also seen (Weiss *et al.*, 2009). Development of HTT assays will allow quantification from a variety of sources (cerebrospinal fluid, blood and peripheral tissues) and will be important for mutant HTT reduction based therapies.

An ultrasensitive single-molecule counting (SMC) mutant Huntingtin (mHTT) immunoassay was used to quantify mHTT levels in CSF samples from individuals bearing the HD mutation and from control individuals. mHTT was undetectable in CSF from all controls but quantifiable in nearly all mutation carriers, which was 3-fold higher in manifest patients than premanifest subjects. mHTT levels also correlated with disease progression and were associated with 5-year onset probability (Wild *et al.*, 2015).

Quantification of huntingtin levels from peripherally, is evidently a much preferred option. Previously mutant HTT (mHTT) was significantly elevated in purified HD patient leukocytes compared with controls. Peripheral blood mononuclear cells (PBMCs) levels of mHTT were measured using a more efficient ELISA-based Meson Scale Discovery (MSD) electrochemiluminescence immunoassay platforms,

confirming previous findings that mHTT increases with advancing disease stage in patient PBMCs (Hensman Moss *et al.*, 2017b).

5.6.5.3 Neurofilament light chain

Neurofilament light (NfL) protein in blood plasma has been proposed as a biomarker of neurodegeneration in general and work recently in HD has found associations between increased NfL levels and reduced brain volume in cortical and subcortical grey matter and within white matter. Longitudinally, NfL predicted subsequent occipital grey matter atrophy and widespread white matter reduction (Johnson *et al.*, 2018) (Byrne *et al.*, 2017).

5.6.5.4 Immune Biology

The pivotal role of the immune system acting as a modifier of disease, has been demonstrated both centrally, mediated through activated microglia in early disease (Tai *et al.*, 2006), and by their monocyte counterparts peripherally, through the innate immune response. In premanifest and early Huntington's disease there is widespread evidence of innate immune activation detectable in plasma throughout the course of HD, starting before the onset of clinical symptoms (Dalrymple *et al.*, 2007). Elevated cytokine levels have been found in post-mortem brain and plasma samples of patients with HD. Concentrations of interleukin 6 were increased in premanifest subjects 16 years from predicted phenocconversion, the earliest biochemical abnormality recognised in gene carriers (Bjorkqvist *et al.*, 2008).

5.6.5.5 Optical Coherence Tomography

Optical coherence tomography (OCT) is a non-ionising imaging modality of the retinal nerve fibre layer (RNFL) at micrometre resolution, with rapid, consistent data acquisition. The speed of sampling, in the order of a few minutes, confers a considerable advantage over MR scanning. It has been employed in clinical use in monitoring of glaucoma, retinal diseases as well as in research studies looking at optic neuritis. This is explored in **Chapter 11**.

6 Aims of Thesis

1. To determine safety and tolerability of selisistat in early Phase trials in Huntington's disease.
2. To determine if the disordered innate immune system in Huntington's disease is a target for selisistat.
3. To determine if a useful motor phenotype segregation method can be developed in Huntington's disease.
4. To utilise the segregation method to see if regional atrophy may underlie the differences in clinical phenotype.
5. To determine the viability of optical coherence tomography as a biomarker in Huntington's disease.

7 Phase 1B Study

7.1 Sirtuins

7.1.1 Selisistat- Biology

SEN0014196 (6-chloro-2, 3, 4, 9-tetrahydro-1*H*-carbazole-1-carboxamide), or selisistat, functions as a sirtuin inhibitor, deacetylating lysine residues. It is a highly selective inhibitor of the silencing information regulator T1 (SirT1), 200 fold greater than SirT2 or SirT3 and likely functions as a nicotinamide mimetic. It is classed as a histone deacetylator (HDAC).

7.1.2 Histone Deacetylators

Table 4- HDAC Orthologs in Yeast, Worm, Fly. Adapted from (Pallos *et al.*, 2008)

		<i>Homo sapiens</i>	<i>Drosophila melanogaster</i>	<i>Caenorhabditis elegans</i>	<i>Saccharomyces cerevisiae</i>
Class I		HDAC1	Rpd3	hda-1, hda-3	Rpd3, Hos1, Hos2
		HDAC2			
		HDAC3	HDAC3	hda-2	
		HDAC8			
Class II	a	HDAC4	HDAC4	hda-4	Hda1, Hos3
		HDAC5			
	HDAC7				
	HDAC9				
b	HDAC6	HDAC6	hda-6		
	HDAC10		hda-5, hda-10		
Class IV		HDAC11	hda-11		
Class III	Ia	SIRT1	Sir2	sir2.1	Sir2 Hst1
	Ib	SIRT2 SIRT3	Sirt2		Hst2
	Ic				Hst3 Hst4
	II	SIRT4	Sirt4	sir2.2 sir2.3	
	III	SIRT5			
	IVa	SIRT6	Sirt6	sir2.4	
	IVb	SIRT7	Sirt7		

Regulation of transcription is controlled by a number of factors, one of which is alteration of the accessibility to DNA. Mechanistically this is achieved through post-translational modification to proteins, including histones, which, through their intimate association with chromatin, control access to DNA. Acetylated histones correspond with gene activity, while deacetylated histones reduce transcription (Kouzarides, 2007). HDACs are highly conserved across species and can be subdivided into the zinc-dependent or classic HDACs and the NAD⁺-dependent Sir2-like group of Sirtuins (class III HDACs) (Table 4).

However, it is clear also that HDACs have targets beyond the histones themselves, including transcription factors and have been implicated in potential roles in ageing, metabolism and lifespan (Finkel *et al.*, 2009).

7.1.3 Sirtuins

Sirtuins are class III, nicotinamide adenine dinucleotide (NAD) dependent HDACs, possessing a conserved catalytic core domain comprised of approximately 275 amino acids. They regulate transcription, cell survival and metabolism.

There are seven human Sirtuins (SirT1–7) and they are differentiated in part by their cellular localisation. SirT1 is located in the nucleus and cytoplasm, SirT6 and SirT7 are found in the nucleus and SirT3-5 within mitochondria (Frye, 2000). With the exception of SirT4 they all possess a deacetylase activity. The coupling of their deacetylase activity to availability of NAD⁺ has led to the suggestion that they may act as metabolically gated sensors of the cell.

7.1.3.1 SirT1

Amongst the seven identified mammalian homologues, SirT1 is believed to most closely resemble yeast Sir2 (silent information regulatory 2). Indeed Sirtuins were first characterised as orthologues of the yeast Sir2 protein, where increased activity of the protein lead to an extended replicative life span in yeast, *C.elegans* and *D.melanogaster* (Kaeberlein *et al.*, 1999; Rogina & Helfand, 2004; Tissenbaum & Guarente, 2001). Conversely, experimental lowering of Sir2 activity in the context of malnutrition increased chronological life-span (Fabrizio *et al.*, 2005).

There is evidence that SirT1 modulates transcription factors such as PGC-1 α , p53 and FOXO 3a (Morris, 2005; Luo *et al.*, 2001; Rodgers *et al.*, 2005). Differences in tissue specific expression are also noted, with SirT1 found in neurones, heart, liver, kidney, blood and spleen (Gao *et al.*, 2012). SirT1 itself has a molecular mass of 81.7kDA and is predominantly found in the nucleus in association with euchromatin but has also been identified in the cytoplasm (Tanno *et al.*, 2007).

7.1.3.2 SirT1 in Health and Disease

SirT1 appears to have important roles in development, DNA repair, cell fate, metabolism and ageing. SirT1 complete knockout mice around birth and suffer with retinal, bone and cardiac defects (Finkel *et al.*, 2009). There is evidence that SirT1 has a role in both pro- and anti-apoptotic processes from a variety of cellular experiments (Cheng *et al.*, 2003; Brunet *et al.*, 2004). SirT1 may have role in mediating vascular tone and vascular disease via augmentation of endothelial nitric oxide through deacetylation of its calmodulin-binding domain (Mattagajasingh *et al.*, 2007). SirT1 may regulate PGC1 α , which is involved in glucose metabolism, lipolysis and mitochondrial biogenesis (Escande *et al.*, 2010; Milne *et al.*, 2007). Expression of SirT1 is reduced with correspondingly increased levels of NF κ B activity, in lungs of smokers and sufferers of chronic obstructive pulmonary disease (COPD) (Rajendrasozhan *et al.*, 2008).

7.1.4 SirT1 and Neurodegenerative Disease

In neuronal tissues a role for SirT1 is implicated in differentiation, abrogating neurodegeneration, with pharmacological and genetic reduction of SirT1 protective in Alzheimer's disease (AD) and amyotrophic lateral sclerosis models (Kim *et al.*, 2007).

Activation of SirT1 via calorie restriction has been shown to decrease the number of amyloid plaques in a mouse model of AD (Qin *et al.*, 2006) while in vitro NF κ B inhibition via SirT1 abrogates amyloid-beta A β toxicity (Chen *et al.*, 2005).

In a mouse AD model, pharmacological enhancement of SirT1 by resveratrol improved functional outcomes and deacetylated p53 and PGC1 α (Kim *et al.*, 2007). SirT1 has also been shown to deacetylate tau and thus aid in its removal, which can improve cognitive function and neuronal loss in mice (Min *et al.*, 2010). However SirT1 inhibition has also been shown to be neuroprotective in AD (Li *et al.*, 2008). In Parkinson's disease, SirT1 overexpression in cellular and animal models reduced formation of α -synuclein aggregates (Donmez *et al.*, 2012).

7.1.5 SirT1 and Huntington's Disease

In a *Caenorhabditis elegans* neuronal model, SirT1 upregulation and augmentation by resveratrol treatment was neuroprotective in the presence of mutant HTT (Parker *et al.*, 2005). Utilising the same compound in the N171-82Q transgenic HD mouse model, no effect was seen on weight loss, motor function or striatal atrophy, though interestingly peripheral markers in adipose tissue and blood glucose levels did show improvement (Ho *et al.*, 2010).

In the HD R6/2 mouse model crossed with a brain-specific SirT1 knockout (BSKO) or knockin (SirT1-KI), the former was associated with a more severe neurodegenerative phenotype, the latter, with an improvement in pathology, lifespan and BDNF levels. Mechanistically, the presence of CREB-regulated transcription co-activator 1 (TORC1) was shown to be required for BDNF expression (Jeong *et al.*, 2012).

Using different mouse models, bacterial artificial chromosome (BAC-HD), a full length HTT model, and a N171-82Q model, overexpression of SirT1 was found to be beneficial in terms of neuropathology and physical attributes, with its deacetylase function key to its function. Interaction with Foxo3a, with improvement in BDNF levels, was suggested as potential mechanism for this effect. Importantly, inhibition of SirT1 deacetylase activity by mutant HTT was also evidenced, suggesting this as a potential pathological step that could be targeted therapeutically (Jiang *et al.*, 2012).

7.1.6 Other HDACs & Huntington's Disease

Expression of mutant HTT exon 1 in PC12 cells results in a global hypoacetylation of histones, which is abrogated by the HDAC inhibitors sodium butyrate, trichostatin A (TSA) and suberoylanilide hydroxamic acid (SAHA). In *Drosophila* bearing Httex1p 93Q that are fed SAHA or sodium butyrate, or subject to genetic reduction of HDAC activity, increased survival and reduced rhabdomere degeneration is seen (Steffan *et al.*, 2001).

In R6/2 mice, sodium butyrate improved longevity while both sodium butyrate and SAHA improved rotarod performance, neuropathology and reversed global hypoacetylation of histones (Ferrante *et al.*, 2003; Hockly *et al.*, 2003).

In HD-N171-82Q mice given phenylbutyrate at 75 days of age, lifespan extension and decreased striatal atrophy and ventricular enlargement was noted, however no effect on motor performance, weight loss or HTT aggregate formation was seen (Gardian *et al.*, 2005).

Treatment with class I histone deacetylase inhibitor prevented initiation of the kynurenine pathway in microglia expressing a mutant huntingtin fragment in a yeast HD model. Application of SAHA blocks production of neurotoxic kynurenine pathway metabolites in microglia of HD mice (Giorgini *et al.*, 2008). Other work has intriguingly suggested that certain HDACs are novel promoting factors for tri-nucleotide repeat expansion in budding yeast and human cells (Debacker *et al.*, 2012).

7.1.7 Histone Deacetylators as Therapeutic Targets

Although several HDAC inhibitors (HDACi) are in trials, largely as anti-cancer therapies, vorinostat (SAHA) is one of only a few used in current clinical practice, in the treatment refractory cutaneous T cell lymphoma. Sodium valproate which has been used for many users as an anti-epileptic drug and mood stabiliser has weak, broad spectrum HDACi activity.

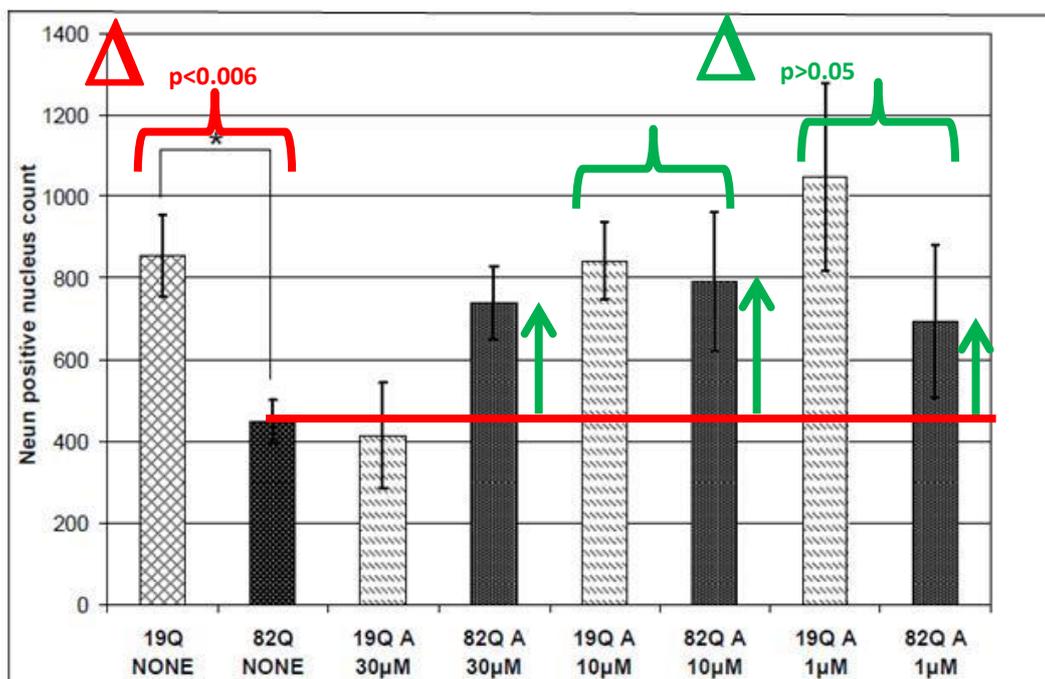
7.2 Selisistat Pre-clinical Data

(Unless stated, all data presented here is confidential data reproduced with kind permission of Siena Biotech SpA.)

7.2.1 Cellular Models- PC12 & Primary Rat Striatal Neurones

Selisistat is neuroprotective in a transfected inducible mutant HTT PC-12 and primary rat striatal neurone cell line (**Figure 3**) (Smith *et al.*, 2014).

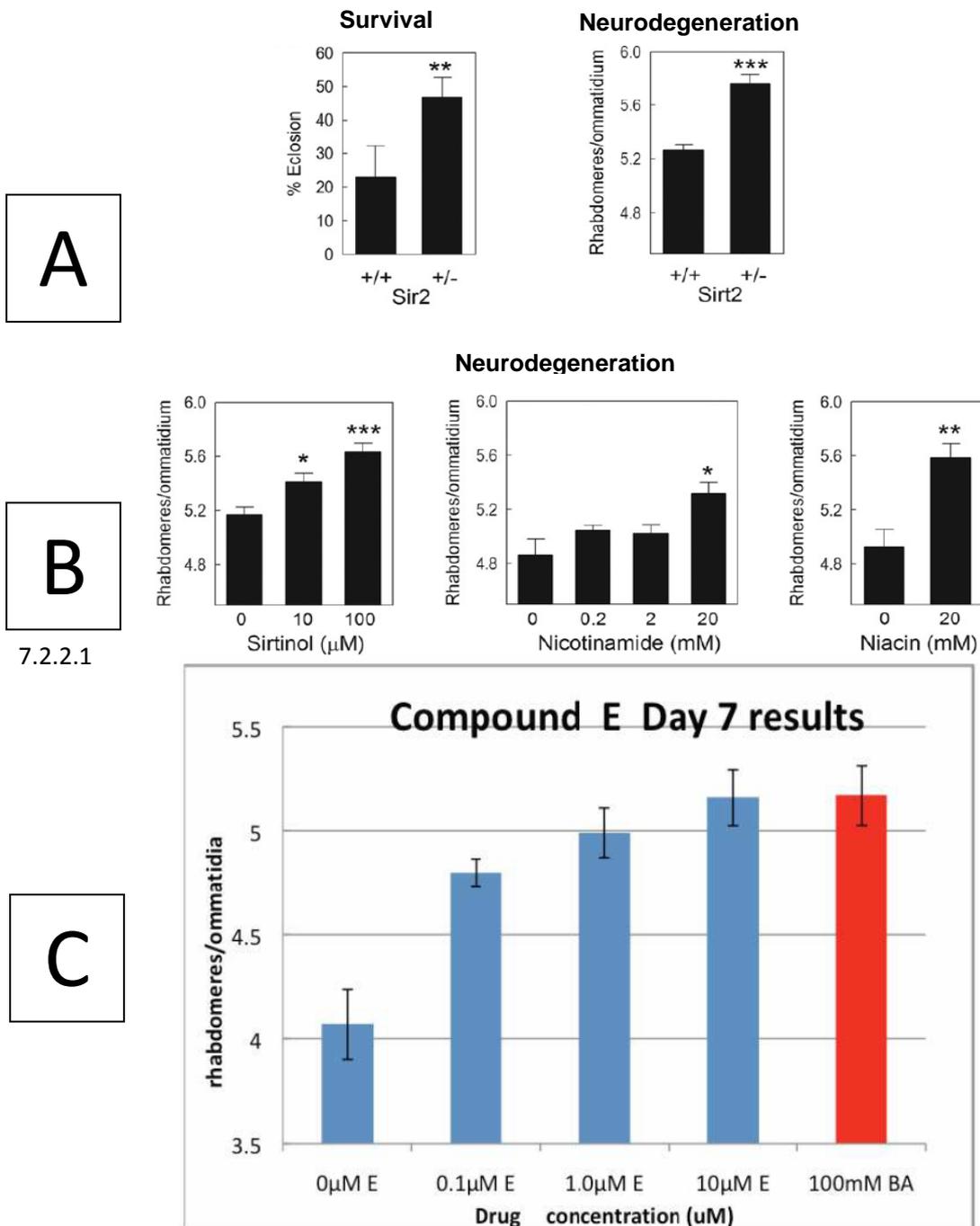
Figure 3- Neuroprotective effects of selisistat in rat striatal neurons challenged with mutant Htt (N171-Q82) or wtHtt (N171-Q19), through lentiviral delivery. Neuronal numbers (NeuN count) are significantly decreased in cultures challenged with mutant Htt (Smith *et al.*, 2014)



7.2.2 Drosophila

In a drosophila model expressing mutant HTT exon 1, Sir2 (SirT1 mammalian orthologue) levels were manipulated, genetically by knockdown and pharmacologically, using inhibitors, with growth, survival and neurodegeneration as outcomes measures (**Figure 4**).

Figure 4- (Pallos et al. 2008 (A & B) & (Smith et al. 2014) (C)) Reduced sirtuin levels are neuroprotective in a drosophila HD model: A- 50% reduction of Sir2 (i.e. heterozygous null) leads to greater survival to adulthood, B- an increased number of photoreceptor neurons of Httex1p Q93-expressing flies and C- selisistat (Compound E) phenocopies Sir2 haploinsufficiency and pharmacological inhibitors used in A.



A neurodegeneration and survival benefit is shown with reduced dose genetically and pharmacologically in a HD drosophila. In the presence of the compound (**Figure 4 Panel C**) the neurodegeneration is abrogated in a similar manner to the above. Overexpression however does not offer any benefit, while complete Sir2 knockouts show more neuronal loss and loss of climbing ability, suggesting a haploinsufficiency or reduced dose effect of Sir2 is beneficial.

7.2.3 R6/2 Mouse Data

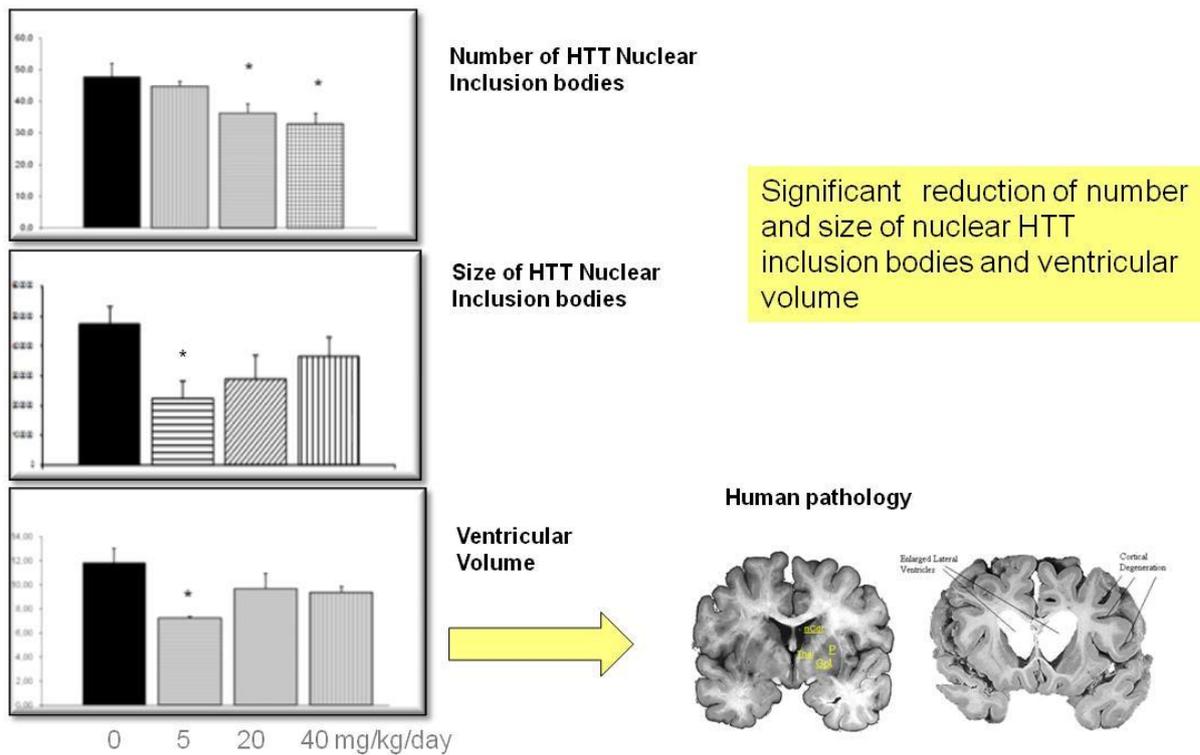


Figure 5- (Smith et al. 2014) A therapeutic study in the R6/2 HD mouse model demonstrated amelioration of pathology- number and size of intranuclear inclusion bodies and ventricular volume.

At differing doses (5, 20 and 40mg/kg), selisistat reduces neuropathology, improves survival, body weight and motor characteristics in R6/2 mice (Figure 5 & Table 5).

Table 5- Heat map showing effects of chronic administration of compounds A and B (Selisistat) on basic physiology tests and tests of motor function performed at PsychoGenics. Probability values above 0.3 are not shown. Trends ($p < 0.3$) are indicated by light pink and blue, significant effects ($p < 0.05$) by darker pink and blue – indicates no effect. Compound A: unrelated compound tested in the same model at the same facility.

		Compound A			Compound B (SEN196)		
		3 mg/Kg	10 mg/Kg	30 mg/Kg	5 mg/Kg	20 mg/Kg	40 mg/Kg
Open Field	Total distance	-			<0.05	-	-
	Distance centerr	-			<0.05	-	
	Rearing Rate (center)	-	-	-	-	-	-
	Total rearing frequency	-	-	-	-	-	-
	Velocity	-	-	-		<0.05	<0.05
Rotarod	-	-			-	<0.05	
Body Weight	-	<0.05	<0.05	<0.05		-	
Grip Strength	-	-	-	-	-	-	
Survival	-	-	-	-	<0.05		

7.2.4 Proposed Mechanism of Action

Dysfunctional protein acetylation is known to be an important factor in HD and the restoration of normal protein acetylation levels is thought to be a potentially efficacious therapeutic approach, as demonstrated by the significant interest in histone deacetylase (HDAC) inhibitors in the HD field (Butler & Bates, 2006).

There is evidence of substantial transcriptional dysregulation and widespread histone de-acetylation in HD (Benn *et al.*, 2008; Cha, 2000). Other work has shown transcriptional dysregulation in caudate samples and whole blood from Huntington's disease patients, occurring in a similar pattern centrally as peripherally (Anderson *et al.*, 2008).

Modulation of acetylation levels through the use of broad-spectrum HDAC inhibitors has been shown to confer benefit in both cellular and animal models of HD, demonstrating at least partial reversal of toxicity, motor and body weight phenotypes, as well as reversal of transcriptional dysregulation (Thomas *et al.*, 2008).

Post-translation modifications of HTT can influence its behaviour, with acetylation at lysine 444 known to activate autophagic removal of the protein (Jeong *et al.*, 2009). In a cellular model, application of selisistat impaired deacetylation at K444 and facilitated macroautophagic clearance of mutant HTT. However, many toxic HTT species do not contain lysine residues and therefore selisistat may be acting therapeutically through alternate pathways (confidential unpublished data from Siena Biotech SpA and Krainc laboratories, MassGeneral Institute for Neurodegenerative Disease, Boston).

Therefore two mechanisms of action are proposed for selisistat:

- 1. Restoration of transcriptional dysregulation seen in HD.**
- 2. Selective reduction in levels of mutant HTT without disturbing levels of wild-type HTT.**

7.3 Selisistat- First in Human Data

A randomised, double-blind, placebo-controlled study involving healthy volunteers was carried out with the aim of establishing the safety, tolerability and pharmacokinetics of selisistat. At single doses of up to 600mg, selisistat was safe and well tolerated in healthy male subjects and by healthy female subjects at a dose of 300 mg. Multiple doses of up to 300 mg for 7 days in male subjects, and 100 mg for 7 days in female subjects, were safe and well tolerated.

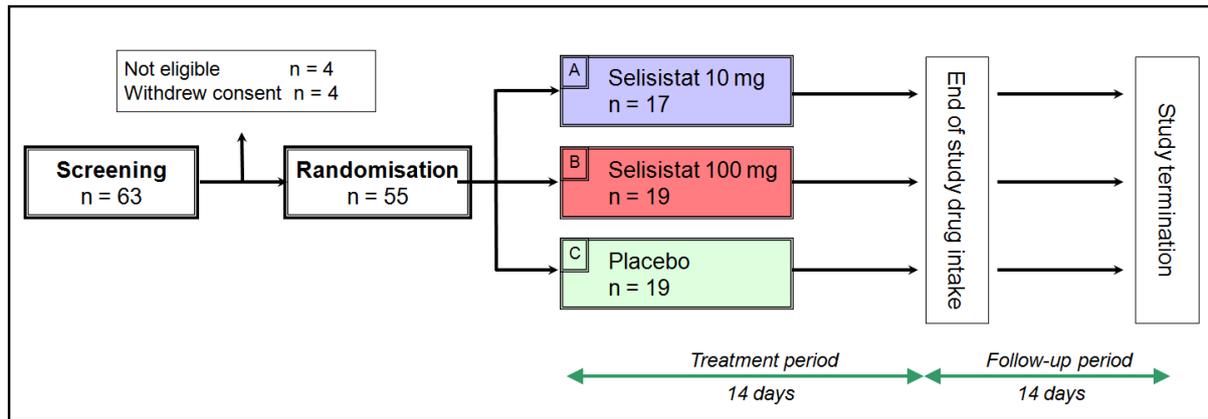
Most adverse events (AEs) were mild in severity and resolved without need for therapy. There was one severe AE with a male subject experiencing postural syncope. No serious adverse events (SAE) occurred during the study and no withdrawals as a result of AEs.

No abnormalities in haematological, liver function and other clinical laboratory evaluations, as well as vital signs, or electrocardiogram (ECG) were seen. There was no evidence that selisistat affects cardiac repolarisation and no evidence of prolongation of the QTc interval was observed at any dose level. There were no clinically significant findings in physical examinations, postural control, or neurological examinations (Westerberg *et al.*, 2015).

7.4 Introduction- A Phase 1B Study (Sussmuth *et al.*, 2014)

A multi-centre, randomised, double-blind, placebo-controlled, parallel group study at 3 dose levels (10 mg, 100 mg and placebo) was conducted. It included 6 visits over a 4-5 week period including a two week treatment period and two week washout (**Figure 6**).

Figure 6- Schematic diagram of design of Phase 1B Study



Patients were required to reside in an in-patient facility from Day -1 (baseline) (the day prior to start of dosing) to the morning of Day 2 (24 hours post-dose) and for 24 hours starting in the morning of Day 14. Patients returned for a visit to the clinic on Day 7 (+/- 1 day). All participants returned for a post-study visit 14 days after their final dose (\pm 3 days).

The objectives of the study:

1. Obtain biosamples to determine effect of selisistat on pharmacodynamic parameters:

- Acetylation status of mutant HTT
- Levels of soluble HTT
- Transcriptional signatures
- Modulation of innate immune markers
- Modulation of BDNF mRNA levels

2. Pharmacokinetic data in HD subjects.

3. Assessment of acute phenotypical effects of selisistat in HD patients.

4. Safety and tolerability of selisistat in HD patients.

7.5 Methods (Sussmuth *et al.*, 2014)

Reproduced from methods as in clinical study protocol and (Sussmuth et al., 2014).

The Study were carried out following the Helsinki protocol (Ethical Principles for Medical Research Involving Human Subjects), with local research ethics committee approval and clinical research organisation supervision including site initiation, monitoring and final visits. All study members were trained in good clinical practice.

7.5.1 Study Population

The study comprised genetically confirmed early stage (I and II Shoulson and Fahn (Shoulson & Fahn, 1979)) HD subjects, aged 18 to 70 years.

7.5.2 Inclusion Criteria

- Patients with early Huntington's disease (age: 18 to 70 years), i.e. genetically confirmed (CAG repeat length ≥ 36) HD, motor signs of HD (motor score of the UHDRS > 5) and a TFC of ≥ 7 .
- All patients will have a body weight greater than 50 kg.
- Female subjects must be surgically sterile or post-menopausal (defined as at least two years post cessation of menses and/or follicular stimulating hormone >18 mIU/mL and serum oestradiol <110 pmol/L), no spontaneous menstruation for at least one year before the first dose, non-lactating and have a negative serum pregnancy test. (Note: For post-menopausal females treated with oestrogen replacement therapy, FSH levels are artificially lowered <40 IU/L. Oestradiol and LH measurements need to be performed to confirm reason for low FSH value. HRT is acceptable, provided the above criteria are respected.)
- Male subjects participating in the trial and their female partners must agree to use a highly effective method of contraception from the time of taking the first dose of the study drug until three months after taking the last dose. This must include a condom or other barrier method (i.e. a diaphragm or cervical/vault cap), with spermicidal cream/gel plus one extra method (i.e. established use of oral, injected or implanted hormonal contraception or IUD or Coil).

- If the female partner is already pregnant, use of condoms is mandatory during sexual intercourse from the time of taking the first dose of Study drug until three months after taking the last dose of study drug.
- All subjects must be capable of providing written informed consent.
- Subjects must have no clinically significant and relevant history that could affect the conduct of the study and evaluation of the data, as ascertained by the Investigator through detailed medical history and screening assessments.

7.5.3 Exclusion Criteria

- Participation in a study of an investigational drug within 30 days of the baseline visit.
- Subjects with presence of psychosis and/or confusional states.
- Subjects with clinically significant laboratory or ECG abnormalities at Screening.
- Subjects with clinically relevant haematological, hepatic, cardiac or renal disease.
- A medical history of infection with human immunodeficiency virus, hepatitis C and/or hepatitis B.
- Any relevant condition, behaviour, laboratory value or concomitant medication which, in the opinion of the Investigator, makes the subject unsuitable for entry into the study.
- Subjects who have previously received histone deacetylase inhibitors e.g. vorinostat or have participated in a clinical trial using a histone deacetylase inhibitor.
- A history of malignancy of any type within 2 years prior to screening.
- A history of surgically excised non-melanoma skin cancers is permitted.
- Subjects with a significant history of drug allergy as determined by the Investigator.
- Subjects who consume more than 28 units (males) or more than 21 units (females) of alcohol per week or who have a significant history of alcoholism or drug/chemical abuse as determined by the Investigator (one unit of alcohol equals 285 mL of beer or lager, one glass [125 mL] of wine, or 25 mL of spirits).

7.5.4 Assessments

1. Motor Assessment using Unified Huntington's Disease Rating Scale (UHDRS)

2. Functional and Quality of Life (QoL) Assessments

- UHDRS Total Functional Capacity (TFC)
- UHDRS Functional assessment (FA) (including Independence Scale)
- Pittsburgh Sleep Quality Index (PSQI)

3. Behavioural Assessments

- European Huntington's Disease Network (EHDN) Problem Behavioural Assessment
 - Short Version (PBA-S)
- Hospital Anxiety and Depression Scale (HADS) with Snaith Irritability Scale (SIS)
- Frontal Systems Behaviour Inventory-Self Rating Test Booklet (FrSBe-self)

Optional

- Frontal Systems Behaviour Inventory-Family Rating Test Booklet (FrSBe-other)

4. Cognitive Assessments

- Trails A
- Trails B
- Symbol Digit Modalities Test (SDMT)
- Stroop Word Test
- Verbal fluency

- Mini Mental State Examination (MMSE)

5. Quantitative Motor Assessment (optional according to availability at the study centre)

- Brainstem motor coordination test
- Upper extremity motor coordination test
- Upper extremity grip force test
- Bradykinesia test
- Neurophysiological chorea analysis

6. Safety Assessments

- Adverse Events (AEs)
- Clinical laboratory tests (haematology, serum chemistry, and urinalysis)
- Electrocardiogram (ECG)
- Vital signs
- Physical examination

7. Pharmacokinetic (PK) Assessments

8. Pharmacodynamic (PD) and Pharmacogenomic Assessments

- Samples for Genotyping
- Samples for Assessing Levels of Soluble Huntingtin
- Samples for Assessing Huntingtin Acetylation Status by enzyme-linked immunosorbent assay (ELISA)

- Samples for Assessing Huntingtin Acetylation Status by liquid chromatography–mass spectrometry (LC-MS/MS)
- Samples for Transcriptional Profiling
- Samples for Markers of Innate Immunity

7.6 Phase 1B- Results

Data presented here is with the kind permission of the study sponsor Siena Biotech SpA. It is the complete data set for all subjects, including 7 London subjects. For the London subjects, I was the site clinical lead at UCLH, carried out all clinical histories, physical examinations, UHDRS, ECG interpretation, TFC & Independence scale Scores, HADS-SIS assessments, cannulation and blood tests. Clinical observations (blood pressure, pulse, temperature, respiratory rate and oxygen saturation levels and blood tests) were carried out by clinical trials nurses at Clinical Research Facility (CRF), University College London Hospital (UCLH) and cognitive assessments by Dr Elin Rees and Dr Gail Owen. Processing of London biosamples was carried out by Dr Ralph Andre and trials nurses at the CRF, UCLH.

7.6.1 Statistical Analysis

Descriptive data analysis was performed by myself from raw data kindly provided by the study sponsor, unless otherwise stated. For the study, Quartesian Clinical Research, US, using SAS Version 9.1.3 (or higher) were employed by the sponsor Siena Biotech SpA in a pre-existing arrangement using descriptive statistics for continuous variables.

7.6.2 Demographics

Table 6- Demographics of Phase 1B Study population (Sussmuth *et al.*, 2014)

Parameter	Selisistat 10 mg (N=17)	Selisistat 100 mg (N=19)	Placebo (N=19)	All Patients (N=55)
Age	46.5 ± 10.2 [33-67]	54.1 ± 10.1 [28-68]	51.9 ± 8.2 [38-64]	51.6 ± 9.8 [28-68]
Gender	M=14, F=3	M=13, F=6	M=12, F=7	M=29, F=16
Age of onset	44.5 ± 10.11	47.6 ± 8.58	46.1 ± 8.83	46.1 ± 9.08

	[28-62]	[28-63]	[28-61]	[28-63]
CAG Repeats	44.1 ± 3.58 [40-53]	42.8 ± 2.48 [40-49]	43.4 ± 2.14 [41-50]	43.4 ± 2.76 [40-53]

Age, age of onset and CAG repeat ranges between the dosing arms were relatively well matched following randomisation. A greater proportion of male subjects were noted most likely due to recruitment bias as a consequence of ineligibility of females of child-bearing age. Enrolled subjects were in good health without significant medical history, examination or laboratory findings. Mean values for vital sign measurements at screening and the day before dosing (baseline) were similar for all treatment groups (**Table 6**).

7.6.3 Study Withdrawals and Completion

There were four screen failures and four consent withdrawals before randomisation. All fifty-five participants completed the study and were included in the safety as well as pharmacokinetic analyses. One patient was excluded from the UHDRS outcome measures due to highly variable clinical presentation before, during and after the study independently from the study drug intake.

7.6.4 Total Motor Score (TMS)

The UHDRS '99 Motor score is a clinical assessment widely employed by HD clinicians to quantify to motor burden of disease (**Appendix 13.1**).

Figure 7- Mean TMS Score by timepoint & treatment arm with no clinically significant difference seen in TMS between timepoints and treatment arms

(Bars represent Standard Deviation).

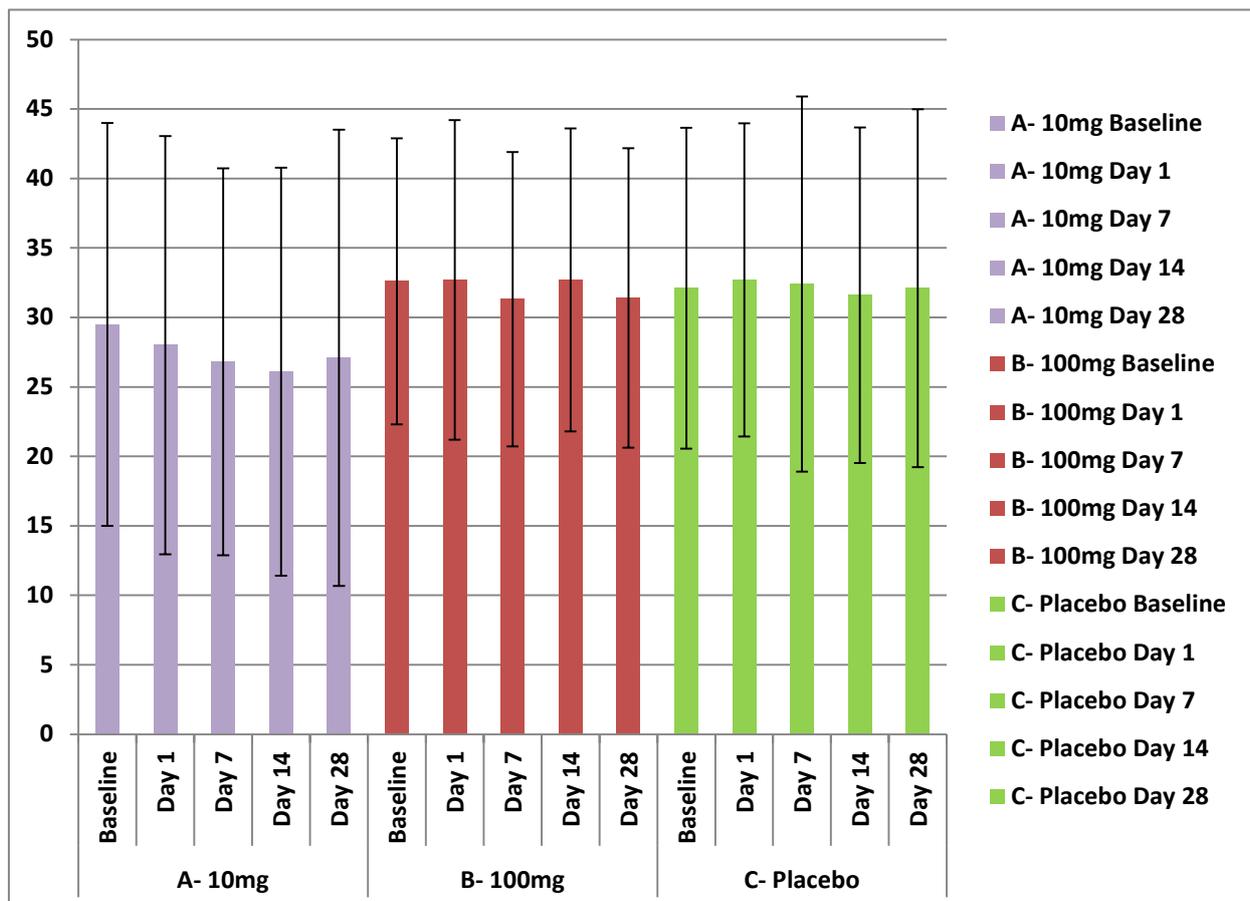
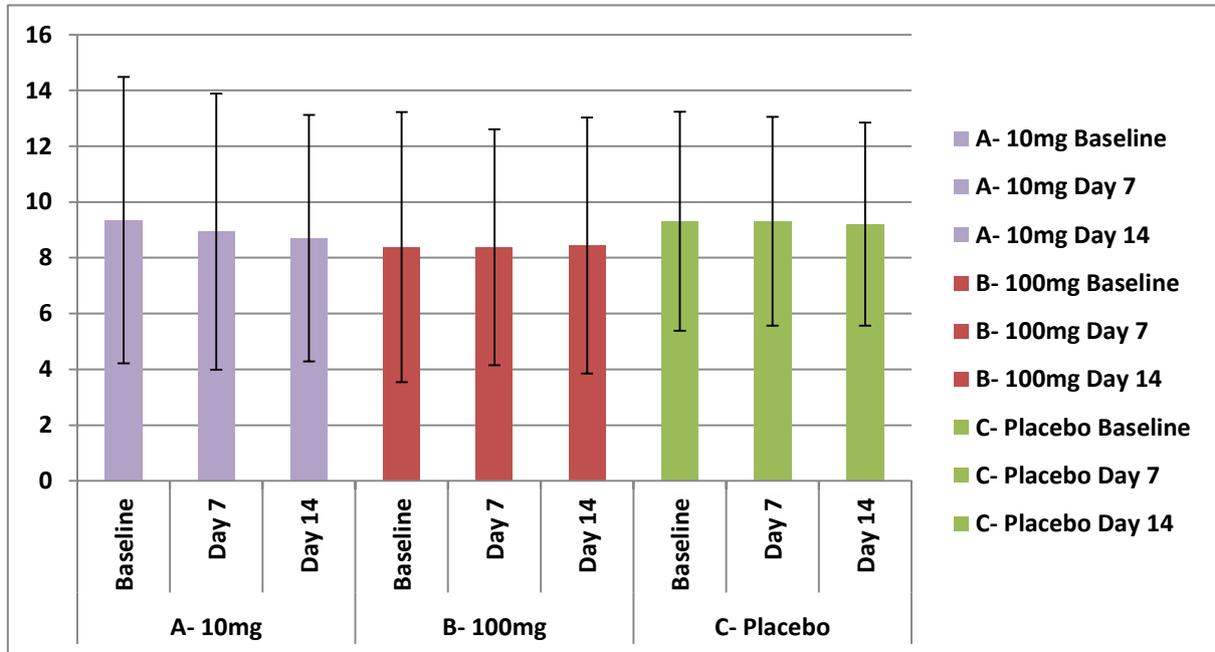


Table 7- Mean change in Total Motor Score between screening and Day 28 for each of the three randomisation groups

	Mean	SD	Min	Max
Placebo n=19	-1.16	5.55	-8	14
10mg n=17	-2.76	4.24	-11	7
100mg n=19	-1.89	8.04	-29	7

Overall, there was good consistency between TMS at Baseline (day before therapy) and Day 1 (first day of therapy) across all treatment groups (**Figure 7 and Table 7**). However, although there were slight differences between groups at various time-points, none were sufficient to merit clinical

Figure 8- Mean chorea Sub-Score by timepoint & treatment arm with no clinically significant difference seen in mean chorea subscore between timepoints and treatment arms (Bars represent Standard Deviation).



significance.

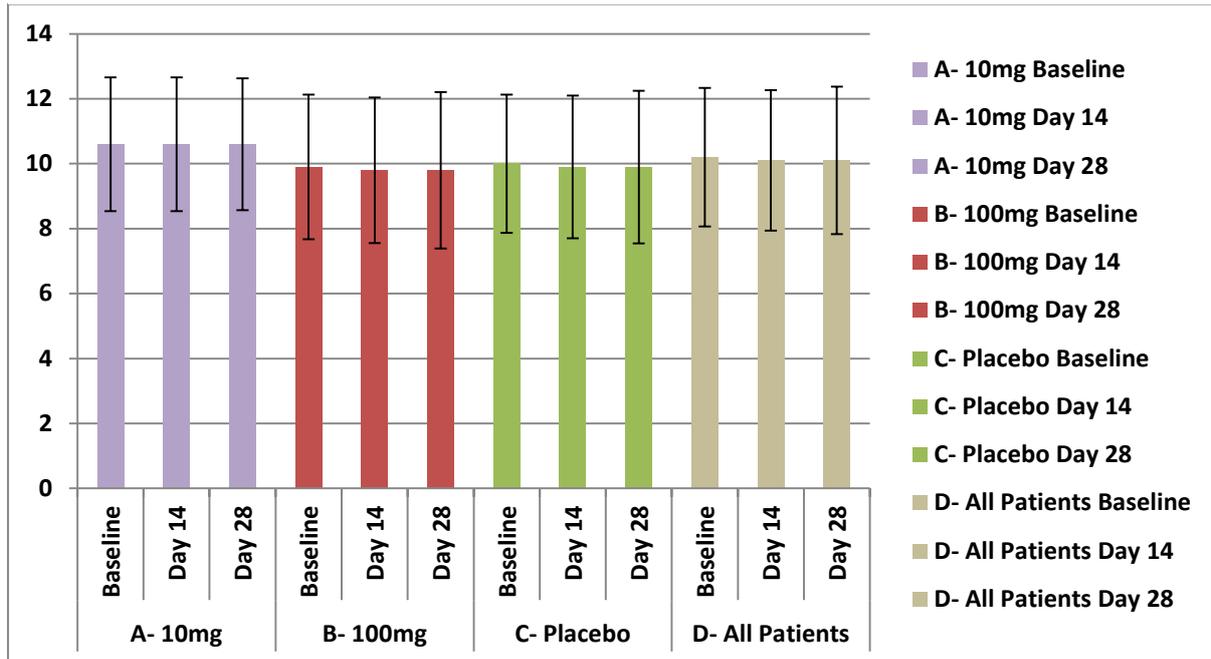
7.6.5 Chorea Sub-score

One of the commonest symptoms reported in HD is chorea. A potential symptomatic improvement therefore would be therapeutically and evidently commercially important. However, there was no obvious change in this score over time and between groups (**Figure 8**).

7.6.6 Total Functional Capacity

This is a clinical staging tool based on functional ability and scored out of a maximum of 13 (Table 2 Page 24).

Figure 9- Mean TFC Score by timepoint & treatment arm with no clinically significant difference seen in TFC between timepoints and treatment arms (Bars represent Standard Deviation).



Overall, there were no differences between the three groups (Figure 9).

7.6.7 UHDRS Functional Assessment (FA) and Independence Scale (IS)

Table 8- Functional Assessment Score (Appendix 13.5) with no clinically significant difference seen in FA between timepoints and treatment arms (represent range) (Sussmuth *et al.*, 2014)

	Group A (N= 17)		Group B (N= 19)		Group C (N= 19)		All Patients (N= 55)	
FA Score (25 max)	Observed	Change from Baseline	Observed	Change from Baseline	Observed	Change from Baseline	Observed	Change from Baseline
Baseline Mean (SD)	21.5 (3.45)	n/a	20.3 (3.78)		20.4 (3.65)		20.7 (3.62)	n/a
Baseline Median (min, max)	23.0 (14, 25)	n/a	20 (12, 25)		21 (13, 25)		21 (12, 25)	n/a
Day 14 Mean (SD)	21.4 (3.78)	-0.1 (0.6)	20.5 (3.47)	0.2 (0.92)	20.3 (3.68)	-0.1 (0.52)	20.7 (3.60)	0.0 (0.71)
Day 14 Median (min, max)	23 (13, 25)	0 (-2,1)	21 (13,25)	0.0 (-2, 3)	21 (13,25)	0.0 (-2, 1)	21 (13, 25)	0.0 (-2, 3)
Day 28 Mean (SD)	21.5 (3.64)	-0.1 (0.56)	20.6 (3.17)	0.3 (1.25)	20.4 (3.59)	0.1 (0.4)	20.8 (3.43)	0.1 (0.83)
Day 29 Median (min, max)	23.0 (14,25)	0.0 (-2, 1)	20.0 (16, 25)	0.0 (-2, 4)	21.0 (13, 25)	0.0 (-1, 1)	21.0 (13, 25)	0.0 (-2, 4)

There were no differences between groups and no significant change over time in FA, a questionnaire based assessment tool, looking specifically at performance in 25 areas of daily life (Appendix 13.5) (Table 8).

Table 9- Independence Scale (IS) (Appendix 13.5) with no clinically significant difference seen in IS between timepoints and treatment arms (Sussmuth *et al.*, 2014).

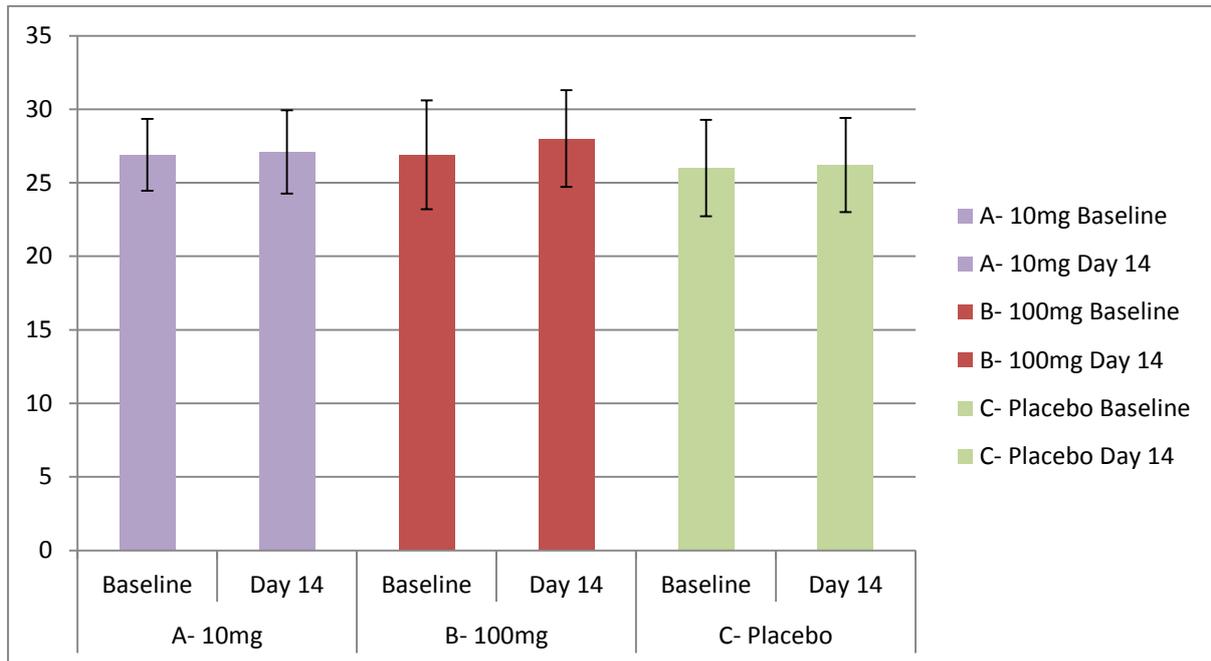
IS Score (100% max)	Group A (N= 17)		Group B (N= 19)		Group C (N= 19)		All Patients (N= 55)	
	Observed	Change from Baseline	Observed	Change from Baseline	Observed	Change from Baseline	Observed	Change from Baseline
Baseline Mean (SD)	86.5 (11.56)	n/a	82.4 (12.18)	n/a	83.7 (10.12)	n/a	84.1 (11.23)	n/a
Day 14 Mean (SD)	85.9 (11, 89)	-0.6 (1.66)	83,2 (11.57)	0.8 (1.87)	83.9 (9.8)	0.3 (1.15)	84.3 (10.95)	0.2 (1.66)
Day 28 Mean (SD)	85.9 (11.49)	-0.6 (1.66)	83.2 (11.57)	0.8 (1.87)	83.9 (9.8)	0.3 (1.15)	84.3 (10.82)	0.2 (1.66)

The independence scale is a percentage based assessment of independence- 100% representing complete independence (**Appendix 13.5**). The lack of significant change from baseline in Groups A & B from placebo group C, suggests that there were no treatment related effects in this measure (**Table 9**).

7.6.8 MMSE

Overall, no significant changes were noted in this 30 point scale of cognitive function (**Appendix 13.6**) (**Figure 10**).

Figure 10- Mean MMSE Score by timepoint & treatment arm with no clinically significant difference seen in MMSE between timepoints and treatment arms (Bars represent Standard Deviation).

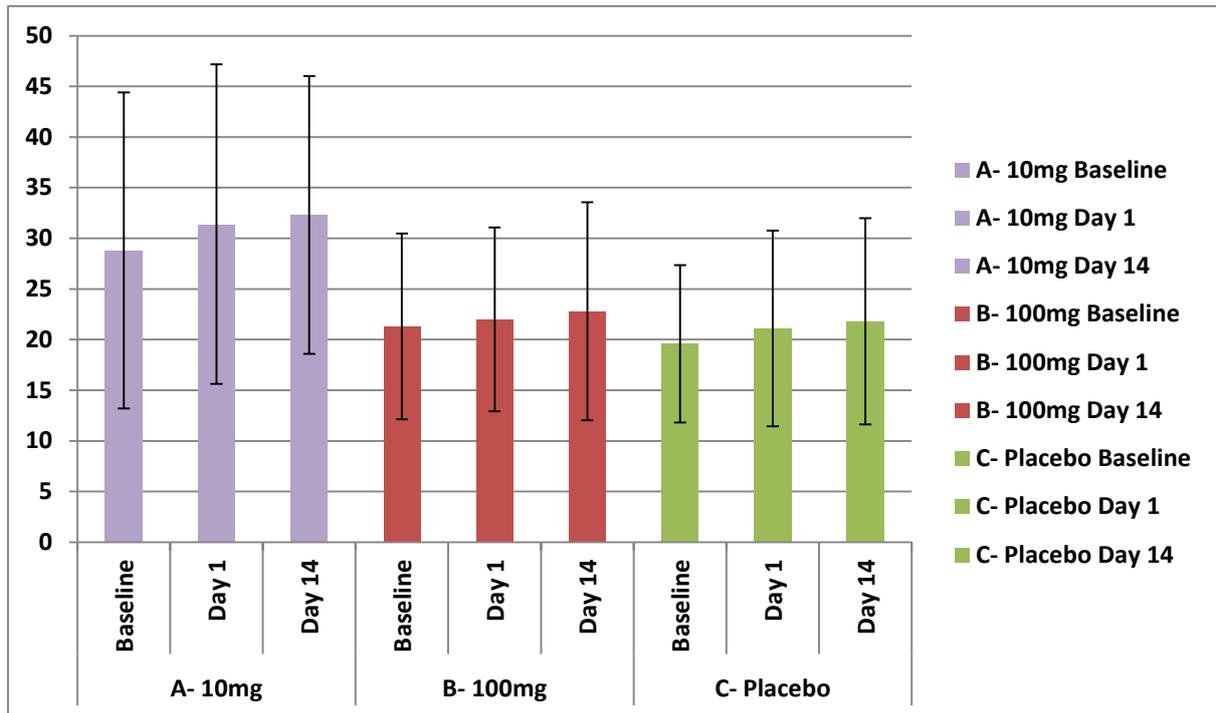


7.6.9 Symbol Digit Modalities Test

This timed cognitive test assesses visuomotor integration as it requires use visual scanning, tracking, and motor speed to complete the task (**Appendix 13.8**).

At baseline and on Day 1, the SDMT data were missing for 1 patient, each, in Groups B. Overall however, no clinically significant changes were noted between groups and time-points. There was however a trend to improvement in clinical scores, which likely reflects practice effects (**Figure 11**).

Figure 11- Mean SDMT Score by timepoint & treatment arm with no clinically significant difference seen in SDMT between timepoints and treatment arms (Bars represent Standard Deviation).



7.6.10 Sleep and Psychiatric Assessments

There were no significant changes between groups during the course of the study on the Pittsburgh Sleep Quality Index, a self-assessment questionnaire, the problem behaviour assessment and the hospital anxiety and depression scale and the Snaith irritability score.

7.6.11 Clinical Efficacy

7.6.11.1 Baseline Phenotypical Characteristics

Table 10- Baseline phenotypical characteristics of Phase 1B study population (Sussmuth *et al.*, 2014)

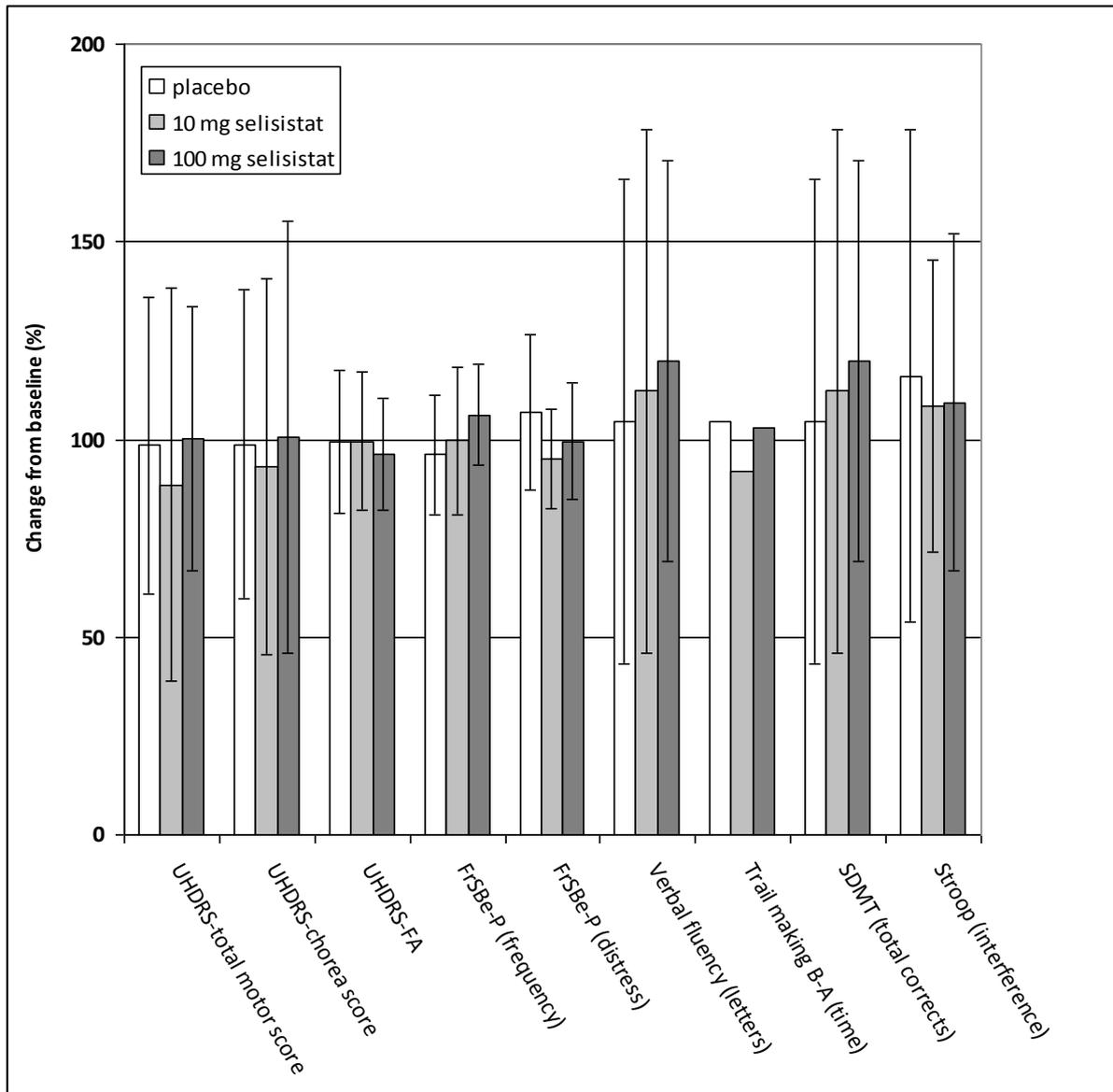
(Values shown are mean +/- Standard Deviation, with range [] given)

Parameter	Selisistat 10 mg (N=17)	Selisistat 100 mg (N=19)	Placebo (N=19)	All Patients (N=55)
UHDRS – TMS	29.5 ± 14.5 [8-64]	35.2 ± 15.37 [17-81]	32.1 ± 11.55 [14-53]	32.4 ± 13.81 [8-81]
UHDRS – TFC	10.6 ± 2.06 [7-13]	9.9 ± 2.23 [7-13]	10.0 ± 2.13 [7-13]	10.2 ± 2.13 [7-13]
UHDRS – FA	21.5 ± 3.45 [14-25]	20.3 ± 3.78 [12-25]	20.4 ± 3.65 [13-25]	20.7 ± 3.62 [12-25]
UHDRS – IS	86.5 ± 11.56 [65-100]	82.4 ± 12.18 [60-100]	83.7 ± 10.12 [65-100]	84.1 ± 11.23 [60-100]
PBA-s	9.6 ± 10.87 [0-42]	10.1 ± 11.95 [0-45]	8.1 ± 7.36 [0-27]	9.3 ± 10.07 [0-45]
MMSE	26.9 ± 2.45 [21-30]	26.9 ± 3.70 [18-30]	26.0 ± 3.53 [17-30]	26.6 ± 3.27 [17-30]
SDMT	28.8 ± 15.6 [12-70]	21.3 ± 9.2 [2-36]	19.6 ± 7.8* [5-32]	23.1 ± 11.7 [2-70]

It is clear that at outset, a wide spread of baseline values were seen, reflecting the phenotypic heterogeneity known in HD. However between the groups a clear difference is not apparent, apart from in the cognitive domain, SDMT, where the 10mg group have a higher SDMT score 28.8 versus the other treatment arms (**Table 10**).

During the comparatively short time period of study drug administration over two weeks, no clinically relevant and obvious changes in the parameters were apparent (**Figure 12**).

Figure 12- Percentage change in clinical parameters on Day 14 compared to baseline of patients receiving placebo, 10mg or 100mg. In a variety of clinical parameters no clear difference is seen between treatment groups. (Bars represent Standard Deviation).



7.6.11.2 Total Motor Score

Formal statistical analysis to look for significant difference between treatment groups in the total motor score results, involved linear modelling of the three treatment groups. The resulting F value 3.49 (Degrees of Freedom (DF) 52) and P value 0.038 did suggest a difference between the groups. However, head to head dose-group post-hoc (Tukey's Studentised Range (HSD) Test for change) comparative analysis failed to demonstrate statistical significance, though the 10mg group did trend in the direction of treatment effect (**Table 11**). Analysis of the chorea sub-

score failed to evidence a treatment effect. (*Analysis carried out at University of Ulm*).

Table 11- Dose-group comparisons of change of motor score between day -1 and day 14 with no evidence of effect noted on Tukey's Studentised range test

Comparisons significant at the 0.05 level are indicated by ***.				
dosegroup Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
100mg - Placebo	0.006	-11.207	11.218	
100mg - 10mg	11.039	-0.499	22.577	
Placebo - 100mg	-0.006	-11.218	11.207	
Placebo - 10mg	11.033	-0.505	22.571	
10mg - 100mg	-11.039	-22.577	0.499	
10mg - Placebo	-11.033	-22.571	0.505	

7.6.11.3 Other Clinical Assessments

Applying this approach to other assessments, TFC, FA, PBA, Verbal fluency, Symbol digit modality, Stroop and Trails A & B showed no statistically significant treatment effect.

The HADS-SIS irritability subscore analysis did suggest some significance, F value 4.30 (DF- 46) P 0.0195. Post-hoc analysis showed a significant effect of 100mg group versus 10mg though not placebo. Intuitively this may suggest that the 100mg dosing was associated with increased levels of irritability amongst subjects. Further analysis suggested this effect was concentrated largely on inward irritability subscore. This however was not sufficient to cause a documented withdrawal from the study or produce a psychiatric adverse event and is unexplained.

7.6.12 Pharmacokinetic

Selisistat was rapidly absorbed with a median t_{max} (time of maximum plasma concentration) of 3 hours. High inter-patient variability was noted in the systemic exposure to selisistat. A pharmacokinetic steady state was reached before Day 14.

7.6.13 Safety & Tolerability

There were no deaths, SAEs, or AEs leading to withdrawal during the study. Overall, 25 patients experienced 44 AEs. 13 patients experienced 15 AEs which, in the opinion of the investigators, were thought to be related to the taking of selisistat. In terms of severity of AEs, 22 patients experienced 40 mild AEs whereas 3 patients experienced 4 moderate AEs during the study. No patient experienced severe AEs.

There was apparent dose-responsiveness to the side-effect frequency between 10mg and 100mg with the number of patients experiencing treatment-related AEs higher in 100mg group B (6) compared to 10mg Group A (4) and placebo Group C (3).

The most commonly seen AE was hypertension (7 patients), followed by Headache (4 patients), anaemia (3 patients) and vomiting, nasopharyngitis, joint injury, neck pain, and tension headache (2 patients). The moderate AEs included anaemia, nasopharyngitis, headache, and circulatory collapse and were reported in 1 patient (1.8%), each. No consistent trends were noted in laboratory values across groups. In 2 patients from Group B high alanine transaminase values were observed on Days 28 and 14, respectively (**Table 12**). Mean changes from baseline of ECG intervals based on safety recordings interpreted at the clinical sites (PR interval, QT interval, heart rate, QTcB interval, and QTcF interval) on Days 1, 7, and 14, were not significantly different.

Table 12- Adverse Events Phase 1B study

SOC/Preferred Term	Selisistat, 10 mg (N=17)		Selisistat, 100 mg (N=19)		Placebo (N=19)	
	Suspected n (%)	Not Suspected n (%)	Suspected n (%)	Not Suspected n (%)	Suspected n (%)	Not Suspected n (%)
Total number of patients with AEs	4 (23.5%)	5 (29.4%)	6 (31.6%)	3 (15.8%)	3 (15.8%)	4 (21.1%)
Blood and lymphatic system disorders	0	0	0	1 (5.3%)	1 (5.3%)	1 (5.3%)
Anaemia	0	0	0	1 (5.3%)	1 (5.3%)	1 (5.3%)
Leukopenia	0	0	0	0	1 (5.3%)	0
Gastrointestinal disorders	1 (5.9%)	0	1 (5.3%)	1 (5.3%)	0	1 (5.3%)
Abdominal pain	1 (5.9%)	0	0	0	0	0
Dyspepsia	0	0	1 (5.3%)	0	0	0
Investigations	0	0	1 (5.3%)	0	1 (5.3%)	0
Alanine aminotransferase increased	0	0	1 (5.3%)	0	0	0
Protein total decreased	0	0	0	0	1 (5.3%)	0
Nervous system disorders	2 (11.8%)	2 (11.8%)	0	2 (10.5%)	1 (5.3%)	1 (5.3%)
Headache	1 (5.9%)	0	0	1 (5.3%)	1 (5.3%)	1 (5.3%)
Paraesthesia	1 (5.9%)	0	0	0	0	0
Renal and urinary disorders	0	0	1 (5.3%)	0	0	0
Leukocyturia	0	0	1 (5.3%)	0	0	0
Vascular disorders	1 (5.9%)	1 (5.9%)	3 (15.8%)	1 (5.3%)	1 (5.3%)	1 (5.3%)

Hypertension	1 (5.9%)	1 (5.9%)	3 (15.8%)	1 (5.3%)	1 (5.3%)	0
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7.6.14 Validation of Mechanism of Action

Preliminary data indicates that selisistat lowers total soluble HTT levels in peripheral blood mononuclear cells (PBMCs) (Westerberg *et al.*, 2012) but has not been fully and definitively established.

7.7 Discussion on Phase 1B Study Results

Data from the Phase 1B study of a novel SirT1 inhibitor, selisistat, administered for the first time in HD patients, at 2 dose levels (10 mg and 100 mg) over 14 days suggests that selisistat is safe and well tolerated in HD patients. No serious side-effects were observed and no dose- or treatment-related trends in terms of clinical laboratory evaluations, vital signs, or ECG parameters were detected.

7.7.1 Sirtuin Inhibition or Activation?

It is clear that aspects of sirtuin biology require further resolution. Inhibition of SirT1 specifically and of other HDACs has been shown to be neuroprotective in *Drosophila* while overexpression and SirT1-KI in mouse models is beneficial (Jeong *et al.*, 2012; Jiang *et al.*, 2012; Pallos *et al.*, 2008; Steffan *et al.*, 2001). Increased gene dosage of Sir2 prevented early neuronal dysfunction, in a polyglutamine *C. elegans* model (Parker *et al.*, 2005). In Type 2 diabetes, cancer and cardiovascular disease a role for SirT1 activators has been advocated from experimental work (Milne *et al.*, 2007).

These findings could be explained in part by experimental differences, namely the lack of equivalence of *Drosophila* models and murine models, and by differences between human HDACs and their cross species orthologs i.e. divergence of genetic function. Even within the same species,

differing effects are seen, with BAC-HD mice showing improvement of inclusion pathology with SirT1 overexpression, decreased aggregation of mutant HTT in R6/2 mice but no effect seen in N171-82Q mice (Jeong *et al.*, 2012;Jiang *et al.*, 2012;La Spada, 2012).

The influence of experimentation has been noted previously in the Sirtuin field. The life-span extending properties of Sir2 is another similarly controversial area where previously found positive results have been called into question and attributed to differences in genetic background arising from the methodology (Burnett *et al.*, 2011;Kaeberlein *et al.*, 1999).

However, selisistat, a SirT1 inhibitor, has been shown to phenocopy genetic and pharmacological inhibition in the same drosophila model as well improving pathology and performance of the R6/2 mouse model (**Chapter 7.2.3**). Therefore although sirtuin biology in general remains unclear, the effect of selisistat is in keeping with the original Drosophila work and in contradiction to more recent mouse model work.

It is suggested from work carried out by Steffan *et al.* 2001 that different HDACs within the same class, and different classes of HDAC, can mediate the same effect. They employed a HDAC inhibitor 4b, which had activity on both HDAC 1 and HDAC 3, within the same class (I). SAHA and butyrate have been utilised as pharmacological HDAC inhibitors but also have effects on multiple classes of HDACs, with the former acting on I, IIa, IIb and IV (Hockly *et al.*, 2003;Rasheed *et al.*, 2007;Steffan *et al.*, 2001).

Sirtinol, nicotinamide and niacin were used similarly to reduce Sir2 levels. Indeed combinatorial reduction of class I and class III HDAC activity, via Rpd3 and Sir2 respectively, was shown to be independent and additive in its neuroprotective effect (Pallos *et al.*, 2008). Finally, HDAC7 has recently been shown to mediate its neuroprotective effective via a mechanism that is *independent* of its deacetylase activity, involving the inhibition of c-jun expression (Ma & D'Mello, 2011).

Therefore the possibility that selisistat could be acting on multiple other targets and exclusive of its HDAC activity seems a reasonable one. However, unpublished data shows no effect of selisistat in mutant HTT challenged flies that have homozygous null alleles for Sir2, with replicated findings in

murine HD models (unpublished data from Siena Biotech SpA and Marsh laboratories, University of California Irvine), suggesting that the major effect is Sirtuin *dependent*. Although the potential for cross-over effects on zinc dependent HDAC classes (I, II & IV) is low as selisistat exhibits over a 1000 fold selectivity for SirT1, the effect on other Sirtuins e.g. SirT4-7 is not known.

A dosing effect may instead be in operation as Sir2 haploinsufficiency in drosophila has been shown to abrogate neurodegeneration while SirT1 knockouts are harmful (Jeong *et al.*, 2012;Pallos *et al.*, 2008). Experiments using heterozygous Sir2/SirT1 models with the compound have not been carried out thus far. Selisistat may thus achieve pharmacologically, what haploinsufficiency does genetically. The ability of SirT1 to mediate multiple different effects in both the central nervous system and periphery may thus be tissue specific and dose dependent. Therefore, at either reduced or overexpressed doses, SirT1 may access undisclosed, complimentary and possibly distinct pathways.

This could be assessed pharmacologically with SirT1 activators and although resveratrol has shown some promise in improving peripheral but not central deficits in a HD mouse model, questions remain as to whether its mode of action is SirT1 dependent (Pasinetti *et al.*, 2011;Ho *et al.*, 2010;Ho *et al.*, 2010). No other highly selective SirT1 activators are available at present.

Evidently direct assessment of SirT1 deacetylase activity may be helpful in calibration of activity, but has only been carried indirectly in vitro through assessment of acetylation of p65 subunit of NFκβ (Yeung *et al.*, 2004). Measurement of SirT1 RNA levels is another possibility but has not been explored.

Finally, activity beyond histone deacetylation is also suggested from pre-clinical and cellular data where selisistat can render mutant HTT to an acetylated status, which could potentially facilitate its clearance. Restoration of transcriptional dysregulation, which may in part be achieved via direct activity on transcription factors, as well as altering the conformational status and accessibility of DNA i.e. a histone deacetylating effect, has also been proposed. In vivo data from healthy volunteers receiving Selisistat does corroborate this potential latter mode of action (Westerberg *et al.*, 2015) but has not been formally evaluated in HD patients.

7.7.2 Mechanism of Action of Selisistat

Although increase in soluble HTT levels was seen during the study, there is no published data at present on amelioration of transcription dysregulation or acetylation related facilitation of HTT clearance. At the time of study, means of measuring the HTT protein were not established but have been subsequently in blood, PBMCs and CSF. Identifying the effects of Selisistat on HTT levels in each of these compartments would have been an essential step in developing a sound justification for its continued study.

7.7.3 Efficacy Measures

Although this study was not designed to determine efficacy, a symptomatic change in chorea for example, might have been a reasonable expectation, though no evidence for this was found. However, clinical experience with anti-chorea medication such as Tetrabenazine would indicate that such an effect may take up to a month to manifest.

For a chronic neurodegenerative condition like Huntington's disease, it is reasonable to postulate a 6 month window in which a treatment may bring about a clinical improvement. This draws parallels from the time taken to recover after stroke or head injury, where a recovery window of up to 2 years is often quoted but is evidently influenced by site of lesion- cortical versus subcortical and premorbid status, environmental and as yet unknown biological and genetic factors.

Similarly, although imaging changes do not always equate to functional changes, deterioration in volumetric measures have been seen in imaging studies over 6 months (Hobbs *et al.*, 2015). By extension therefore, a clinical improvement may be expected over a similar sort of time frame, though mechanistically this may be achieved through a combination of diminution of pathology and activation of compensatory mechanisms.

Evidently, data from later phase trials (II and III) are required together with validation of mechanism of action from pharmacodynamic analyses, before a disease modifying label can be justifiably applied. Additionally, data on ability of HD patients to access compensatory networks and relearn tasks would be important to quantify recovery and thus set reasonable expectations for disease modifying therapies.

8 Selisistat & Innate Immune System

8.1 Introduction

SirT1 and the Immune System

Due to their effects on various transcription factors, SirT1 plays a role in thymocyte and lymphocyte activation. In addition, SirT1 may have effects in both the innate and adaptive immune response via their regulation of NFkB and AP-1 pathways respectively. SirT1 knockout mice are more susceptible to inflammatory disorders including a lupus-like phenotype (Sequeira *et al.*, 2008).

SirT1, NFkB & AP-1

NFkB is a key component in the pathway that leads to cytokine production and lymphocyte activation. SirT1 is proposed to reduce NFkB activity through acetylation of p65 at multiple lysine residues (Chen *et al.*, 2002). AP-1 activity is associated with production of an immune response, particularly in T cells comprising proliferation, interleukin (IL) -2 release and differentiation.

SirT1 & Macrophage Function

Macrophages are the main source of pro-inflammatory cytokines TNF- α , IL-6 and IL-1 secreted in response to infection and inflammation. SirT1 restricts the pro-inflammatory phenotype of macrophages via inhibition of the NFkB pathway (Gao *et al.*, 2012). Intraperitoneal macrophages in a mouse SirT1 knockdown model, showed increased tumour necrosis factor (TNF) alpha levels on lipopolysaccharide (LPS) stimulation (Yoshizaki, 2011).

However, in a LPS stimulated J774 macrophage model, use of sirtinol and cambinol as Sirtuin inhibitors attenuated the release of pro-inflammatory cytokines, TNF α and IL-6, via a decrease in NFkB activity (Fernandes *et al.*, 2012).

Sirtuins- Pro or Anti-inflammatory?

As discussed earlier (**7.7.1**), these conflicting results may reflect in part experimental differences, knockouts versus pharmacological inhibition, but also the fact that sirtinol and cambinol have effects on SirT2 levels. Inhibition of SirT2 via genetic manipulation or pharmacological inhibition has been shown to be neuroprotective in striatal neurone and drosophila HD models, with reduction in sterol biosynthesis proposed as a mechanism (Luthi-Carter *et al.*, 2010). Finally their effect on SirT4, SirT6 & SirT7, which have competing effects, is not known (Fernandes *et al.*, 2012). By way of example, SirT6 has been shown to positively influence TNF- α levels via a post translational mechanism (Van & Veenstra, 2009). In the absence of a full understanding of the wider effect of Sirtuin inhibition, caution must be taken in interpreting results.

8.1.1 The Immune System and Huntington's Disease

There is substantial evidence of the deleterious effects of the HD gene mutation on the immune system of both the periphery and that of the brain. HD lymphoblasts show a variety of deficits in mitochondrial morphology and calcium handling capacity, autophagy and transcriptional dysfunction (Borovecki *et al.*, 2005; Mormone *et al.*, 2006; Panov *et al.*, 2002).

LPS stimulation of HD monocytes reveals an inherent hyper-reactivity that is similar to that seen in microglia, suggesting a cell-autonomous effect of mutant huntingtin in peripheral myeloid cells as well as in the CNS. A pattern of pro-inflammatory cytokine elevation has been observed in plasma in HD, with IL-6 significantly elevated in a group of subjects predicted to be, on average, 16 years from disease (**Figure 13**). A parallel post-mortem cytokine expression profile is seen in HD striatum (Tai *et al.*, 2006; Bjorkqvist *et al.*, 2008).

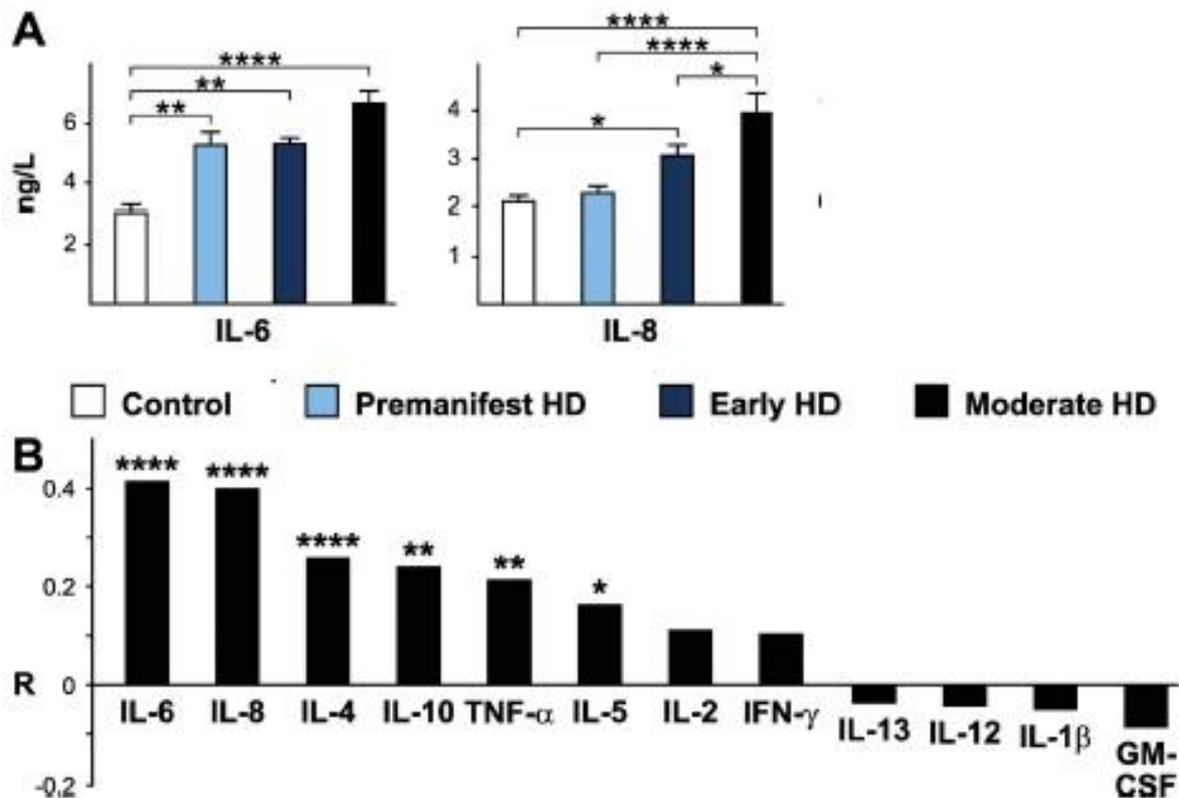


Figure 13- (Bjorkqvist *et al.*, 2008) Altered immune profile peripherally in HD. (A) Multiplex ELISA quantification of cytokine levels in plasma from HD patients (premanifest, early and moderate HD stages) compared with control subjects. Graphs show mean concentrations with standard error bars. Significant differences between individual groups are shown (ANOVA with post-hoc Tukey HSD test). (B) The overall trend for increasing levels of cytokines across all groups, analysed using linear regression, was highly significant for IL-6 and IL-8 and significant for IL-4, IL-10, TNF- α , and IL-5. R-values (partial correlation coefficients) are corrected for age and sex. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$.

Correlations between regional sites of neuronal loss and levels of microglial as well as astrocytic activity have been noted. It is unclear whether microglia are functioning as primary modulators of disease or simply appearing in reaction to neuronal loss, or both. Indeed microglia can mediate free radical production, excitotoxicity and initiation of caspase pathways (Kim *et al.*, 2005; Tikka & Koistinaho, 2001; Wang *et al.*, 2005).

PET studies demonstrating activation of microglia in early HD patients may support their involvement in the preliminary pathological stages of disease (Tai *et al.*, 2006; Politis *et al.*, 2011). HD striatum also demonstrates significant complement deposition versus normal controls and gene expression studies revealed significantly higher levels of complement pathway components in HD brains (Singhrao *et al.*, 1999).

Evidence therefore suggests simultaneous dysfunction of CNS and peripheral inflammatory pathways in HD. Sirtuins have a potential regulatory role in the innate immune system through modification of both pro and anti-inflammatory transcription factors. The relative impermeability of the blood brain barrier and the potential morbidity as well as practical difficulties associated with administration of therapies directly into the cerebrospinal fluid or brain parenchyma, makes the peripheral immune system an attractive therapeutic target.

Notwithstanding, the PET studies detailed above, the role of the immune system as a modifier of disease is buoyed further by the finding that bone marrow transplantation partially ameliorated motor abnormalities in HD mouse models (Kwan *et al.*, 2012a). Additionally, administration peripherally of JM6, an inhibitor of the kynurenine 3-monooxygenase (KMO) tryptophan degradation pathway found on microglia, enhances levels of the neuroprotective, kynurenic acid, augments longevity, reduces synaptic loss and microglial reactivity in a HD mouse model (Zwilling *et al.*, 2011). Genetic deletion of CB₂ receptors, which are involved in down-regulation of the immune system, in a R6/2 mouse model foreshortens the motor onset and worsens the phenotype. Application of a CB₂ receptor agonist peripherally, with no central microglial action, abrogates these effects (Bouchard *et al.*, 2012).

Experimental evidence using HD primary myeloid cells also suggests that their hyper-reactivity is directly caused by the expression of mHTT via the NFκβ pathway (Trager *et al.*, 2014), which is itself modulated by SirT1. This provides a potential mechanistic link between immune dysregulation and Sirtuins.

Given the known immune abnormalities in HD and the role that Sirtuins play in regulating the immune system, comparative analysis of the plasma cytokine profile of Huntington's disease patients at four time points- screening, baseline, day 14 and day 28, in each of the three treatment arms of the study was carried out, testing the hypothesis that SirT1 inhibition via Selisistat restores the altered cytokine profile seen in HD subjects.

8.2 Methods

The experimental laboratory work was carried out solely by myself with initial supervision by Dr Ralph Andre. The reading of the multiplex plates was carried out under the initial supervision of Dr Ulrike Traeger and Dr Ralph Andre and using equipment at the UCL Institute of Child Health, Department of Haematology, London. Analysis plans were formulated by myself while statistical work carried out by Ruth Farmer & Professor Chris Frost, School of Hygiene and Tropical Medicine.

The Study were carried out following the Helsinki protocol (Ethical Principles for Medical Research Involving Human Subjects), with local research ethics committee approval and clinical research organisation supervision including site initiation, monitoring and final visits. All study members were trained in good clinical practice.

8.2.1 Cytokine Assays

Assays were carried out using the Mesoscale Discovery (MSD) multiplex platform (Human ProInflammatory II 4-plex ultra-sensitive kit) as per the manufacturer's instructions. Blood samples for cytokine assays were taken pre-dose at screening, Day 1 & 14 of dosing, and finally at Day 28, two weeks after the last dose from all 55 Phase 1B study participants. The operator was blinded to the disease state of each sample during processing and statistical analysis was performed independently.

Cytokines of interest included IL-1, IL-6, IL-8 and TNF- α , anti-bodies for which are pre-coated into 4 spots per well. To this labelled detection anti-bodies are added. This label is an electroluminescent compound. The sample of interest is thus bound by the pre-coated antibodies at one end, and by the detection antibodies at the other. Application of a current causes light emission- the signal.

Each sample representing one visit point of one patient was repeated in triplicate. Where samples failed to produce a result, they were repeated. A mean of the three values produced was used for data analysis. Where one value appeared aberrant versus the other two it was excluded. A calibrator solution with a graduated series of known concentrations generates a standard signal-concentration curve, against which the analysed samples are compared. Typically 8 dilutions are employed in producing a calibrator solution. However, previous work in our laboratory had suggested that a 9th dilution may be necessary to pick up signal for cytokines at low levels.

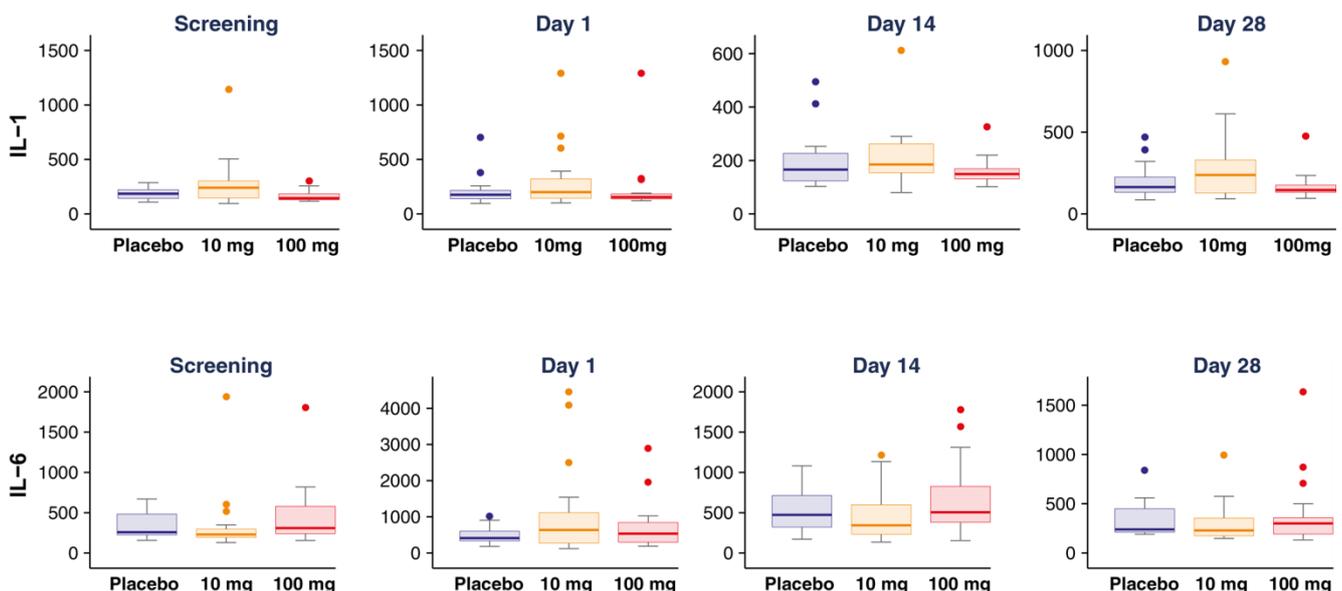
8.2.2 Statistical Analysis

The model used for statistical analysis of these data is an extension of the usual ANCOVA model for randomised controlled trials with a single follow-up and baseline measures, allowing simultaneous estimation of treatment differences at all follow-up visits adjusted for baseline. This is achieved using a linear mixed model that allows for correlated responses at all timepoints, including the screening visit. The model enforces a zero treatment effect at screening, but imposes no restrictions from day 1 onwards. The model was fitted to log transformed data for each cytokine type due to improve normality assumptions.

8.3 Selisistat & Innate Immune System Results

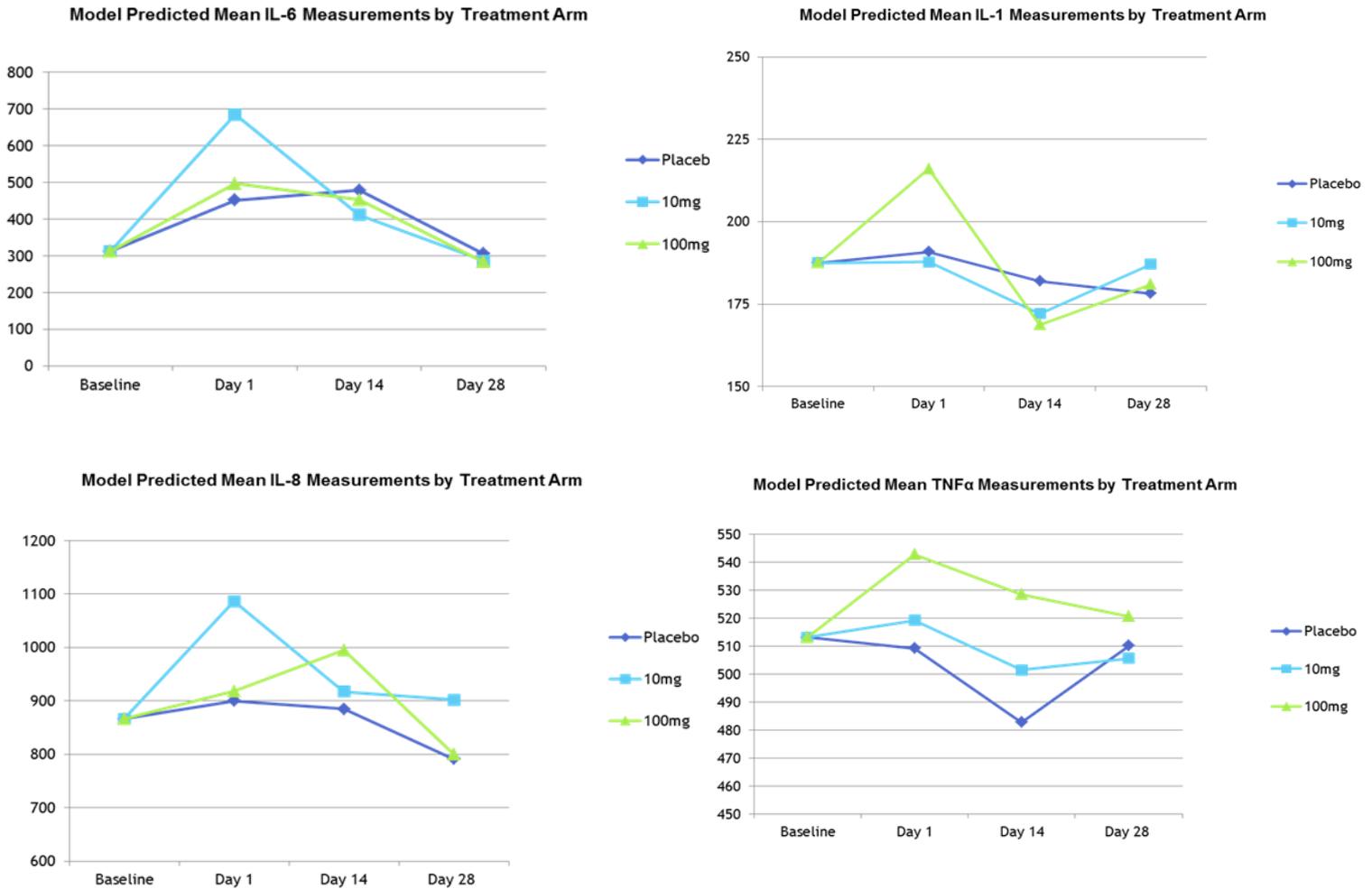
There is no clear evidence of cytokine alteration across time-points and in different treatment arms for IL-1, IL-6, IL-8 and TNF α (**Figure 14**). A few outlying values are seen for each of the cytokines tested and are explored further in **Chapter 8.4**.

Figure 14- Box-plot Distribution of Cytokine Levels (pg/ml) by Treatment Arm & Time Point with no apparent difference seen in cytokine levels- IL-1, IL-6, IL-8 and TNF α between timepoints and treatment arms.



Using the data obtained to generate a statistical model, no apparent change in cytokine levels over time is noted in each of the three treatment arms. The influence of the outliers in this model is also noted (**Figure 15**).

Figure 15- Model Predicted Mean Cytokine Levels (pg/ml) for each Treatment Arm. Despite some mild evidence of outlier effects, no clear evidence of treatment group on cytokine levels of IL-1, IL-6, IL-8 & TNF α is seen. ANCOVA modelled log transformed data.



Finally, analysis of the data looking for any evidence of statistically significant effect of treatment with 10mg or 100mg of selisistat on cytokine levels, fails to support this hypothesis (**Table 13**).

Table 13- P-values indicating evidence of treatment effect (10 mg & 100 mg) at each time-point with no evidence of statistically significant difference (joint Wald test) of treatment on cytokine levels.

Time Point	P Values for Cytokines			
	IL-1	IL-6	IL-8	TNF α
Day 1	0.4196	0.1741	0.1867	0.4083
Day 14	0.7540	0.6916	0.5460	0.2282
Day 28	0.8718	0.8635	0.2975	0.8925

8.4 Outlier Analysis

A handful of subjects with outlying values were noted during these analyses (**Figure 14**). A retrospective, observational analysis of these subjects, looking for alternative explanations such as illness e.g. flu or infection, new medications or presence of any other change to pre-treatment state, was undertaken.

8.4.1 IL-1 Outlier

One subject was noted to have values greater than 500 pg/ml at Day 1, 14 and 28, the latter time point being 14 days after dosing. This subject was on 10mg of selisistat. No obvious changes were noted on review of the clinical database aside from transitory hypertension on day 1.

8.4.2 IL-6 Outlier

In one subject, in the 10mg treatment group, a screening measurement of 1940 pg/ml was noted followed by subsequent readings of about 600 pg/ml. Non-specific headache was noted at screening which resolved by the next visit. No other explicatory factors were identified.

8.4.3 IL-8 Outliers

One subject in the 10mg treatment group had a value above 3000 pg/ml at baseline. At screening he had a non-serious fall on his way back home and reported some migraine, which was pre-existing at baseline. Another subject in 10mg treatment arm showed a high reading throughout the study period but with no obvious explanation. Finally a subject in 100mg treatment group had a peak at Day 14. However detailed investigation of the clinical record revealed no helpful findings.

8.5 Discussion- Selisistat & Innate Immune System

Analysis of data indicates that selisistat is unlikely to have biologically significant effects on modulation of IL-1, IL-6, IL-8 and TNF α levels. Furthermore, results here suggest that SirT1 inhibition is not acutely pro-inflammatory, in contrast to work previously carried out in macrophages, adipose and myocardial tissues, as well as in autoimmune disease (Kong *et al.*, 2012).

Experimental work in a macrophage-based cellular model, using Glucan encapsulated siRNA particles (GeRPs), has shown a significant decrease in IL-6 and TNF α production in both HD patient and control cells. This suggests that HTT is necessary for the hyper-reactive phenotype in HD myeloid cells (Trager *et al.*, 2014) and therefore that selisistat may not be having a HTT lowering effect in myeloid cells. This hypothesis is eminently testable through application of the compound to a human macrophage cell model using the above paradigm and by measurement of HTT levels in PBMCs from treatment groups. Although SirT1 expression is thought to be widespread and sirtuins are heavily

implicated in an immune modifying role, it is also possible that SirT1 is not expressed at significant levels in myeloid cells to abrogate the hyper-reactive phenotype.

Secondly, the duration and/or strength of dosing may not have been sufficient to provoke an effect. One notes the PK absorption data, which showed that a steady state was not achieved until day 4, and further pharmacokinetic analysis suggested the induction of other absorption mechanisms by day 14. The phase 2 study where treatment occurred over 3 months may be able to shed some light on this issue. Correlation of drug concentration levels with cytokine levels could also be looked at directly.

The original work carried out by Bjorkqvist & Wild et al. 2008 comprised 194 subjects including 60 controls and demonstrated a convincing, statistically significant elevated cytokine profile in HD patients. Sirtuins mediate the balance of pro- and anti-inflammatory action and therefore the absence of effect in either direction could reflect an equilibrated state. Although Selisistat appears to be highly selective for SirT1 above SirT2-3, counter-balancing effects on other Sirtuins, or indeed other pathways, are not excluded by current work. However the absence of a difference between the placebo group and treatment arms would suggest selisistat has no effect on the cytokine profile of HD patients.

In Huntington's disease it is proposed that the immune system may be functioning as a peripheral modifier of disease, acting peripherally on muscle and adipose tissue and potentially centrally, though this is not yet evidenced in humans. Moreover, it is not clear that the innate immune system of *all* HD patients is hyper-reactive or indeed remains consistently so. By extension, the Phase 1B HD population may not exhibit this phenotype. Similarly, phenotypic diversity within HD is not unusual at all. Indeed it is clear that different motor sub-types of HD exist, akinetic rigid and classical hyperkinetic and that cognitive or psychiatric disease may be manifest strongly in some and be absent or mild in others. A control, HD gene negative population, would have been required for this set of experiments, to test this hypothesis adequately. Comparison to historical controls used in the work by Bjorkqvist & Wild et al. 2008 was considered, but was felt to be unviable given differences in methodology between studies.

The work that first demonstrated innate immune hyperactivity in HD monocytes employed lipopolysaccharide (LPS) stimulation as part of the experimental paradigm (Bjorkqvist *et al.*, 2008). LPS is derived from the outer membrane of gram negative bacteria and interacts with the innate immune system via the Toll-like receptors (TLR) which in turn leads to transcriptional changes. It is believed to play a role in mediation of septic shock and the inflammatory response to infection. Indeed recent work in HD myeloid cells has shown a pathologically altered pro-inflammatory transcriptome even in the absence of LPS stimulation, suggesting that myeloid cells may be primed to overreact on receipt of the correct stimulus (Miller *et al.*, 2016). However a clinical manifestation of this phenomenon is not apparent, although has never been carefully looked for. One would speculate that immune hyper-reactivity would lead to lower rates of infection, which would evidently not be reported. Conversely, one may expect a higher prevalence of auto-immune conditions.

It may however explain the often noted clinical scenario of decompensation of a chronic neurodegenerative condition in the context of infection, where the disease progression of the patient appears to accelerate seemingly over days and then returns over subsequent months. Additionally some patients report a preceding medical illness prior to onset of a neurodegenerative disorder, as well as other chronic disorders in general (De *et al.*, 2012; Deleidi & Isacson, 2012).

Thus in the phase 1B study, no such stimulation was given, and therefore it may be that in some HD patients the baseline state is altered, while in others it remains at normal levels, but upon stimulation, this hyper-reactivity emerges, and there may be a final group which are no different from controls.

The outlier analysis implies that cytokine levels may also be subject to inherent variability. Although substantial data is lacking, one study examined cytokine profiles of health controls and fibromyalgia patients over a 24hr period. They noted some clustering of peak values around the time of first sampling, despite having had time to acclimatise to the research facility and sampling itself (Togo *et al.* 2009). Further work has shown evidence of the pulsatile secretion of IL-1 suggesting variability may be inherent (Licinio *et al.* 1994). In addition, the influence of other factors such as physical activity as well as time of sampling is not accounted for in this study.

9 Phase 2 Study of Selisistat

With kind permission of the study sponsor Siena Biotech SpA, data presented here is the complete data set for all subjects, including 6 London subjects, where I was the site clinical lead. In this role, I undertook all clinical histories, physical examinations, UHDRS, ECG interpretation, TFC & Independence scale Scores, HADS-SIS assessments, cannulation and blood tests. Routine clinical observations were performed by clinical trial nurses at Clinical Research Facility (CRF), University College London Hospital (UCLH) and cognitive assessments by Miss Monica Lewis. Processing of London biosamples was carried out by Miss Nicola Robertson at the CRF, UCLH.

9.1 Introduction

Selisistat, a SirT1 inhibitor, has been specifically developed for Huntington's disease, with a mechanism of action based on acetylation of mutant Htt, activating cell based autophagy. As presented earlier, the first-in-human study showed Selisistat to be safe and well tolerated in healthy volunteers, with the majority of adverse events determined as mild in severity and not requiring treatment and no SAE. The phase 1B study revealed no SAEs and no subjects were withdrawn from the study as a result of adverse events.

Herein is described a phase 2, double-blind, placebo-controlled parallel group design study with three treatment groups to assess the safety and tolerability of selisistat in male and female patients between 30 and 70 years of age with Stages I to III HD.

9.2 Methods (Reilmann *et al.*, 2013)

*The Study were carried out following the Helsinki protocol (Ethical Principles for Medical Research Involving Human Subjects), with local research ethics committee approval and clinical research organisation supervision including site initiation, monitoring and final visits. All study members were trained in good clinical practice. Below are the methods reproduced from the clinical study report (Reilmann *et al.*, 2013) unless otherwise indicated.*

9.2.1 Summary of Design

The study was also designed to evaluate short-term clinical outcomes and the pharmacokinetics of Selisistat over a 12 week dosing period, involving 18 centres and approximately 144 patients randomised in order to achieve 120 completed patients (40 patients per treatment group) in three countries: Italy (four sites), Germany (seven sites), and the United Kingdom (UK) (seven sites).

Screening took place within 5 to 21 days prior to the Baseline Visit (Day 1) when the randomisation and first dose administration took place. All Baseline assessments (including verification of patient eligibility) had to be completed before randomisation and dosing with study drug. Determination of standardised CAG repeat length at Baseline was required for participation in the study. CAG repeat number assessment for eligibility had to be done prior to screening and was not a study procedure. Dose changes were not permitted.

Patients, who in the opinion of the Investigator had intolerable AEs on their assigned dose, could have a 3- to 5-day drug 'holiday'. However, after the drug holiday, the study drug had to be restarted at the same dose level as previously administered (same supply). If intolerable AEs reappeared after reintroducing the study drug, the patient was to be withdrawn, rather than undergoing further drug-free periods.

9.2.2 Primary Objectives

To assess safety and tolerability of 12 weeks of treatment with selisistat at doses of 50 mg and 200mg in HD patients.

Primary outcome measures were:

- Type and frequency of AEs.
- Clinical laboratory tests (haematology, serum biochemistry, and urinalysis)
- 12-lead electrocardiogram (ECG).
- Physical and neurological examination findings.

- Vital signs.
- Suicide risk and suicide-related events (behaviour and/or ideation) as assessed by the Columbia-Suicide Severity Rating Scale (C-SSRS).

9.2.3 Secondary Objectives

To assess short-term clinical effects, modulation of candidate pharmacodynamic (PD) markers and the pharmacokinetic (PK) profile of Selisistat at 50 mg and 200 mg in patients with HD treated for 12 weeks, through the following outcomes:

- Clinical Impression.
- Global Clinical Impression (GCI) (patient and Investigator-based).
- Unified Huntington's Disease Rating Scale (UHDRS) Total Motor Scale (TMS).
- Functional Assessment.
- Independence Scale Assessment.
- Problem Behaviours Assessment.
- Cognitive Battery (Symbol Digit Modalities Test, Stroop Word Test, Verbal fluency, Mini-Mental State Examination [MMSE]).
- Change from Baseline levels of soluble Huntingtin protein (HTT)
- Change from Baseline in acetylation status of mutant HTT
- Pharmacokinetics of selisistat at steady state with the maximum observed plasma concentration (C_{max}), time of maximum observed plasma concentration (t_{max}), AUC from time zero to the length of the dosing interval (τ) ($AUC_{0-\tau}$), AUC from time zero to the last quantifiable concentration (AUC_{0-last}), and inter-patient variability.

9.2.4 Inclusion Criteria

Male and female HD patients as defined by the following inclusion criteria were eligible to enrol in the study:

1. Genetically confirmed, manifest HD (CAG repeat length 36) and motor signs of HD (motor score of the UHDRS \geq 5).
2. Clinical Stages I to III (TFC of \geq 4).

3. Anticipated to be ambulatory and able to attend outpatient visits for the duration of the study.
4. Aged ≥ 30 years and ≤ 70 years.
5. Body mass index between 18 and 31 kg/m² inclusive and a body weight > 50 kg.
6. Able to give informed consent or have a legal representative who could consent on their behalf. Patients had to be able to comply with trial procedures.
7. No clinically significant and relevant medical or psychiatric history that could affect the conduct of the study and evaluation of the data, as ascertained by the Investigator through detailed medical history and screening assessments.
8. Male patients had to agree to use condoms during the entire duration of the study and for 3 months following the last dose of study drug.
9. Females of childbearing potential (last menses less than 1 year prior to enrolment):
 - Negative pregnancy test at Screening and at Baseline (Day 1).
 - Could not be breastfeeding.
 - Had to be either be surgically sterile (hysterectomy, bilateral oophorectomy, or tubal ligation), postmenopausal (cessation of menses for at least 1 year), or agree to use a medically accepted, highly effective method of contraception during the entire duration of the study and for 3 months after the last dose of the study drug. An effective method of birth control was defined as those methods, either alone or in combination, which has a low failure rate when used consistently and correctly, such as implants, injectables, combined oral contraceptives, non-hormonal intrauterine devices, sexual abstinence, a vasectomised partner or use of a double-barrier contraception method (e.g., condom with diaphragm/occlusive cap and spermicide cream).

9.2.5 Exclusion Criteria

A patient was not eligible for inclusion in the study if any of the following criteria were met:

1. Participation in a study with an investigational drug within 30 days of the Baseline Visit.
2. Any prior or concomitant use of Class I or Class II HDAC inhibitors such as Zolinza[®]/vorinostat or belinostat.
3. Clinical evidence of significant or unstable medical illness in the Investigator's judgement, including screening transaminases (aspartate aminotransaminase [AST] or alanine

aminotransaminase [ALT]) ≥ 1.5 times the upper limit of normal (ULN), or an estimated glomerular filtration rate (GFR) (modification of diet in renal disease [MDRD] equation) of < 60 mL/min, or unexplained proteinuria or microscopic haematuria in an uncontaminated sample obtained at Screening and confirmed on repeat testing.

4. Corrected QT interval using Fridericia's formula (QTcF) interval > 450 ms in men and 470 ms in women or PR > 220 ms, or other clinically relevant abnormal ECG findings that, in the Investigator's judgement, would present an unacceptable risk to patient safety.
5. Women who were pregnant or breastfeeding.
6. Clinically significant abnormalities in the screening laboratory studies which, in the opinion of the Investigator, would have interfered with participation in the study.
7. Current evidence or history (within 1 year of baseline) of psychosis, hallucinations or delusions, including major depression with psychotic features, as defined in the Diagnostic and Statistical Manual, Fourth Edition, Text Revision (DSM-IV-TR). Patients experiencing mild depression, or moderate depression, which is adequately and appropriately treated, in the judgement of the Investigator, could participate if depression was not expected to interfere with study participation.
8. Suicide risk, as determined by meeting any of the following criteria:
 - A suicide attempt within the past year or suicidal ideation within 60 days of the Baseline Visit (Day 1).
 - Significant risk of suicide, as judged by the Principal Investigator, based on the psychiatric interview or information collected in the C-SSRS.
9. Current diagnosis or history (within 1 year of baseline) of any alcohol or substance abuse (except nicotine and caffeine-related disorders) as defined in the DSM-IV-TR.
10. Known allergy to any ingredient in the study drug (active and/or placebo).
11. A history of malignancy of any type within 2 years prior to screening. A history of surgically excised non-melanoma skin cancers was permitted.
12. Any relevant condition, behaviour, laboratory value, or concomitant medication which, in the opinion of the Investigator, made the patient unsuitable for entry into the study.
13. Following pre-dose assessments, patients could be excluded from the study also for the following reasons:
 - Intercurrent illness or medically significant AEs.
 - Positive urine drug screen or alcohol breath test result, which suggested substance abuse and was not explained on the basis of prescription or over-the-counter medication that was used according to instruction.

9.2.6 Patient Withdrawal

Patients could be withdrawn after discussion with the Sponsor's Medical Monitor, if any of the following criteria were met:

- Any clinically relevant signs or symptoms which, in the opinion of the Investigator, warranted patient withdrawal.
- Positive urine drug screen or alcohol breath test result that suggested substance abuse and was not explained on the basis of prescription or over-the-counter medication used according to instruction.
- Intercurrent illness or any clinically relevant signs or symptoms which, in the opinion of the Investigator, warranted patient withdrawal.
- Clinically significant AEs which, in the opinion of the Investigator, warranted patient withdrawal.
- In case of an AE, study drug could be stopped for up to 5 days without the patient being withdrawn. If deemed safe and justifiable by the Investigator and by the Sponsor's medical representative, study drug could be reinstated at the previous dose within 5 days of discontinuation and the patient continued in the study. If intolerable AEs reappeared after reinstating medication at the previous dose, the patient was to be withdrawn rather than undergoing further drug-free periods.
- If a patient's corrected QT interval using Bazett's formula (QTcB) increased > 60 ms compared to Baseline or to an absolute QTcB value > 500 ms. In such cases, two repeat ECGs were to be immediately obtained, at least 2 minutes apart. A mean QTcB value was calculated from the three ECGs; if this mean value confirmed the increase in QTcB, then the mean QTcF was calculated for all three ECGs. If the stopping criterion was also met by QTcF, then the ECGs were manually read by a cardiologist, to confirm the QTc increase. If confirmed, the patient was withdrawn from further dosing (i.e., early termination) and would begin the safety Follow-up period.
- Liver function tests (either AST, ALT, or both) ≥ 4 times ULN reference range.
- If a patient had severe nausea and vomiting for an extended period.
- If a patient had severe diarrhoea for an extended period.
- If during treatment, patients had microscopic haematuria or proteinuria, in two uncontaminated samples at two consecutive visits during the study, the patient was to be

referred for general medical evaluation. If the assessment suggested renal dysfunction attributable to another cause, such as infection, treatment with study drug could continue if deemed appropriate by the Investigator and the other cause was not to be treated and the patient reassessed. If the microscopic haematuria or proteinuria was not attributable to another cause and, in the opinion of the Investigator was related to the study drug, the patient was discontinued and followed until resolution or an alternative cause was found.

- A patient was free to withdraw his or her consent to participate in the study, at any time, without explanation. In addition, the Investigator could decide, for reasons of medical prudence, to withdraw a patient. In either event, the Sponsor was notified and the date and reasons for the withdrawal clearly stated in the patient's CRF. If the randomisation code needed to be broken for a patient, the date, time and reason was recorded in the patient's CRF.
- If a patient was withdrawn, or chose to withdraw, the relevant post-study assessment was performed wherever possible according to the Schedule of Assessments outlined for the safety Follow-up period.
- Patients who prematurely withdrew from the study were not replaced.

9.2.7 Drug Dosing

The active study drug was orally administered as tablets of either 50 mg or 100 mg in strength with a glass of water in the morning. Placebo was administered in the same manner. To maintain blinding, each patient took two tablets each day of the study according to the scheme below:

Dose level (mg) selisistat Placebo

50mg: 1 x 50 mg tablets + 1 (placebo) tablet

200mg: 2 x 100 mg tablets

Placebo (0mg) - 2 tablets

Placebo was of identical pharmaceutical composition and appearance aside from containing no active drug substance. All tablets were both white, round, bi-convex coated tablets, 10 mm in diameter and 5 mm in thickness i.e. identical in size and appearance.

The treatment period was planned to last 12 weeks and comprised 7 visits (Day 1 [Baseline], Day 7 [Germany only], Week 2 [UK and Italy only], Week 4, Week 6, Week 8, Week 10, and Week 12). The

visit on Day 7 was scheduled within a time window of +/- 1 day, and the visits at Weeks 4, 8, and 12 were scheduled within a time window of +/- 4 days in relation to the intended date of the visit, with reference to Day 1. Similarly, the visits at Weeks 2, 6 and 10 should be scheduled within a time window of +/- 4 days in relation to the intended date of the visit, with reference to Day 1.

9.2.8 Blinding

An independent, unblinded statistician prepared the randomisation and pack lists based on a randomisation scheme. The active and placebo tablets were identical in size and appearance. The packaging for each respective preparation was identical with the same labelling (apart from the batch number and medication identification number) to ensure that neither the investigator nor the patient were aware of the treatment allocated.

Blinding was only to be broken in the case of a medical emergency where drug identification was necessary. In such an event, the Investigator was able to obtain details of the treatment assigned to an individual patient via the IVRS. Where possible and safe to do so, the Investigator could discuss the need to break the blind with the Medical Monitor. If an individual's treatment was unblinded, the patient was withdrawn from the trial.

9.2.9 Drug Group Assignment

Each patient was assigned to one of the three treatment groups (50 mg, 200 mg, or placebo) in a 1:1:1 ratio. Randomisation to treatment group, according to the patient randomisation list, and assignment of the study drug was managed by an interactive voice response system (IVRS).

9.2.10 Prior and Concomitant Therapy

With the exception of HDAC inhibitors (such as Zolinza®/vorinostat, belinostat), concomitant medications were allowed during the treatment phase. It was preferred that, whenever possible, the doses of concomitant medications remained stable during the trial, in order to prevent confounding of results. However, changes in concomitant medications that were medically necessary were not prohibited.

Medications taken within 21 days before the first dose of trial medication were documented as prior medication. Medications taken after the first dose of trial medication were documented as concomitant medications. Patients were questioned about concomitant medication at each visit to the clinic and the concomitant medication was entered into the CRF.

9.2.11 Treatment Compliance

To enhance treatment compliance, study drug was dispensed and instructions to the patient given under the supervision of suitably qualified site staff. Patients were instructed to return all study drugs packaging (empty, used and partially used) at each Visit (or End-of-Treatment [EOT] Visit).

9.2.12 Schedule of Assessments & Blood Tests

This is summarised below in **Table 14 and 15**.

Table 14- Schedule of assessments. Adapted from (Reilmann *et al.*, 2013)

Period (duration)	Screening (up to 21 days)	Treatment ^a (up to 12 weeks)					Follow-up ^b (4 weeks +/- 4 days)
		Day 1 (Baseline)	Day 7 (+ /- 4 days)*	Week 4 (+/- 4 days)	Week 8 (+/- 4 days)	Week 12/ EOT ^c (+/- 4 days)	
Study Day/Week Activity	Days -21 to -5						Week 16 (+/- 4 days)
Informed consent	X						
Inclusion/exclusion criteria	X						
Demographic data	X						
Medical/medication history	X						
Urine drugs of abuse screen and alcohol breath test	X	X	X	X	X	X	
Urine pregnancy test	X	X	X	X	X	X	X
CAG repeat length		X					
Randomisation (IVRS)		X					
Study drug dispensing		X		X	X		
Concomitant Medications Recording		X	X	X	X	X	X

Study drug reconciliation				X	X	X	X
Safety Parameters							
AE recording		X	X	X	X	X	X
Clinical laboratory tests^d	X	X	X	X	X	X	X
12-lead ECG^e	X	X*	X	X*	X	X	X
Physical examination	X	X	X	X	X	X	X
Neurological examination	X	X	X	X	X	X	X
Vital signs^f	X	X	X	X	X	X	X
UHDRS^g	X	X	X	X	X	X	X
C-SSRS^h	X	X	X	X	X	X	X
Efficacy Parameters							
Clinical (GCI)ⁱ	X	X		X	X	X	X
UHDRS Total Motor^j	X	X		X	X	X	X
Scale and functional Assessments							
Cognitive Battery tests^k	X	X		X	X	X	X
Pharmacokinetics		X		X		X	
Pharmacodynamic		X		X		X	

Abbreviations: CAG = cytosine, adenine, guanine; C-SSRS = Columbia-Suicide Severity Rating Scale; ECG = electrocardiogram; EOT = End of Treatment; GCI = Global Clinical Impression; Htt = Huntingtin protein; IVRS = Interactive Voice Response System; and UHDRS = Unified Huntington's Disease Rating Scale.

** Germany only.

a Unscheduled visits were possible at the discretion of the Investigator, for reason of safety or evaluation of AEs, or to repeat protocol-required procedures if necessary.

b The Follow-up Visit was done 4 weeks (+/- 4 days) after the last dose of study drug. The C-SSRS, 12-lead ECG, clinical laboratory tests (serum biochemistry, haematology and urinalysis), and physical and neurological examinations were only performed at the Follow-up Visit if there were any clinically significant abnormalities (from Baseline) at Week 12/EOT Visit, as judged by the Investigator.

c An EOT Visit was to be performed for patients who discontinued prematurely, whenever possible. Patients were instructed to return study drug at the EOT Visit.

d Clinical laboratory tests included haematology (white blood cell count, differential white blood cell count, red blood cell count, neutrophils, platelets, haemoglobin, haematocrit, mean cell volume, and mean cell haemoglobin); serum biochemistry (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, gamma glutamyl transferase, electrolytes, glucose, urea, total bilirubin, creatinine, total protein, and albumin); and urinalysis (colour, specific gravity, pH, protein, glucose, ketones, blood, nitrate, and

examination of urinary sediment for crystals, casts, red blood cells, white blood cells, and bacteria or yeast). Additional blood samples for serum biochemistry (safety monitoring of liver function tests) were collected and analysed at a local or central laboratory on 3 additional visits (UK and Italy) at Week 2 (+/- 4 days), Week 6 (+/- 4 days) and Week 10 (+/- days) (2 additional visits at Week 6 (+/- 4 days) and Week 10 (+/- 4 days) in Germany).

e ECGs were recorded after at least 5 minutes supine rest. At Screening, Day 1; at Weeks 4, 8, and 12; and at Follow-up, single recordings for safety evaluation were performed. (*) In addition, triplicate ECGs for analysis by central ECG laboratory were to be performed at Baseline (pre-dose) and at the Week 4 visit (both pre-dose and 3 hours post-dosing).

f Vital signs included supine and standing blood pressure and pulse rate, respiration rate, oral or tympanic body temperature and body weight.

g UHDRS tests included motor, cognitive, behavioural, independence, functional and total functional capacity assessments. Those individuals determining UHDRS ratings had current valid certification by the EHDN.

h The C-SSRS was done by certified staff members.

i GCI included both patient and clinician-based assessments.

j UHDRS efficacy assessment test consisted of the total motor scale. Those individuals determining UHDRS ratings had current valid certification by the EHDN.

k Cognitive Battery tests consist of the symbol digit modalities, Stroop word, verbal fluency and Mini-Mental State Examination tests. The verbal fluency test was described within the UHDRS form.

l A genetic test using the same Baseline genotyping blood sample was done to assess CYP2C19 and CYP2D6 polymorphisms in samples from patients who provided separate written consent for this analysis. Using the same sample, cytochrome (CYP) P450 2C19 and 2D6 polymorphisms will be assessed in patients that provide separate written consent.

Table 15- Blood Tests Schedule. (Reilmann *et al.*, 2013)

Study Day	Screening	Baseline (Day 1)	Day 7 (+/- 4 days)**	Week 4 (+/- 4 days)	Week 8 (+/- 4 days)	Week 12 (+/- 4 days)	Follow-up (+/- 4 days)
Visit	1	2		3	4	5	6
Assessment	Blood Volume (mL)						
CAG repeat length	-	6.0 ^d		-	-	-	-
Biochemistry ^a	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Haematology ^a	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Pharmacodynamics							
Soluble Htt ^b	-	16		8	-	8	8
Acetylated Htt ^b	-	16		8	-	8	8
Pharmacokinetics ^c	-	10		4	-	12	-
Total	7.5	55.5	7.5	27.5	7.5	35.5	23.5

Abbreviations: CAG = cytosine, adenine, guanine.

** Germany only.

a Serum biochemistry and haematology tests were performed pre-dose on Day 1, Day 7 (Germany only), at 4, 8 and 12 weeks and at Follow up. For safety monitoring of liver function tests, 2 additional in Germany and 3 in Italy and UK 5-mL blood samples (i.e., an additional 10 mL (Germany) or 15 mL (UK and Italy) of blood to that shown in the above table) for serum biochemistry were collected and analysed at a local or central laboratory on additional visits at Week 2 (+/- 4 days) (Italy and UK only), Week 6 (+/- 4 days), and Week 10 (+/- 4 days).

b Soluble Htt and acetylated Htt blood collection occurred at pre-dose and 4 hours post-dose on Day 1, but only at 4 hours post-dose at the visits at 4 and 12 weeks and at the Follow-up Visit. Blood collection was to be as close as possible to the specified timing (\pm 10 minutes) and the actual time of collection with regard to dosing was to be clearly noted in the CRF. No analysis was conducted. Development of a GCLP-validated and robust method is ongoing and it is planned that the collected samples will be analysed at a later date.

c PK blood collections were performed at pre-dose, 1, 2, 3, and 4 hours on Day 1; at pre-dose and 4 hours at Week 4; and at pre-dose, 1, 2, 3, 4 and 6 hours at Week 12. Blood collection was to be as close as possible to the specified timing (\pm 10 minutes) and the actual time of collection with regard to dosing w be clearly noted in the CRF. Blood samples for soluble HTT and acetylated HTT analysis will be collected on Day 1 (Baseline) at pre-dose and 4 hours post-dose, and during the Week 4, Week 12 and Follow-up visits at 4 hours post-dose.

d A genetic test using the same Baseline genotyping blood sample was done to assess CYP2C19 and CYP2D6 polymorphisms in samples from patients who provided separate written consent for this analysis.

9.2.13 Safety and Tolerability Assessments

Every effort was made to schedule and perform the procedures as closely as possible to the same time of day, giving consideration to appropriate posture conditions, practical restrictions and the other procedures to be performed at the same time point. The order of priority for scheduling procedures at each time point was:

1. Dosing.
2. Blood samples.
3. Urine samples.
4. Any other procedures.

Electrocardiograms were recorded before vital sign measurements.

9.2.13.1 Adverse Events

An AE was defined as any untoward medical occurrence in a patient, or clinical investigation patient administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE could, therefore, be any unfavourable and unintended sign, symptom, or disease temporally associated with the use of a drug (investigation) product, whether or not related to the drug (investigational) product.

For the purposes of this study, any detrimental change in the patient's condition, after signing the informed consent form and up to completion of the 4-week Follow-up period after the last administration of the study drug, could be considered an AE. Adverse event recording began on the first day of study drug intake (Day 1, Baseline).

All AEs that were on-going at the end of the study were to be followed up for 30 days after the last administration of the study drug, with the exception of any on-going study drug related AEs, which were to be followed until resolution, unless, in the Investigator's opinion, the AE was unlikely to resolve due to the patient's underlying disease. All AEs were tracked in the CRF.

The Investigator assessed the intensity of AEs based on the following definitions:

- Mild (awareness of sign or symptom, but easily tolerated)
- Moderate (discomfort sufficient to cause interference with normal activities)
- Severe (incapacitating, with inability to perform normal activities).

For an AE to be a suspected drug-related event, there had to be at least a reasonable possibility of a causal relationship between the study drug and the AE.

9.2.13.2 Serious Adverse Events

An SAE was defined as, but was not limited to, an event that:

- Resulted in death
- Was life-threatening
- Required inpatient hospitalisation or prolonged existing hospitalisation
- Resulted in persistent or significant disability/incapacity
- Was a congenital anomaly/birth defect
- Resulted in an important medical event that required medical intervention to prevent any of the above outcomes or was a medically important event or reaction according to Investigator's judgement.

Important medical events that did not result in death, were life-threatening or required hospitalisation could be considered a serious adverse drug experience, when based on appropriate medical judgement, when jeopardising the patient or resulting in the patient requiring medical or surgical intervention to prevent one of the outcomes listed in this definition. Serious AEs were recorded from the date when informed consent was signed if the Investigator considered the event to be related to a study procedure.

9.2.13.3 Pregnancy

Pregnancy itself was not regarded as an AE unless there was a suspicion that the study drug may have interfered with the effectiveness of a contraceptive medication. However, the outcome of all pregnancies that occurred during paternal

or maternal exposure to study drug (spontaneous miscarriage, elective termination, normal birth, or congenital abnormality) had to be followed up and documented even after the patient had been withdrawn from the study.

All reports of congenital abnormalities/birth defects were to be considered SAEs. Spontaneous miscarriages were also to be reported and handled as SAEs. Elective abortions without complications were not to be considered as AEs. All outcomes of pregnancy were to be reported to the Sponsor's Drug Safety vendor (Covance DSS) on a pregnancy outcomes report form.

9.2.13.4 Laboratory/Vital Sign/ECG Abnormalities

Laboratory/vital sign/ECG abnormalities were to be reported as AEs if any one of the following criteria were met:

- Any criterion for an SAE was fulfilled.
- The laboratory/vital sign abnormality caused the patient to discontinue study drug or discontinue from the study.
- The laboratory/vital sign abnormality caused the patient to interrupt study drug treatment.
- The laboratory/vital sign abnormality caused the patient to modify the dose of study drug.
- The Investigator believed that the abnormality should be reported as an AE.
- If an abnormal laboratory value or vital sign was associated with clinical signs and symptoms, the sign or symptom was to be reported as an AE and the associated laboratory result or vital sign was to be considered additional information that had to be collected on the relevant CRF.

In case of accidental overdosing of Selisistat, vital functions were to be monitored carefully. Special attention was to be given to cardiovascular (heart rate, blood pressure, and ECG parameters) and central nervous system functions.

9.2.13.5 Laboratory Safety Assessments

A central laboratory was used for the analysis of all urine and blood samples. A local or central laboratory was used for the additional serum biochemistry blood samples collected at Week 2 (the UK and Italy only), Week 6, and Week 10. These were collected, processed, and stored according to instructions that were provided to sites by the relevant laboratory.

The following clinical laboratory parameters were evaluated:

Haematology:

- White blood cell count (WBC) $10^9/L$.
- Differential WBC $10^9/L$ & %.
- Neutrophils $10^9/L$.
- Platelets $10^9/L$.
- Red blood cell count (RBC) $10^{12}/L$.
- Haemoglobin (Hb) g/dL.
- Haematocrit (packed cell volume [PCV]) %.
- Mean cell volume (MCV) fL.
- Mean cell haemoglobin (MCH) pg.

Serum biochemistry:

- AST IU/L.
- ALT IU/L.
- Alkaline phosphatase (ALP) IU/L.
- Gamma glutamyl transferase (GGT) IU/L.
- Electrolytes (sodium, potassium, chloride, calcium, and inorganic phosphate).
- Inorganic phosphate mmol/L.
- Glucose mmol/L.
- Urea mmol/L.
- Total bilirubin (total) $\mu\text{mol}/L$.
- Creatinine $\mu\text{mol}/L$.
- Total protein g/L.
- Albumin g/L.

Urinalysis:

- Colour.
- Specific Gravity.
- pH.
- Protein.
- Glucose.
- Ketones.
- Blood.
- Nitrate.

Examination of urinary sediment:

- Crystals, casts, RBC, WBC, and bacteria or yeast.

9.2.13.6 Electrocardiogram Assessments

The same equipment was to be used to collect standard 12-lead ECG recordings at the Baseline Visit and throughout the study. For safety monitoring, 12-lead ECGs were recorded at each visit after the patient had been supine for at least 5 minutes. A repeat ECG was then be performed after at least 5 additional minutes supine. A copy of these recordings was retained in the study site files.

In addition, triplicate ECGs for analysis by the central ECG laboratory were performed at Baseline (pre-dose) and at Week 4 (both pre-dose and 3 hours post-dosing).

A site physician performed a clinical assessment of each 12-lead safety ECG. The ECG machine computed the PR, QT and QTcF intervals, QRS duration, and heart rate (HR). Reference ranges were applied to all ECG parameters determined throughout the study. Patients were to be withdrawn from the study if abnormal QTcF intervals were recorded meeting the withdrawal criterion.

9.2.13.7 Physical Examination

A full physical examination (including body weight) was performed by a trained physician during each study visit (Screening, Day 1 [Baseline], Week 4, Week 8, and Week 12) and during Follow-up.

The physical examination included the following:

- General appearance.
- Abdomen.
- Cardiovascular systems.
- Lungs.
- Lymph nodes.
- Musculoskeletal and neurological systems.
- Skin.
- Extremities.
- Head, ears, eyes, nose, throat, mouth.
- Thyroid gland.
- Body weight (kg).

9.2.13.8 Neurological Examination

A standard neurological examination by a trained neurologist was performed as indicated in the Schedule of Assessments (**Table 14**). The frequency of the assessments could be increased if required in the opinion of the Investigator.

9.2.13.9 Vital Signs

At the Screening Visit and Baseline Visit (pre-dose), blood pressure, and pulse rate were measured in triplicate at approximately 2-minute intervals. The median value was used as the baseline value in the data analyses.

Vital signs were also assessed at other times if judged to be clinically appropriate or if the on-going review of the data suggested a more detailed assessment of vital signs was required. Patients had to be supine for at least 5 minutes before blood

pressure and pulse rate measurements. For the standing assessment, patients were asked to sit for 1 minute and then stand for 2 minutes prior to the measurement. Temperature was measured once either orally or tympanically as detailed.

9.2.13.10 Unified Huntington's Disease Rating Scale

The UHDRS, a clinician's assessment of the capacity to perform in each of five functional domains, was carried out for each patient and included the following:

- Motor.
- Cognitive.
- Behavioural.
- Independence.
- Functional.
- TFC (including occupation, finances, domestic chores, activities of daily living, and care level).

9.2.13.11 Columbia-Suicide Severity Rating Scale

The C-SSRS is a questionnaire used for suicide assessment (Columbia University Medical Centre website [<http://cssrs.columbia.edu/>]). It was developed by leading experts to assess both behaviour and ideation, appropriately assesses and tracks suicidal all events, and uniquely addresses the need for a summary measure of suicidality (FDA Advisory Committee Meeting, 2007).

9.2.13.12 Drug and Alcohol Screening

Patients were asked to provide urine samples for drugs of abuse screen, and undergo an alcohol breath test at the times indicated in the Schedule of Assessments (**Table 14**). The urine was screened for the presence of the following drugs of abuse: methadone, benzodiazepines, cocaine, amphetamines, methamphetamines, opiates, and barbiturates.

9.2.14 Clinical Assessments

9.2.14.1 Clinical Impression (Patient and Investigator)

A clinical impression was performed using a Global Clinical Impression (GCI) scale (patient and Investigator based).

9.2.14.2 Cognitive Battery Assessments

The effect of fatigue was minimised by conducting cognitive assessment as early as possible in the study visit day and was controlled for by keeping the time of day of the assessments as stable as possible across patients. The cognitive battery included:

- Symbol Digit Modalities Test.
- Stroop Word Test.
- Verbal fluency.
- MMSE.

9.2.14.3 DNA Genotyping

DNA genotyping samples were collected at Baseline to ascertain HD genotype and to identify genetic modifiers of HD, in particular genetic modifiers of age of onset, rate of progression, and phenotypic characteristics.

9.2.14.4 Pharmacokinetics

Blood samples for PK analysis of selisistat were collected at Baseline (Day 1) and during the Week 4 and 12 visits. *(Only descriptive pharmacokinetic data is presented as further detail is beyond the scope of this work.)*

9.2.14.5 Pharmacodynamic Measurements

Blood samples for PD assessments were collected at Baseline (Day 1) and during the Week 4, Week 12 and Follow-up visits. Pharmacodynamic samples were processed on-site without delay, to extract high quality peripheral blood mononuclear cell (PBMC) pellets for freezing. Samples were collected to assess the effect of selisistat on candidate pharmacodynamic markers (levels of soluble mutant Htt); however, no

analysis was performed as the previously developed methods were found to be suboptimal.

9.2.14.6 Biomarker Measurements

Samples for the PD biomarkers, the level of soluble HTT and the acetylation status of mutant HTT, were collected at pre-dose and 4 hours post-dose on Day 1, at 4 hours post-dose at both Weeks 4 and 12, and at the follow-up visit.

9.2.14.7 Genetic Assessments

The HD genotype and genetic modifiers of HD, and CYP2C19 and CYP2D6 isoenzymes will be listed by treatment and patient based on the safety analysis set.

9.2.14.8 Data Quality Assurance (QA)

The Investigators were provided with standardised CRFs and ensured that all data from patient visits were promptly entered into the CRFs in accordance with the specific instructions given. Investigators had to sign each CRF to verify the integrity of the data recorded. The Investigators maintained source documents, such as laboratory reports, X-rays, ECGs, consultation reports and complete medical history and physical examination reports.

The Sponsor/ICON implemented and maintained QC and (QA) procedures with written standard operating procedures (SOPs) to ensure that the study was conducted and data were generated, documented and reported in compliance with the protocol, GCP, and applicable regulatory requirements.

9.2.15 Statistical and Analytical Plans

The study sponsor indicated that due to the exploratory first in disease nature of the study, no formal statistical comparisons were planned or performed. *Further comparative statistical sub-analysis of change in clinical parameters between treatment groups was undertaken using an unpaired t-test, by myself with oversight from Dr Mariam Shoai, UCL, where indicated.*

9.2.15.1 Data Handling

For safety parameters, an end of treatment visit was defined as the last non-missing post-baseline record for each patient. There was no imputation for partial dates or times. However, imputation of partial dates was used to determine the treatment-emergent flag for AEs and the previous/concomitant flag for medications.

For efficacy analyses, and in case of missing post-baseline records occurring during the treatment, the last non-missing post-baseline observation was carried forward. If the first post-baseline observation was missing, a missing value was imputed. Records occurring after the end of the treatment date/ time were not imputed. Patients who withdrew from the study were summarised by treatment group according to their reason for withdrawal.

9.2.15.2 Demographic and Baseline Characteristics

All demographic and baseline characteristic data recorded at the Screening Visit were summarised by treatment group. Patient disposition was also summarised.

9.2.15.3 Drug Compliance

Compliance was calculated as: $100\% * [(sum\ of\ Number\ of\ tablets\ dispensed - sum\ of\ Number\ of\ tablets\ returned) / (overall\ exposure * 2)]$. Compliance was assessed both between visits and overall.

9.2.15.4 Safety Analysis

The primary objective was to assess the safety and tolerability of selisistat in HD patients following treatment for 12 weeks.

Adverse events volunteered throughout the study were classified per the Medical Dictionary for Regulatory Activities (MedDRA 14.0). Treatment-emergent adverse events (TEAEs) were defined as signs or symptoms that emerged during treatment or within 30 days of the last dose of study drug, including those signs and symptoms absent at pre-treatment or those that had worsened relative to pre-treatment status. No formal statistical comparisons were conducted.

Laboratory parameters were described by time point and treatment using descriptive statistics for continuous data. The change from baseline was summarised for each post baseline visit with descriptive statistics by treatment and time.

Vital signs (including supine and standing blood pressure), pulse rate, respiratory rate, body temperature, weight, height (Screening only), and body mass index [BMI] were summarised descriptively by treatment and time point.

All data from the physical and neurological examinations were presented in a listing. ECG parameters (HR, PR, QRS interval, QT, QTcF) were summarised descriptively by treatment and time point. Changes from baseline were summarised for all scheduled post baseline visits. Summary statistics also included the number and percentage of patients with Normal, Abnormal non-clinically significant (NCS) or Abnormal clinically significant (CS), by parameter and visit.

Continuous variables were summarised with the following descriptive statistics: n (Number of observations), mean, standard deviation (SD), median, minimum, and maximum. Categorical data were summarised with frequencies and percentages. Summary statistics were presented for each question of the C-SSRS separately at each time point.

9.2.15.5 Efficacy Analysis

All efficacy summaries were presented by treatment group and visit/time point not including the EOT visit.

Unified Huntington's Disease Rating Scale

Scores and changes from baseline scores (for post-baseline visits only) for each domain were summarised by treatment group using descriptive statistics at every planned visit.

Global Clinical Impression

The GCI scores (assessed by the patient and the clinician) for each of the three items ("How ill is the subject at this time", "GCI assessed by clinician", "GCI assessed by subject") were summarised for each assessor by treatment group using categorical

descriptive statistics at every planned visit. The same analysis were performed separately for each of the TFC subgroups (defined above) using the FAS only.

Mini-Mental State Examination

The MMSE score, as well as the change from baseline, were summarised at every planned Visit. The same analysis were performed separately for each of the TFC subgroups (defined above) using the FAS only.

The PD variables were:

- Change from Baseline levels of soluble HTT
- Change from Baseline in acetylation status of mutant HTT

The variables for genetic assessments are:

- HD genotype and genetic modifiers of HD
- CYP2C19 and CYP2D6 isoenzyme genotype

9.2.15.6 Determination of Sample Size

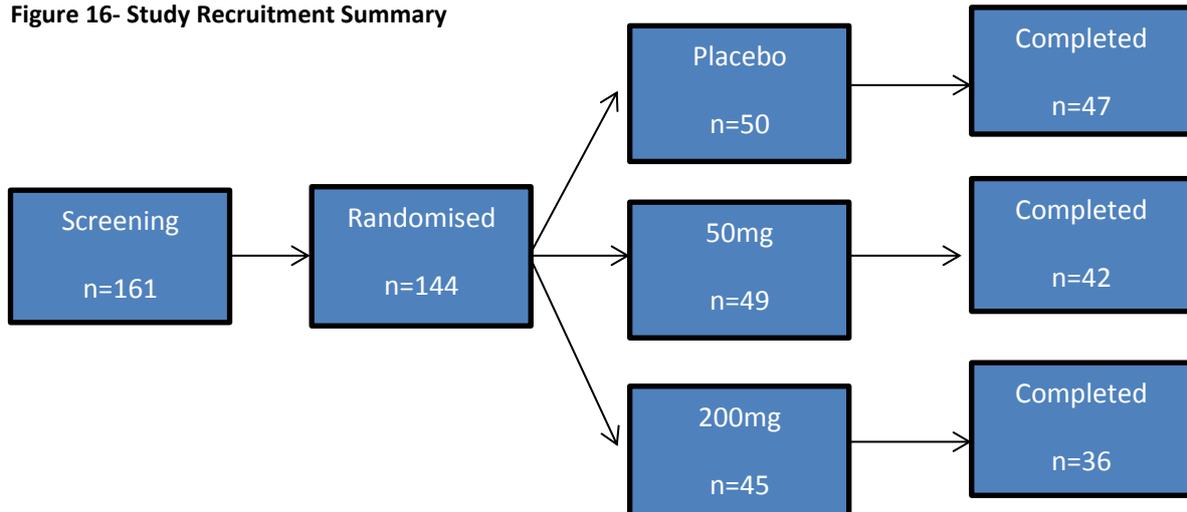
The sample size of 120 patients (40 patients per treatment group) was based on clinical judgement and/or practical considerations and not on statistical considerations. However, with 40 patients per treatment group, there was an 80.0% probability of detecting an AE with an underlying incidence rate of 0.04. The proposed number of patients was also chosen to generate PD data to support dose selection for further studies.

9.3 Results (Reilmann *et al.*, 2013)

9.3.1 Study Summary

161 patients were screened and 144 randomised to the three treatment arms, placebo, 50mg and 200mg of the study drug with 125 patients (86.8%) completing the study (**Figure 16**). The dropout

Figure 16- Study Recruitment Summary



rate between these different arms did not reach statistical significance though one notes that the percentage of patients completing the study in the higher dose group was lower than the others. This dropout was related to higher incidence of adverse effects though again this did not reach statistical significance.

9.3.2 Demography

Patient demography data collected at the Screening Visit are presented below (**Table 16 & Figure 17**).

Table 16- Demographic characteristics at screening visit. Adapted from (Reilmann *et al.*, 2013)

	Placebo	SEN0014196 50 mg	SEN0014196 200 mg	Total
	N=50	N=49	N=45	N=144
Age (years)^a				
n	50	49	45	144
Mean (SD)	50.9 (10.29)	52.4 (9.98)	48.0 (7.50)	50.5 (9.50)
Sex				
Male	32 (64.0)	24 (49.0)	24 (53.3)	80 (55.6)
Female	18 (36.0)	25 (51.0)	21 (46.7)	64 (44.4)
Race^b				
Asian	1 (2.0)	0 (0.0)	1 (2.2)	2 (1.4)
White	49 (98.0)	49 (100.0)	44 (97.8)	142 (98.6)
Height (cm)				
n	50	49	45	144
Mean (SD)	172.29 (9.063)	169.85 (10.093)	172.24 (9.322)	171.44 (9.508)
Weight (kg)				
n	49	49	45	143
Mean (SD)	70.09 (11.954)	73.03 (13.841)	71.94 (11.986)	71.68 (12.613)
BMI (kg/m²)^c				
n	49	49	45	143
Mean (SD)	23.61 (3.423)	25.23 (3.766)	24.18 (2.949)	24.34 (3.451)

Abbreviations: BMI = Body mass index; SD = standard deviation.

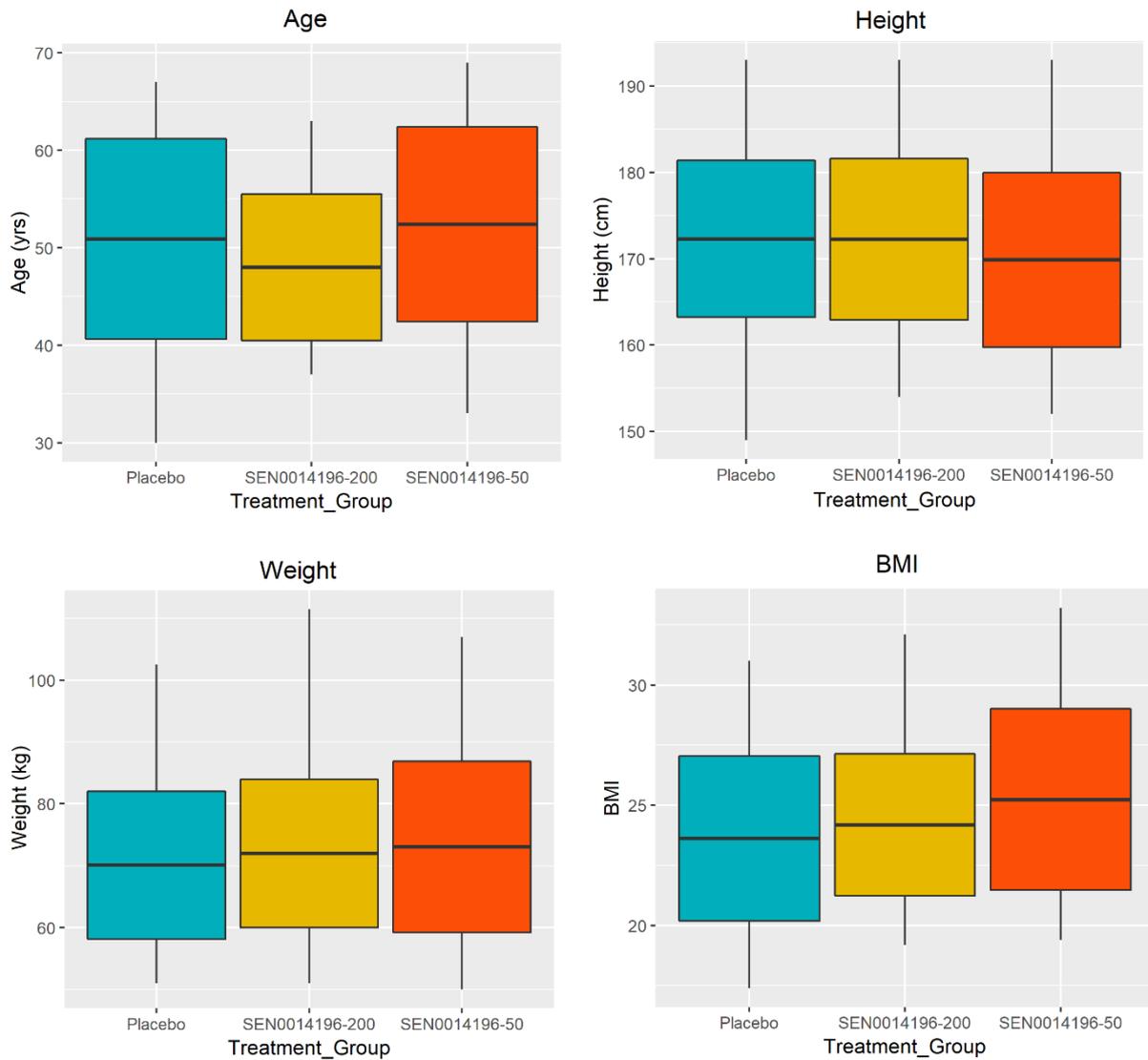
a Age was defined as the integer value of the number of years between the date of screening and the date of birth, divided by 365.25.

b Multi-racial patients were counted in each race category they listed.

c BMI was calculated as weight (kg) / [(height (cm)/100)²].

While no major differences were seen between treatment arms in relation to age, race, height, weight or BMI, one notes that a higher proportion of males were present in the study (55.6% to 44.4%) with this most pronounced in the placebo group (64% to 36%). Additionally only two non-Caucasian patients were enrolled.

Figure 17- Boxplot of Selected Demographic Data across Treatment Groups



9.3.3 Other Baseline Characteristics

All patients had a CAG repeat length of ≥ 36 . The most frequent medical history was surgical and medical procedures (125 patients [86.8%] in total). The most frequent procedures were appendectomy (23 patients [16.0%] in total), tonsillectomy (11 patients [7.6%] in total) and hysterectomy and vasectomy (both nine patients [6.3%] in total). There were no notable differences between the treatment groups.

The next most frequently experienced medical history was the psychiatric disorders, affecting 85 patients (59.0%) in total, of which 36 patients were in the placebo group, compared to 25 patients and 24 patients in the selisstat 50 mg and 20 mg groups, respectively. The most frequent disorders were depression (50 patients [34.7%] in total) and sleep disorder (11 patients [7.6%] in total). There were no other notable differences between the treatment groups.

9.3.4 Prior and Concomitant Medications

Prior medications were those medications taken within 21 days before the first dose of study drug. The most frequently taken of these medications were antidepressants, pain relief medications and dietary supplements. Concomitant medications taken during the treatment period by more than 5% of patients in any treatment group are summarised below (**Table 17**).

Table 17- Prior and concomitant medications. Adapted from (Reilmann *et al.*, 2013)

	Placebo	SEN0014196	SEN0014196	Total
	N=50	50 mg N=49	200 mg N=45	N=144
Any concomitant Medication	48 (96.0)	4 6 (93.9)	42 (93.3)	136 (94.4)
Citalopram	16 (32.0)	9 (18.4)	12 (26.7)	37 (25.7)
Vitamins, other Combination	9 (18.0)	7 (14.3)	7 (15.6)	23 (16.0)
Tetrabenazine	10 (20.0)	5 (10.2)	6 (13.3)	21 (14.6)
Tiapride hydrochloride	10 (20.0)	5 (10.2)	6 (13.3)	21 (14.6)
Mirtazapine	7 (14.0)	3 (6.1)	7 (15.6)	17 (11.8)
Olanzapine	7 (14.0)	5 (10.2)	4 (8.9)	16 (11.1)
Etizolam	4 (8.0)	3 (6.1)	7 (15.6)	14 (9.7)
Lorazepam	5 (10.0)	4 (8.2)	4 (8.9)	13 (9.0)
Paracetamol	2 (4.0)	7 (14.3)	4 (8.9)	13 (9.0)
Risperidone	5 (10.0)	3 (6.1)	3 (6.7)	11 (7.6)
Levothyroxine sodium	2 (4.0)	4 (8.2)	4 (8.9)	10 (6.9)
Ascorbic acid	5 (10.0)	3 (6.1)	2 (4.4)	10 (6.9)
Ubidecarenone	4 (8.0)	4 (8.2)	2 (4.4)	10 (6.9)
Ibuprofen	4 (8.0)	4 (8.2)	1 (2.2)	9 (6.3)
Sertraline	3 (6.0)	3 (6.1)	3 (6.7)	9 (6.3)
Sulpiride	3 (6.0)	0	6 (13.3)	9 (6.3)
Acetylsalicylic acid	3 (6.0)	3 (6.1)	2 (4.4)	8 (5.6)

Anti-depressant medications in the form of Citalopram and anti-chorea drugs in the form of Tetrabenazine and tiapride were the most common class of medications used in the study, alongside multivitamins. Tetrabenazine and tiapride hydrochloride occurred more commonly

in the placebo group (both 20.0%) than in the selisstat 50 mg group (10.2%) or the selisstat 200 mg group (both 13.3%). There was no notable difference between the proportions of patients taking vitamins in each treatment group.

In terms of potential impact of concomitant drugs on the study drug, there were no patients taking drugs with a similar mode of action to the study drug or on enzyme inducing medications.

Both Citalopram and Tetrabenazine were taken more frequently within the placebo arm of the trial and a difference here could affect comparative analysis of motor and mood readouts. However on sub-analysis via unpaired t-test, no statistically significant difference in concomitant medication use was found between the various arms of the trial (**Table 18 & 19**).

Table 18- Comparative Analysis of Citalopram Use across treatment groups

Test	Difference of percentage	Std Error	t-value	p-value
placebo vs 50mg	9.8	0.0712	1.376	0.1704
placebo vs 200mg	6.7	0.0734	1.21	0.2278
200 vs 50mg	3.3	0.0666	-0.47	0.6406

Table 19- Comparative Analysis of Tetrabenazine/Tiapride Use across treatment groups

Test	Difference of percentage	Std Error	t-value	p-value
placebo vs 50mg	9.8	0.0712	1.376	0.1704
placebo vs 200mg	6.7	0.0734	1.21	0.2278
200 vs 50mg	3.3	0.0666	-0.47	0.6406

9.3.5 Treatment Compliance

Table 20- Treatment Compliance. Adapted from (Reilmann *et al.*, 2013)

	Placebo N=50	SEN0014196 50 mg N=49	SEN0014196 200 mg N=45	Total N=144
Compliance (%)				
N	50	49	45	144
Mean (SD)	100.31 (2.360)	99.73 (1.465)	99.66 (2.714)	99.91 (2.232)
Compliance (%)				
80% - 120%	50 (100.0)	49 (100.0)	45 (100.0)	144 (100.0)

Compliance with all arms was above 90% in all groups with no apparent significant differences between groups (**Table 20**).

9.3.6 Efficacy Analysis

9.3.6.1 Introduction

Data is presented here using descriptive statistics carried out by the study sponsor as per study report (Reilmann *et al.*, 2013). *Further comparative statistical sub-analysis of change in means scores between treatment groups was undertaken by myself using an unpaired t-test where indicated.*

9.3.6.2 United Huntington's Disease Motor Rating Scale

The UHDRS '99 Motor score is a clinical assessment widely employed by HD clinicians to quantify to motor burden of disease (**Appendix 13.1**).

Table 21- Change in UHDRS across treatment groups and time-points. Adapted from (Reilmann *et al.*, 2013)

Visit	Descriptive Measure	Placebo		SEN0014196 (50 mg)		SEN0014196 (200 mg)		Total	
		N=50		N=49		N=45		N=144	
		Value at visit	ΔUHDRS	Value at visit	ΔUHDRS	Value at visit	ΔUHDRS	Value at visit	ΔUHDRS
Baseline	N	50	-	49	-	45	-	144	-
	Mean (SD)	41.2 (16.69)	-	40.9 (19.26)	-	39.7 (16.51)	-	40.6 (17.44)	-
Week 4	N	49	49	48	48	43	43	140	140
	Mean (SD)	41.8 (16.48)	0.4 (4.88)	41.8(18.78)	0.3 (4.58)	41.4 (16.49)	0.8 (5.17)	41.7 (17.18)	0.5 (4.84)
Week 8	N	49	49	48	48	43	43	140	140
	Mean (SD)	42.3 (17.51)	0.9 (5.49)	42.3 (19.51)	0.8 (6.59)	41.2 (17.20)	0.7 (6.44)	42.0 (18.01)	0.8 (6.13)
Week 12	N	49	49	48	48	43	43	140	140
	Mean (SD)	42.9 (17.98)	1.5 (7.15)	42.3 (19.21)	0.8 (5.67)	41.4 (18.38)	0.8 (6.99)	42.2 (18.41)	1.0 (6.59)

On a descriptive level, the scores were generally comparable across treatment groups and time-points (Table 21 & Figure 18).

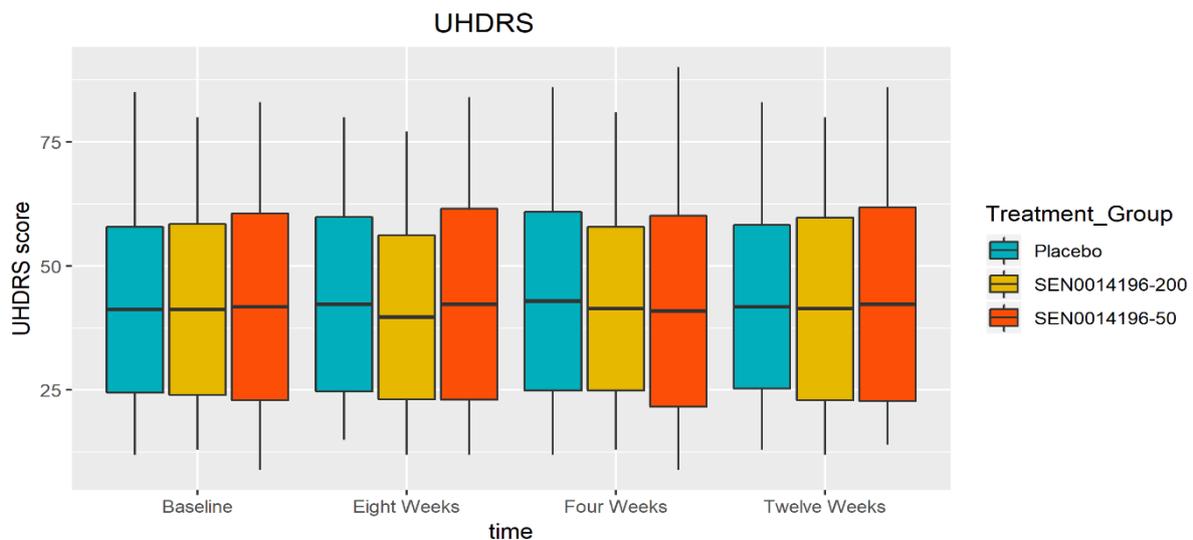


Figure 18- Mean UHDRS Motor Score across Treatment Groups and Time-points

Table 22- Comparative analysis of difference in UHDRS Score in treatment groups at Baseline and Week 12

t	Test	Difference of means	Std Error	t-value	p-value
Baseline	placebo vs 50mg	0.3000000	3.62511809	0.08275592	0.93422065
	placebo vs 200mg	1.5000000	3.4100524	0.4398759	0.6610569
	200 vs 50mg	-1.2000000	3.6915708	-0.3250649	0.7458734
Week 12	placebo vs 50mg	0.6000000	3.7796245	0.1587459	0.8742086
	placebo vs 200mg	1.5000000	3.8018338	0.3945464	0.6941332
	200 vs 50mg	-0.9000000	3.9426366	-0.2282736	0.8199595

A sub-analysis performed confirms that there were no significant differences in UHDRS motor score of the three arms of the trial at baseline. Unfortunately, there were also no significant changes in the motor symptoms of those treated for 12 weeks with either 200mg or 50mg of selisistat (**Table 22**).

9.3.6.3 Cognitive Assessment

Although the verbal fluency test score improved modestly from Baseline to Week 12 in all treatment arms, this did not reach significance.

The SDMT tests visuomotor integration, visual scanning, tracking and motor speed. The examinee is given 90 seconds to match symbols and digits as quickly as possible. The key (specifying which number corresponds to each symbol) is located at the top of the page. There was no discernibly significant change in symbol digit modality score (**Table 23 & 24 & Figure 19**) between treatment groups and across time-points with sub-analysis not revealing any significant differences.

Table 23- Change in SDMT scores across treatment groups and timepoints.

Adapted from (Reilmann *et al.*, 2013)

Visit	Placebo		SEN0014196 50 mg		SEN0014196 200 mg		Total	
	N=50		N=49		N=45		N=144	
	Value at visit	Change from Baseline	Value at visit	Change from Baseline	Value at visit	Change from baseline	Value at visit	Change from Baseline
Symbol Digit Modalities Test								
Baseline								
N	50		49		43		142	
Mean (SD)	21.2 (11.64)		24.3 (15.10)		25.5 (10.97)		23.6 (12.80)	
Week 4								
N	49	49	48	48	41	41	138	138
Mean (SD)	21.8 (11.66)	0.8 (3.73)	23.4 (13.80)	-0.3 (4.03)	24.0 (10.38)	-0.7 (3.84)	23.0 (12.05)	-0.0 (3.89)
Week 8								
N	49	49	48	48	41	41	138	138
Mean (SD)	21.9 (11.83)	0.8 (4.27)	23.8 (16.01)	0.0 (4.95)	24.1 (11.79)	-0.7 (4.53)	23.2 (13.36)	0.1 (4.60)
Week 12								
N	49	49	48	48	41	41	138	138
Mean (SD)	21.7 (10.65)	0.6 (4.37)	24.4 (15.88)	0.6 (4.23)	24.3 (12.41)	-0.4 (5.10)	23.4 (13.14)	0.3 (4.54)

Figure 19- SDMT Score between treatment groups across time-points

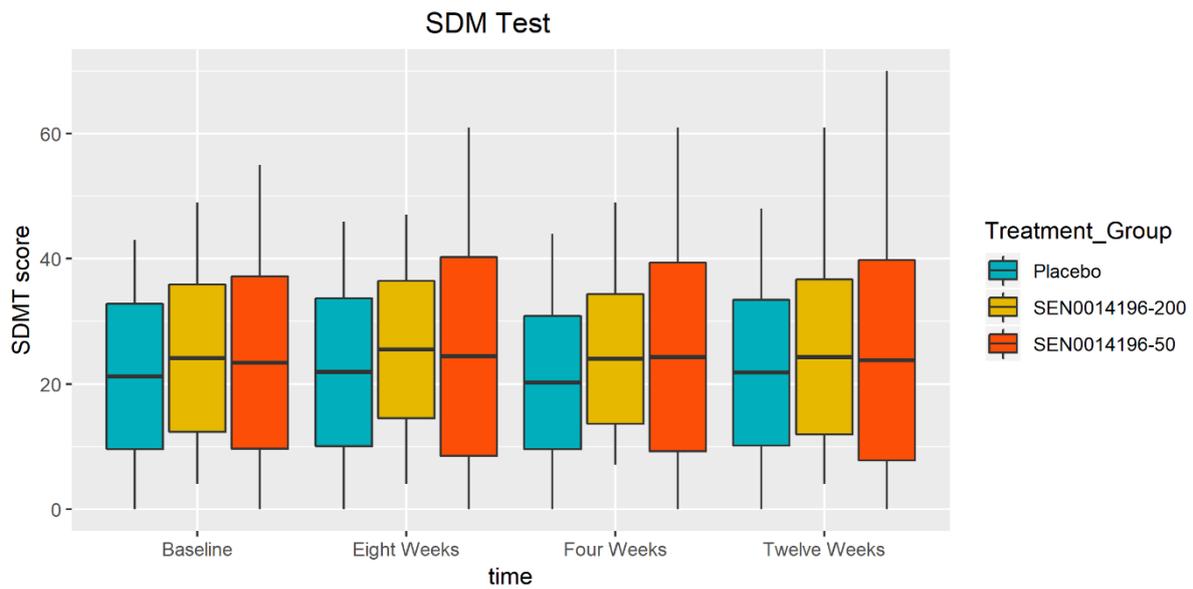


Table 24- Comparative Analysis of change in SDMT between treatment groups at baseline and week 12

t	Test	Difference of means	Std Error	t-value	p-value
Baseline	placebo vs 50mg	-3.1000000	2.7134954	-1.1424379	0.2562951
	placebo vs 200mg	-4.3000000	2.32035218	-1.85316696	0.06703632
	200 vs 50mg	1.2000000	2.7069369	0.4433055	0.6586390
Week 12	placebo vs 50mg	-2.7000000	2.7510686	-0.9814368	0.3292656
	placebo vs 200mg	-2.600000	2.462720	-1.055743	0.294287
	200 vs 50mg	-0.1000000	3.00064614	-0.03332616	0.97349141

The Stroop Test has three conditions that require visual scanning, cognitive control and processing speed. Subjects are given a card on which the names of colours are printed in black ink and must read as many words as they are able in 45 seconds. The sub- analysis confirms that there were no significant differences in stroop word test of the three arms of the trial at baseline. However, the results do suggest that the two treatment arms of the trial are tending towards significance. That is, those treated had a higher score than those in the placebo arm at baseline.

However, after 12 weeks, their scores are less different (unpaired t-test). There were no significant changes in the score for those treated for 12 weeks with either 200mg or 50mg of selisstat (**Table 25 & 26 & Figure 20**).

Table 25- Change in Stroop scores across treatment groups and time-points.

Adapted from (Reilmann *et al.*, 2013)

Visit	Descriptive Measure	Placebo		SEN0014196 (50 mg)		SEN0014196 (200 mg)		Total	
		N=50		N=49		N=45		N=144	
		Value at visit	Δ stroop	Value at visit	Δ stroop	Value at visit	Δ stroop	Value at visit	Δ stroop
Baseline	N	50	-	49	-	45	-	144	-
	Mean (SD)	52.0 (21.04)	-	57.9 (24.75)	-	58.8 (21.51)	-	56.1 (22.56)	-
Week 4	N	49	49	48	48	43	43	140	140
	Mean (SD)	53.1 (20.76)	1.7 (8.89)	56.1 (22.96)	-1.4 (9.47)	54.6 (21.28)	-2.5 (6.89)	54.6 (21.57)	-0.7 (8.66)
Week 8	N	49	49	48	48	43	43	140	140
	Mean (SD)	51.0 (22.17)	-0.4 (12.47)	54.8 (25.87)	-2.7 (13.08)	55.1 (20.54)	-1.9 (9.44)	53.6 (22.96)	-1.7 (11.81)
Week 12	N	49	49	48	48	43	43	140	140
	Mean (SD)	50.3 (21.47)	-1.1 (11.97)	53.3 (24.59)	-4.2 (11.06)	54.4 (22.63)	-2.6 (9.17)	52.6 (22.84)	-2.6 (10.86)

Table 26- Comparative Analysis of change in Stroop Score in treatment groups at Baseline and 12 weeks

t	Test	Difference of means	Std Error	t-value	p-value
Baseline	placebo vs 50mg	-5.9000000	4.6211370	-1.2767421	0.2048411
	placebo vs 200mg	-6.8000000	4.3744042	-1.5544974	0.1235185
	200 vs 50mg	0.9000000	4.7731599	0.1885543	0.8508586
Week 12	placebo vs 50mg	-3.0000000	4.6909079	-0.6395350	0.5240513
	placebo vs 200mg	-4.1000000	4.6170402	-0.8880148	0.3769802
	200 vs 50mg	1.1000000	4.9504492	0.2222021	0.8246661

Figure 20- Stroop Score between treatment groups and across time-points.



9.3.6.4 Problem Behaviours Assessment

The goal of the Problem Behaviours Assessment (PBA) is to assess the following ten symptoms: low mood (i.e. depression), suicidal ideation, anxiety, irritability, angry out-bursts and aggressive behaviour, lack of motivation (apathy, social and household activities, enthusiasm/spontaneity), perseveration, paranoid thinking/delusions, hallucinations and behaviour suggesting disorientation. Each symptom is rated for severity and frequency on a scale from 0 (absent and never) to 4 (severe and always).

The behavioural assessment scores at Baseline were comparable cross-sectionally, and were modestly better longitudinally from Baseline to Week 12 (**Table 27**).

Table 27- Change in Problem Behaviour Assessment across treatment groups and time-points.

Adapted from (Reilmann *et al.*, 2013)

Visit	Placebo		SEN0014196 50 mg		SEN0014196 200 mg		Total		
	N=50		N=49		N=45		N=144		
	Value at visit	Change from baseline	Value at visit	Change from Baseline	Value at visit	Change from Baseline	Value at visit	Change from Baseline	
Problem Behaviour Assessment									
Baseline									
n		50		49		45		144	
Mean (SD)		10.8 (9.72)		11.3 (10.50)		8.3 (8.24)		10.2 (9.59)	
Week 4									
n		49	49	48	48	43	43	140	140
Mean (SD)		9.6 (8.60)	-0.9 (4.83)	10.4 (10.19)	-1.1 (4.44)	7.8 (7.36)	-0.7 (5.03)	9.3 (8.84)	-0.9 (4.73)
Week 8									
n		49	49	48	48	43	43	140	140
Mean (SD)		9.8 (8.95)	-0.7 (4.46)	9.9 (9.95)	-1.7 (6.43)	6.7 (7.17)	-1.8 (5.55)	8.9 (8.87)	-1.4 (5.51)
Week 12									
n		49	49	48	48	43	43	140	140
Mean (SD)		10.0 (8.90)	-0.4 (5.07)	10.1 (10.15)	-1.4 (5.87)	7.7 (7.39)	-0.9 (6.10)	9.4 (8.94)	-0.9 (5.65)

A sub-analysis of PBA scores across treatment groups at baseline and week 12 failed to identify a statistically significant difference (**Table 28**).

Table 28- Comparative analysis of difference of PBA score across treatment groups at baseline and week 12

t	Test	Difference of means	Std Error	t-value	p-value
Baseline	placebo vs 50mg	-0.5	2.03459	-0.2451	0.806
	placebo vs 200mg	2.5	1.8434	1.3561	0.17835
	200 vs 50mg	-3	1.9387	-1.547	0.125
Week 12	placebo vs 50mg	-0.1	1.9398	-0.05155	0.95899
	placebo vs 200mg	2.3	1.689	1.3537	0.1792
	200 vs 50mg	-2.4	1.848	-1.298	0.1976

9.3.6.5 Independence & Functional Assessment

The independence and functional assessment scale is a percentage based assessment of independence- 100% representing complete independence, looking specifically at performance in 25 areas of daily life (**Appendix 13.5**). There was no apparent change in the mean independence scale and functional assessment scores from Baseline to Week 12 (**Table 29 & 30 & Figure 21**).

Table 29- Change in Independence Scale across treatment groups and time-points.

Adapted from (Reilmann *et al.*, 2013)

Visit	Placebo		SEN0014196 50 mg		SEN0014196 200 mg		Total	
	N=50		N=49		N=45		N=144	
	Value at visit	Change from Baseline	Value at visit	Change from Baseline	Value at visit	Change from baseline	Value at visit	Change from baseline
Independence Scale Assessment								
Baseline								
n	50		49		45		144	
Mean (SD)	5.1 (2.89)		5.0 (3.25)		4.3 (2.28)		4.8 (2.85)	
Week 4								
N	49	49	48	48	43	43	140	140
Mean (SD)	5.0 (2.80)	0 (0.87)	5.0 (3.31)	0 (0.71)	4.3 (2.33)	-0.1 (0.78)	4.8 (2.85)	-0.1 (0.78)
Week 8								
n	49	49	48	48	43	43	140	140
Mean (SD)	5.2 (2.92)	0.1 (1.08)	4.9 (3.23)	-0.1 (0.76)	4.3 (2.23)	-0.1 (0.86)	4.8 (2.85)	-0.0 (0.91)
Week 12								
n	49	49	48	48	43	43	140	140
Mean (SD)	5.0 (2.94)	-0.1 (0.97)	4.9 (3.20)	-0.1 (0.81)	4.4 (2.31)	0.0 (0.95)	4.8 (2.85)	-0.1 (0.90)

Table 30- Change in Functional Assessment Scores across treatment groups and time-points.

Adapted from (Reilmann *et al.*, 2013)

Visit	Placebo N=50		SEN0014196 50 mg N=49		SEN0014196 200 mg N=45		Total N=144	
	Value at visit	Change from baseline	Value at visit	Change from baseline	Value at visit	Change from baseline	Value at visit	Change from baseline
Functional Assessment								
Baseline								
n	50		49		45		144	
Mean (SD)	19.2 (5.60)		19.2 (5.97)		20.5 (4.55)		19.6 (5.43)	
Week 4								
n	49	49	48	48	43	43	140	140
Mean (SD)	19.0 (5.79)	-0.2 (1.60)	19.3 (5.97)	0.2 (0.87)	20.4 (4.50)	0.0 (0.65)	19.5 (5.49)	0.0 (1.14)
Week 8								
n	49	49	48	48	43	43	140	140
Mean (SD)	19.0 (5.98)	-0.3 (1.73)	19.5 (5.72)	0.4 (1.20)	19.6 (5.48)	-0.7 (3.44)	19.3 (5.71)	-0.2 (2.30)
Week 12								
n	49	49	48	48	43	43	140	140
Mean (SD)	19.1 (5.80)	-0.1 (1.74)	19.3 (5.79)	0.2 (1.21)	20.3 (4.37)	-0.0 (0.91)	19.6 (5.38)	0.0 (1.35)

Figure 21- Functional Assessment Score between treatment groups and across time-points



9.3.6.6 Total Functional Capacity Assessment

This is a clinical staging tool based on functional ability and scored out of a maximum of 13 (Table 2 Page 24). The functional capacity assessment scores were comparable from Baseline to Week 12 (Table 31 & Figure 22).

Table 31- Total Functional Capacity change from baseline at each visit. Adapted from (Reilmann *et al.*, 2013)

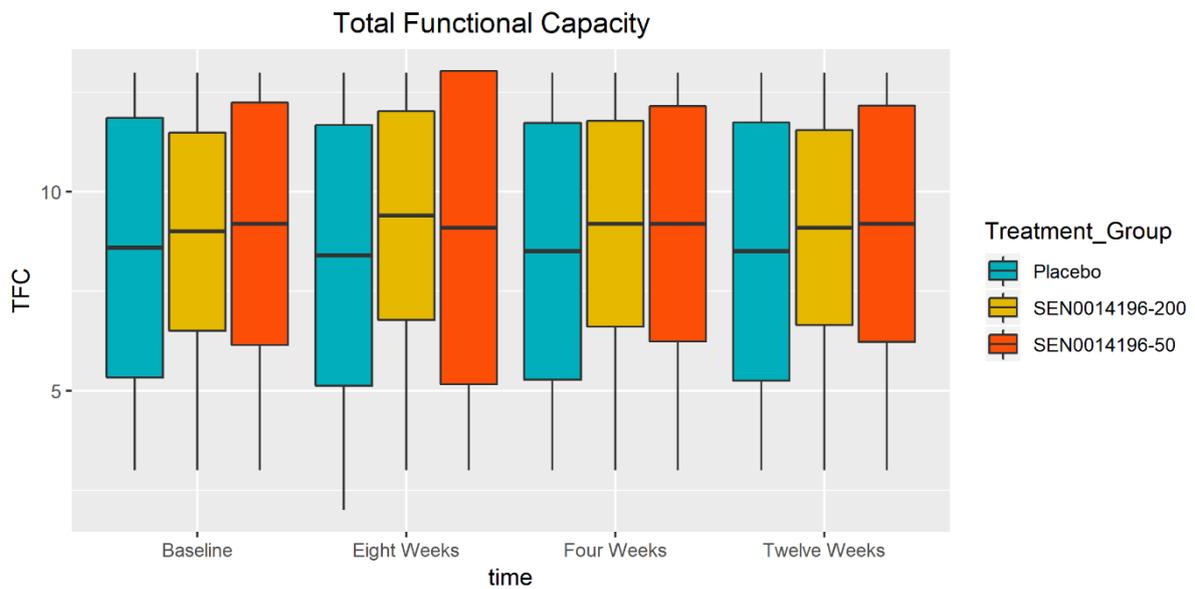
Visit	Placebo		SEN0014196 50 mg		SEN0014196 200 mg		Total	
	N=50		N=49		N=45		N=144	
	Value at visit	Change from baseline	Value at visit	Change from baseline	Value at visit	Change from baseline	Value at visit	Change from baseline
Total Functional Capacity								
Baseline								
n	50		49		45		144	
Mean (SD)	8.6 (3.27)		9.2 (2.96)		9.4 (2.63)		9.0 (2.98)	
Week 4								
n	49	49	48	48	43	43	140	140
Mean (SD)	8.5 (3.25)	-0.1 (0.62)	9.2 (3.05)	0.0 (0.62)	9.2 (2.59)	-0.0 (0.67)	9.0 (2.99)	-0.0 (0.63)
Week 8								
n	49	49	48	48	43	43	140	140
Mean (SD)	8.4 (3.28)	-0.2 (0.79)	9.2 (2.97)	0.0 (0.83)	9.0 (2.49)	-0.2 (0.89)	8.9 (2.95)	-0.1 (0.83)
Week 12								
n	49	49	48	48	43	43	140	140
Mean (SD)	8.5 (3.23)	-0.1 (0.62)	9.1 (2.94)	-0.0 (0.77)	9.1 (2.45)	-0.2 (0.92)	8.9 (2.90)	-0.1 (0.77)

Sub-analysis of difference between means of TFC scores across treatment arms at baseline and week 12 failed to identify any statistically significant change (**Table 32**).

Table 32- Comparative analysis of difference of TFC scores across treatment groups at baseline and week 12

t	Test	Difference of means	Std Error	t-value	p-value
Baseline	placebo vs 50mg	-0.6	0.6266	-0.9575	0.3407
	placebo vs 200mg	-0.8	0.606	-1.3195	0.19027
	200 vs 50mg	0.2	0.5766	0.3468	0.7295
Week 12	placebo vs 50mg	-0.6	0.62689	-0.957	0.34096
	placebo vs 200mg	-0.6	0.5937	-1.0105	0.3149
	200 vs 50mg	0	0.5639	0	1

Figure 22- TFC Score between treatment groups and across time-points



9.3.6.7 GCI Assessment

Throughout the study, the GCI did not alter to a meaningful extent.

Clinician Assessment

Overall there was no change in the clinician assessment of most of the patients during the course of the study.

Patient Assessment

The majority of patients themselves overall reported no change to their condition.

9.3.6.8 MMSE

Similar MMSE scores were noted across treatment groups and timepoints (**Table 33**).

Table 33- Change in MMSE score across treatment groups and time-points.

Adapted from (Reilmann *et al.*, 2013)

Visit	Placebo N=50		SEN0014196 50 mg N=49		SEN0014196 200 mg N=45		Total N=144	
	Value at visit	Change from baseline	Value at visit	Change from baseline	Value at visit	Change from baseline	Value at visit	Change from baseline
Baseline								
n	50		49		45		144	
Mean (SD)	25.2 (3.70)		25.2 (4.85)		26.3 (2.88)		25.5 (3.93)	
Week 4								
n	49	49	48	48	43	43	140	140
Mean (SD)	25.9 (4.21)	0.7 (2.20)	25.6 (4.38)	0.5 (1.40)	26.5 (3.26)	0.3 (2.38)	26.0 (3.99)	0.5 (2.02)
Week 8								
n	49	49	48	48	43	43	140	140
Mean (SD)	25.4 (3.73)	0.2 (1.85)	25.4 (4.55)	0.3 (1.82)	26.4 (3.05)	0.2 (2.33)	25.7 (3.85)	0.2 (1.99)
Week 12								
n	49	49	48	48	43	43	140	140
Mean (SD)	26.1 (3.90)	0.9 (1.72)	25.3 (4.69)	0.2 (2.10)	26.4 (3.11)	0.2 (2.53)	25.9 (3.98)	0.5 (2.14)

9.3.6.9 Pharmacokinetic Data

Rapid peak concentrations were achieved after 1 to 2 hours post dosing at both drug doses. Higher drug levels were noted in females versus males across time at both dose levels on Day 1 and Week 12.

9.3.6.10 Safety

Table 34- Adverse Events. Adapted from (Reilmann *et al.*, 2013)

	Placebo N=50		SEN0014196 50 mg N=49		SEN0014196 200 mg N=45		Total N=144	
	Events	Patients	Events	Patients	Events	Patients	Events	Patients
Any TEAEs	54	25 (50.0)	87	37 (5.5)	64	32 (71.1)	205	94 (65.3)
TEAEs leading to study discontinuation	1	1 (2.0)	7	7 (14.3)	12	8 (17.8)	20	16 (11.1)
Any related TEAEs	8	5 (10.0)	25	16 (32.7)	29	18 (40.0)	62	39 (27.1)
Any SAEs	3	2 (4.0)	3	3 (6.1)	3	3 (6.7)	9	8 (5.6)

Abbreviations: SAE = serious adverse event; TEAE = treatment-emergent adverse event. Data source: TEAEs were defined as signs or symptoms that emerged during treatment or within 30 days of the last dose of study drug, including those signs and symptoms absent pre-treatment or that worsened relative to pre-treatment status.

Descriptively a trend to an increased number of adverse events were noted in the active drug treatment arms versus placebo- 25 patients (50.0%) patients in the placebo group, 37 patients (75.5%) in the selisistat 50 mg group, and 32 patients (71.1%) in the selisistat 200 mg group (**Table 34**). A statistically significant difference was noted in the percentage difference of adverse events occurring in the active treatment groups versus placebo, confirming the above observation (**Table 35**).

Table 35- Comparative Analysis of Difference in Percentage of Adverse events across treatment groups

Test	Difference of percentage	Std Error	t-value	p-value
placebo vs 50mg	25.5	0.0937	-2.723	0.0074
placebo vs 200mg	21.1	0.0978	-2.159	0.0334
200 vs 50mg	4.5	0.0913	-0.482	0.6316

In terms of sub-typing the adverse events into organ systems, infections (27 patients), abnormalities in investigations (27 patients) and gastrointestinal disorders (24 patients) were the most common TAEs reported.

For infections and infestations, there was not a clear difference across treatment groups, while for investigational abnormalities a significantly greater preponderance of patients receiving active compound was noted with 9 patients in 50mg and 12 patients in 200mg group respectively afflicted. This pattern was repeated for gastrointestinal side-effects with 13 patients in the 50mg group, 7 in the 200mg and 4 in the placebo group. Diarrhoea was reported by 12 patients in the active treatment group and one in the placebo group.

Table 36- Adverse Events leading to withdrawal by system organ class.

Adapted from (Reilmann *et al.*, 2013)

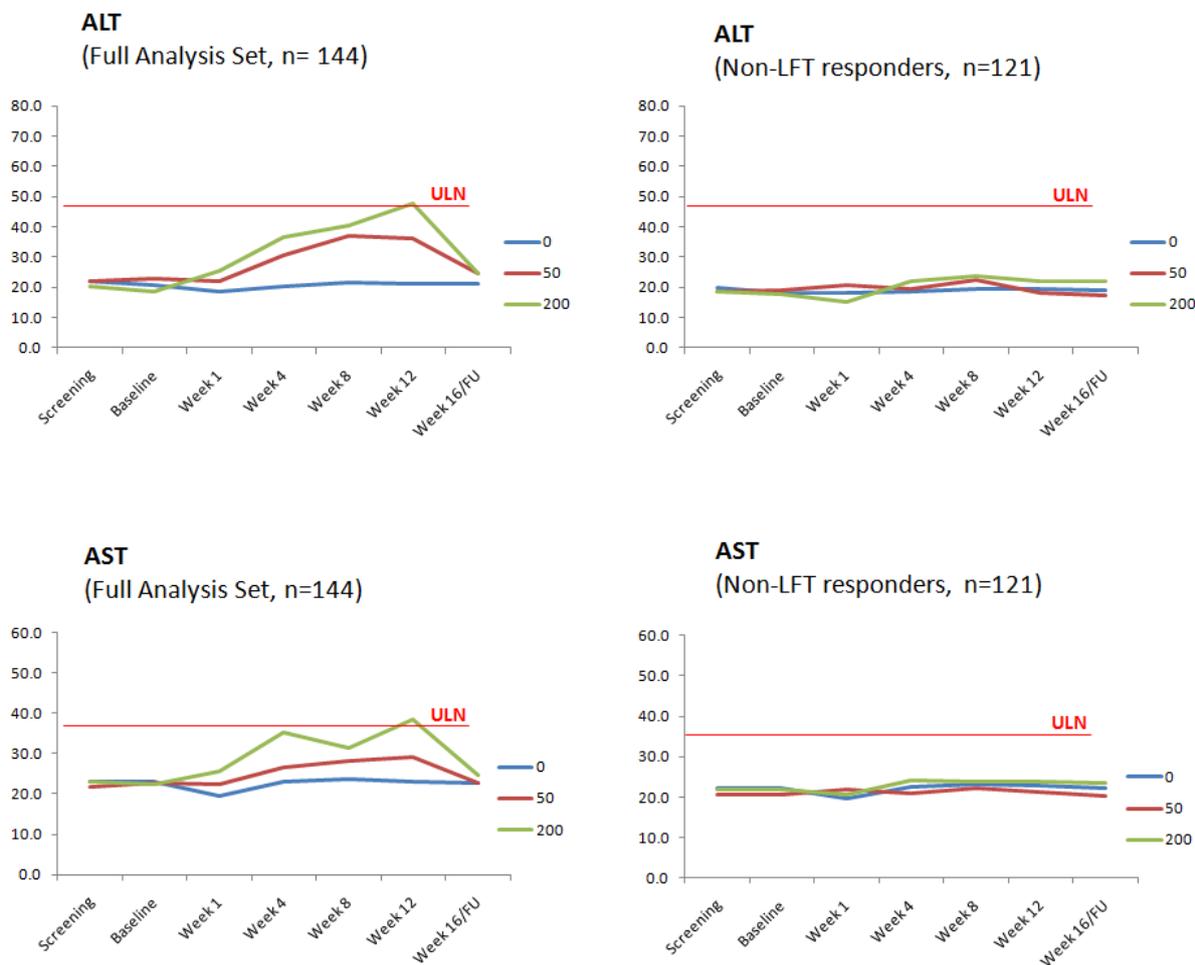
System Organ Class Preferred Term	Placebo		SEN0014196 50 mg		SEN0014196 200 mg		Total	
	N=50		N=49		N=45		N=144	
	Events	Patients	Events	Patients	Events	Patients	Events	Patients
Gastrointestinal disorders	0	0	0	0	1	1 (2.2)	1	1 (0.7)
Diarrhoea	0	0	0	0	1	1 (2.2)	1	1 (0.7)
Hepatobiliary disorders	0	0	1	1 (2)	0	0	1	1 (0.7)
Hepatitis	0	0	1	1 (2)	0	0	1	1 (0.7)
Infections and infestations	0	0	1	1 (2)	0	0	1	1 (0.7)
Gastroenteritis	0	0	1	1 (2)	0	0	1	1 (0.7)
Investigations	0	0	5	5 (10.2)	9	6 (13.3)	14	11 (7.6)
ALT increased	0	0	0	0	4	4 (8.9)	4	4 (2.8)
AST increased	0	0	0	0	2	2 (4.4)	2	2 (1.4)
GGT increased	0	0	0	0	1	1 (2.2)	1	1 (0.7)
Hepatic enzyme increased	0	0	3	3 (6)	1	1 (2.2)	4	4 (2.8)
Liver function test abnormal	0	0	2	2 (4)	1	1 (2.2)	3	3 (2.1)
Psychiatric disorders	1	1 (2.0)	0	0	2	2 (4.4)	3	3 (2.1)
Apathy	0	0	0	0	1	1 (2.2)	1	1 (0.7)
Depression	0	0	0	0	1	1 (2.2)	1	1 (0.7)
Suicide attempt	1	1 (2.0)	0	0	0	0	1	1 (0.7)

9.3.6.11 Safety Conclusions

20 events in 16 patients prompted withdrawal from the drug trial, with one patient in the placebo, 7 in the 50mg and 8 in 200mg group (**Table 36**), with rise in ALT and

hepatic enzymes the most commonly occurring event, which was only in the active drug arms. A dose dependency was suggested as ALT & AST increases were seen only in 200mg group (**Figure 23**).

Figure 23- Hepatic Enzyme (ALT/AST) Changes in Treatment Groups



Individual descriptions of serious adverse events can be found in **Appendix 13.10**. In summary however, the one death and one suicide attempt that occurred was not thought to be related to study drug as both patients were in the placebo group. In 15% (n=22) of patients abnormalities in liver function disturbance were observed, with 20 patients in the active drug arm, 50mg (n=9) and 200mg (n=11) while three were in the placebo group. All patients recovered aside from one, in whom blood tests deteriorated despite previous serial improvements 9 weeks following cessation of study drug. This patient had to be followed up outside the end of the study window by the local investigator.

9.3.7 Clinical Laboratory Evaluation

9.3.7.1 Haematology, Biochemistry & Urinalysis

While there were elevations in monocytes, basophils and neutrophils through the course of the study, none reached statistical significance. No other haematological abnormalities were noted. Liver function disturbance is covered elsewhere and there were no changes in renal function and electrolytes. No significant changes in urinalysis parameters were noted.

9.3.7.2 Vital Signs, Physical Findings and Electrocardiogram

There were no significant abnormalities noted in these assessments throughout the study.

9.3.7.3 Columbia-Suicide Severity Rating Scale

Aside from one serious adverse event with a suicide attempt, only transient suicidal ideation was noted in the study. Overall the intensity of suicidal ideation was low and no treatment initiation related severe suicidal ideation.

9.4 Discussion

Selisistat, a Sirtuin and selective inhibitor of silencing information regulator T1 (SirT1), may have a therapeutic role in preventing deacetylation of mutant Htt thereby enabling its extrusion.

The aim of the present study was to assess safety and tolerability of 12 weeks of treatment with selisistat at doses of 50 mg and 200 mg in HD patients, and secondarily to assess short-term clinical effects, modulation of candidate pharmacodynamic markers and the pharmacokinetic profile of Selisistat.

In summary the average age of the study participants was 50.5 years and a BMI of 24.34 kg/m² with a preponderance of Caucasian patients. An unequal gender distribution was noted but this was not statistically significant. The most commonly declared medical history was depression (50 patients) and sleep disorder (11 patients). In terms of the drug history the most frequent medications were antidepressants, dietary supplements and chorea suppressing medications. Compliance of 80-120% was achieved across all treatment groups. Nineteen patients were excluded with the most common reason being that they did not complete the study due to abnormal liver function test results. There were no statistically significant effect of disease relevant clinical parameters such as motor score, stroop, PBA and TFC scores.

9.4.1 Demographics

Two clear differences between treatment groups were apparent. Firstly there is a significant under-representation of Asian or other racial groups. It is established that there can be racial differences in bio-absorption of drugs and therefore evidently the applicability of this research to other racial groups would need to be investigated further (Bjornsson *et al.*, 2003;Chen, 2006).

9.4.2 Adverse Event Profile

The mean exposure to the study drug was 80.2 days (SD 15.79 days), with no apparent difference between the treatment arms. Of the 205 treatment related adverse events TEAEs, there were 177

deemed of mild severity, 24 moderate and 4 were severe. More events were recorded in active treatment groups, 37 patients in 50mg group and 32 in the 200mg group.

ALT increase was the most frequent treatment related adverse event with all 8 patients occurring in the active drug arms. AST increase was seen in 6 patients again with no patients in the placebo group affected. There were 9 serious adverse events in total, with 2 in the placebo group (aspiration leading to death, and suicide attempt) and 3 patients in 50mg (hepatitis, liver function test abnormality, and choking) and 200mg groups (furuncle, clavicle fracture and liver function test abnormality) respectively.

It was notable that adverse events concerning abnormal liver function tests occurred as the most frequent related TEAEs, and with the exception of elevated gamma-glutamyltransferase, occurred only in the selisistat groups, suggesting that this is a drug related effect.

These results suggest that selisistat may cause abnormalities in liver function. Bi-weekly monitoring of LFTs allowed timely identification of these events, which were all reversible, except in one case. For related TEAEs, there was a tendency for more liver function-related TEAEs to occur in the higher selisistat dose group. Additionally one notes this was a 12 week study. It is not clear whether liver function disturbance would have occurred over a longer duration. An escalating dose design study could answer this question.

Most patients had no suicidal thoughts throughout the study. As mentioned above, one patient in the placebo group made a suicide attempt that was considered not related to the study drug. Up to two patients in each of the placebo and selisistat 50 mg groups and no patients in the selisistat group had suicidal thoughts at any visit, suggesting that these thoughts did not have a relationship with selisistat.

9.4.3 Comparison to Phase 1B Study

There were also no treatment related changes to clinical efficacy parameters in the shorter 14 day Phase 1B study. Interestingly, despite the much shorter duration, one patient did experience disturbance in ALT levels. This emphasises the merited concern over potential hepatic enzyme derangement.

9.4.4 Efficacy

Although there were some minimal changes to the parameters throughout the study, there were no clinically significant results. In this short term study, no effect of selisistat was seen on clinical parameters in the patient population. The study was not powered to reveal any efficacy readouts and moreover, in this 12 week study, finding a statistically significant effect would be less probable.

9.4.5 Pharmacokinetics

Pharmacokinetic data has already suggested that the drug reaches higher concentrations in females than males which itself may be a reflection of the relative difference in body mass index. Thus the larger number of male subjects in the placebo groups could potentially skew pharmacokinetic comparisons between groups. However this difference did not reach statistical significance on sub-analysis.

9.4.6 Pharmacodynamics- Target Engagement

Levels of soluble mutant huntingtin in peripheral blood mononuclear cells showed borderline statistically significant ($p = 0.058$, $p = 0.075$) increases of similar magnitude at 12 weeks compared to placebo in the 50mg and 200mg groups respectively, that reverted to levels consistent with the placebo group at follow-up (Reilmann *et al.*, 2014). The clinical significance of mutant Huntingtin levels in PBMCs is not clear and thus the effect of any modulation thereof is not known. The effect on CSF mutant Huntingtin levels, which would evidently be more relevant to a potential CNS effect of the compound, is also not known.

9.4.7 Summary

While the compound does appear generally safe and tolerated, two major concerns exist. To date no validated clinical data has been published to demonstrate pharmacodynamic efficacy and as such

leaves large question marks over the future of the compound. This may well be largely due to the closure of this arm of drug development by the study sponsor.

Secondly there remains significant disquiet about the liver function disturbance observed despite the relatively short study period. The prohibitive cost of phase III studies, which could potentially last two years, may have effectively curtailed critical pharmacodynamic work that could have addressed both these concerns.

At the time of writing therefore, further work on selisistat for Huntington's disease has been discontinued although the compound has been recently acquired by AOP Orphan Pharmaceuticals who do focus on rare diseases, though no clear plans have been announced at present.

10 Characterisation of Motor Phenotypes in Huntington's disease and Correlation with Imaging Measures of Regional Atrophy

This study conception and motor segregation methodology was developed by myself with supervisory input from Professor Tabrizi, UCL & Dr Ralf Reilmann, University of Munster. Statistical advice was given by Ruth Keogh, London School of Hygiene & tropical Medicine. The VBM analysis parameters were suggested by myself but finalised following discussions between myself, Ruth Keogh, Dr Rachel Scahill, UCL and Dr Elin Rees, UCL. Dr Rees kindly performed the VBM analysis. Secondary analysis was discussed with and carried out by Dr Mariam Shoaib, UCL. The Study was carried out using subjects from TRACK-HD and PADDINGTON studies, both of which followed the Helsinki protocol (Ethical Principles for Medical Research Involving Human Subjects), with local research ethics committee approval and all study members were trained in good clinical practice.

10.1 Introduction

There is well-established consensus amongst experts that distinct motor phenotypes exist in HD. While the classical adult onset motor presentation is that of a hyperkinetic movement disorder with chorea predominating over bradykinesia and rigidity, in juvenile HD subjects bradykinesia, rigidity and dystonia are clearly more prominent, described as the Westphal variant. This more parkinsonian phenotype has also been noted in a minority of adult onset subjects. A further significant percentage fall into a mixed motor phenotype with both hypokinetic and hyperkinetic features. Finally, a minority group are those who appear to have clearly symptomatic disease but with relatively lower motor scores, though care must be taken to control for disease stage. A classification system for the subdivision of these varying phenotypes has not been established and thus the spectrum of motor features in HD while clinically evident, remains uncharacterised formally.

The underlying neuroanatomical and pathological basis for these phenotypes also remains unexplored. With the advent of imaging biomarker studies, they can be tested for the first time in HD. The importance of such a correlation resides in the ability to fully reflect the heterogeneity that exists in HD, which will in turn facilitate accurate, relevant biomarkers tailored to the patient group in question. It would also facilitate a more detailed understanding of the natural history of the evolution of motor symptoms in HD. There is a valid impression based on clinical experience, that after years of hyperkinetic movements, in the moderate to latter stages of disease, a more akinetic

picture images, reflecting presumably more widespread degeneration. Such a rating system would better facilitate sub-analysis of this motor evolution, an individualised disease pathway for the patient, and in the setting of an effective intervention, form part of longer-term efficacy measures.

The Unified Huntington's Disease Rating Scale (UHDRS) is a multi-item assessment tool used in HD patients, which includes motor evaluations- Total Motor Score (TMS) and functional tools- Total Functional Capacity (TFC) (Huntington Study Group, 1996;Huntington Study Group, 1999). Although the TMS does suffer from floor and ceiling effects, at either end of disease states, it has shown a trend to distinguish between disease stages (Franciosi *et al.*, 2013) and remains the sole motor rating scale in HD. A natural history study of HD over 3 years, demonstrated that the TMS worsened by 3 points per year and TFC by 0.6 per year (Dorsey *et al.*, 2013).

Indeed recent work utilised the TMS to develop a means of segregating patients into these distinct motor phenotypes. Interestingly, choreic subjects performed better on functional and cognitive measures than akinetic ones. For the choreic subtype, chorea subscore were used totalling 28 and for hypokinetic-rigidity, finger taps, pronate-supinate hands, bradykinesia, rigidity, items measuring speed of movement and rigidity, out of 28. A 4 point difference between the two subtype scores was required to be assigned to either group. If this was not met the mixed motor type was used (Hart *et al.*, 2013).

A sub-analysis of cortical thickness in HD patients found that compared to controls, subjects with hypokinetic features showed greater cortical loss in anterior frontal, pre-motor and supplementary motor areas compared to those subjects whose motor phenotype was more hyperkinetic. Interestingly, striatal volumes did not differ between the two phenotypes (Rosas *et al.*, 2008).

In Parkinson's disease, cognitive impairment seems to be associated with the akinetic rigid phenotype, tremor dominant disease correlates with a slower course of disease and gait failure with rapid disease progression (van Rooden *et al.*, 2009;Jankovic *et al.*, 1990).

Although the JHD group represents arguably the phenotypically purest grouping, unfortunately, imaging studies using Juvenile HD populations are rare and no direct comparison with adult onset imaging has ever been carried out. There is a suggestion from cases reports and small case series, of cerebellar atrophy, as well more florid putaminal atrophy alongside the cortical and subcortical volumetric loss amongst JHD patients (Comunale, Jr. *et al.*, 1995; Ribai *et al.*, 2007; Ruocco *et al.*, 2006).

Thus using specific sub-scores of the TMS, segregation criteria were developed for the major motor groups outlined above. Using these HD motor phenotypes, a whole brain VBM analysis was undertaken for the first time in HD research, to see whether regional differences in atrophy exist between these groups that might, based on their neuroanatomical function, plausibly explain the clinical heterogeneity seen in HD.

10.2 Methods

10.2.1 Participants

A combined PADDINGTON Imaging biomarker study and TRACK-HD cohort of 147 early stage HD subjects were utilised for this study. Initially COHORT subjects, from the London longitudinal study were included raising total number to 191. However due to the technical differences in imaging between this group and the PADDINGTON and TRACK-HD subjects, that would prevent VBM analysis, they had to be excluded.

10.2.2 Development of Motor Classification System

A motor classification system was developed based on sub-scores of the UHRDS Total motor score (**Appendix 13.1**) to segregate HD patients. The development of this classification system and scientific rationale is covered in the results sections.

10.2.3 VBM Analysis

A voxel-wise structural brain image analysis was conducted, in SPM8 on a Matlab 2012b platform, to examine between-phenotype volumetric differences, using Unified segmentation (Ashburner & Friston, 2005) and a study-specific DARTEL template (Ashburner, 2007). Results were adjusted for multiple comparisons using False Discovery Rate (FDR) correction at the $p < 0.05$ level.

10.3 Results

10.3.1 Development of Motor Phenotype Criteria

Clinical experience in managing early stage HD patients would indicate that there would be far more hyperkinetic adult patients than hypokinetic. However, perhaps the greatest portion would constitute those with so called mixed motor phenotypes encompassing bradykinesia as well as chorea. One must make a clinical distinction here between true bradykinesia with decrement, bradyphrenia and motor slowing, which the UHDRS is not sophisticated enough to detect. For pragmatic reasons therefore, bradykinesia is used hereafter to define slowness of movement which may arise from extra-pyramidal, pyramidal and inter-pyramidal sources. Dystonia unless fixed is classified as a hyperkinetic movement disorder. However, due to the fact that is notoriously difficult to gain consensus on the presence and/or extent of dystonia, this score was left out of the hyperkinetic group.

The UHDRS motor score was thus sub-divided into those calibrating hyperkinetic and akinetic clinical signs. The starting composition was as follows: Hyperkinetic= chorea subscore from all 4 limbs, face, mouth and trunk + finger tapping + pronation/supination from upper limbs; Akinetic= Bradykinesia subscore from all 4 limbs. It is important to note that leg tapping does not form part of the assessment of leg bradykinesia whereas finger tapping is assessed in upper limb assessments.

From preliminary review of motor scores data, a number of helpful observations emerged that could inform segregation criteria. Firstly, it was apparent that patients could score in both groups to

differing extents, or not at all, giving rise to hyperkinetic, akinetic, mixed motor and minimal motor groups. Additionally a small score in either hyperkinetic or akinetic or both probably would be unlikely to reflect a truly hyperkinetic or akinetic state. Therefore a minimum score in each of these scores, hyperkinetic and akinetic, must be reached in order to be classified as one or the other or mixed motor. If that minimum score was not met or there was a low total motor score, this could be assumed to reflect a low motor burden consistent with the minimal motor group.

Criteria 1

In the further development of the scoring system, finger-tapping and pronation/supination were removed from the hyperkinetic score, as although they do assess speed of movement, they are not as definitively extra-pyramidal as bradykinesia and rigidity are, as they probably tap into corticospinal and cerebellar pathology as much as they do striatal/basal-ganglia.

Alongside the clinically established hyperkinetic and akinetic groups, when examining the raw data, mixed motor and minimal motor groups emerged. In retrospect these groups probably reflect the predominant clinical experience. Moreover, there were subjects with high and low mixed motor burdens, which prompted a further subdivision of mixed motor into severe and mild groups.

In order to obtain maximum discriminatory value, a stricter segregation method was applied, removing finger tapping and pronation, and increasing the cut-offs in the various groups. To ensure better separation in seemingly mixed motor groups, I also subtracted the AkR score from the HK score (Hk-AkR) to better identify predominantly hyperkinetic groups or akinetic groups or truly mixed groups, and worked this into the definitions below.

If the TMS score was greater than 28 this merited admission into the mixed motor group and there was clearly a high motor burden.

Methods:

TMS subscores:

Hyperkinetic (Hk): Chorea scores from 7 body parts; max score of 28

Akinetic Rigid (AkR): Bradykinesia & Rigidity (Left & Right); max score of 12

Segregation Criteria:

191 participants were divided into groups by the following criteria:

Hyperkinetic: HK score (total chorea) ≥ 14 & HK-AkR score ≥ 7 & total motor score (TMS) >10 & ≤ 28 .

Akinetic Rigid: AkR ≥ 4 & HK-AkR score ≤ -4 & TMS > 10 & ≤ 28 .

Mixed Motor Severe (MixMtrSev): TMS >28 .

Mixed Motor Mild (MixMtrMld): TMS ≤ 28 and Hk-AkR score ranges from -3 to 7.

Minimal Motor (MinMtr): TMS ≤ 10 .

Results:

Group	Hyperkinetic	Akinetic Rigid	Mixed Motor Severe	Mixed Motor Mild	Minimal Motor
Number (%TOT)	9 (5%)	7 (4%)	72 (38%)	82 (43%)	20 (10%)

Conclusion:

Overall it was felt that these criteria utilised too many groups and thus may leave too few numbers in either extremes to render an analysis viable. It was also felt that the higher total score for the hyperkinetic group versus akinetic group may also skew data analysis.

Criteria 2a:

Rationale

The small numbers of akinetics in criteria 1 prompted consideration to re-introduce finger taps and pronation into this assessment. The bradykinesia assessment could similarly have components not only of extra-pyramidal pathology but evidently pyramidal and cerebellar. Ultimately they are all motor pathways. The comparative assessment also seemed more mathematically eloquent given both groups was now being scored out of 28, as opposed 28 vs 12.

In order to select out any clear hyperkinetics and akinetics from the large mixed motor group, the criteria were revised by seeking to quantify the proportion that the Hk or AkR score contributes to the total motor score, using the *Hk/TMS and AkR/TMS* method.

e.g. Hk score 4, TMS 10, therefore $Hk/TMS = 0.4$

In order to tease out from those with a high total motor score (>28) into those who are truly mixed and those where HK or AkR is responsible for a significant part of the high total motor score, I employed a cut-off of ≥ 0.4 , whereby the Hk or AkR score must comprise ≥ 0.4 of the TMS.

Regarding the Hyperkinetics, I also decided to remove the absolute score ≥ 14 also and focus solely on the difference between the phenotypes i.e. the Hk-AkR score.

From the end goal point of view, which is imaging correlation, there did not seem to be much value in subdividing the mixed motor group and thus, this was abandoned.

Methods:

TMS subscores:

Hyperkinetic (HK): Chorea scores from 7 body parts; max score of 28

Akinetic Rigid (AkR): *Finger Taps, Pro/Sup, Bradykinesia & Ridigity (left & Right)*; max score of 28

(Finger-tapping and pro/supination re-added)

Segregation Criteria:

191 participants were divided into groups by the following criteria:

Hyperkinetic: HK-AkR score ≥ 7 & TMS >10. If TMS >28, then Hk/TMS must be >0.4 (to 1 decimal space).

AkR (*re-includes finger taps and pronation supination*): HK-AkR score ≤ -4 . If TMS >28 then AkR/TMS must be ≥ 0.4 (to 1 decimal space).

Mix Mtr (*merging of Max and Mild Mix Motor*): TMS >10 **and** Hk-AkR score ranges from -3 to 6. Or does not fit in any other group.

Min Mtr: TMS ≤ 10 .

Results

Group	Hyperkinetic	Akinetic Rigid	Mixed Motor	Minimal Motor
Number (%TOT)	14 (8%)	18 (9%)	139 (72%)	20 (10%)

Conclusions

Hyperkinetics and akinetic numbers have risen further as expected but is not really reflective of true clinical experience as there are more akinetic patients than hyperkinetic.

Criteria 2b

Rationale

In light of the above, it was decided to relax the Hk-AkR criteria to ≥ 4 for the hyperkinetic group, to the same parameter as one uses in AkR criteria, which is also more appropriate from an analytical perspective.

Methods:

TMS subscores:

Hyperkinetic (HK): Chorea scores from 7 body parts; max score of 28

Akinetic Rigid (AkR): *Finger Taps, Pro/Sup, Bradykinesia & Ridigity (left & Right)*; max score of 28

(Finger-tapping and pro/sup re-added)

Segregation Criteria:

191 participants were divided into groups by the following criteria:

Hyperkinetic: HK-AkR score ≥ 4 & TMS >10 . If TMS >28 , then Hk/TMS must be >0.4 (to 1 decimal space).

AkR (*re-includes finger taps and pronation supination*): HK-AkR score ≤ -4 . If TMS >28 then AkR/TMS must be ≥ 0.4 (to 1 decimal space).

Mix Mtr (*merging of Max and Mild Mix Motor*): TMS >10 and Hk-AkR score ranges from -3 to 3. Or does not fit in any other group.

Min Mtr: TMS ≤ 10 .

Results

Group	Hyperkinetic	Akinetic Rigid	Mixed Motor	Minimal Motor
Number (%TOT)	26 (14%)	18 (9%)	127 (66%)	20 (10%)

Conclusion

Further increase in Hyperkinetic numbers.

Criteria 3a

Rationale

Previously, we have used the Hk/TMS and AkR/TMS calculations to sift further patients out from the larger mixed motor group. However, a fair part of the TMS cannot be attributed to either of the phenotypes and it could be argued therefore, masks the true proportions.

Therefore I developed the *phenotype motor score (pMS)* which is Hk and AkR scores combined and applied the Hk/pMS and AkR/PMS calculation in those subjects with a high TMS, using a higher cut-off of ≥ 0.8 .

Methods

TMS Subscores:

Hyperkinetic (HK): Chorea scores from 7 body parts; max score of 28

Akinetic Rigid (AkR): Finger Taps, Pro/Sup, Bradykinesia & Rigidity (left & Right); max score of 28

Phenotype Motor Score (pMS) is the combination of the above two scores, out of 56.

Segregation Criteria:

191 participants were divided into groups by the following criteria:

Hyperkinetic: HK-AkR score ≥ 7 & TMS >10 . If TMS >28 , then Hk/pMS must be ≥ 0.8 (to 1 decimal space).

AkR (re-includes finger taps and pronation supination): HK-AkR score ≤ -4 . If TMS >28 then AkR/pMS must be ≥ 0.8 (to 1 decimal space).

Mix Mtr (merging of Max and Mild Mix Motor): TMS >10 **and** Hk-AkR score ranges from -3 to 6. Or does not fit in any other group.

Min Mtr: TMS ≤ 10 .

Results

Group	Hyperkinetic	Akinetic Rigid	Mixed Motor	Minimal Motor
Number (%TOT)	11 (6%)	9 (5%)	151 (79%)	20 (10%)

Conclusion

A corresponding reduction in group numbers has occurred, so I go on to refine this further below.

Criteria 3b

Rationale

Criteria 3a but relax Hk criteria to ≥ 4 and Hk or AkR/pMS to 0.7

Methods

TMS Subscores:

Hyperkinetic (HK): Chorea scores from 7 body parts; max score of 28

Akinetic Rigid (AkR): Finger Taps, Pro/Sup, Bradykinesia & Rigidity (left & Right); max score of 28

Phenotype Motor Score (pMS) is the combination of the above two scores, out of 56.

Segregation Criteria:

191 participants were divided into groups by the following criteria:

Hyperkinetic: HK-AkR score ≥ 4 & TMS >10 . If TMS >28 , then Hk/pMS must be >0.7 (to 1 decimal space).

AkR (re-includes finger taps and pronation supination): HK-AkR score ≤ -4 . If TMS >28 then AkR/pMS must be ≥ 0.7 (to 1 decimal space).

Mix Mtr (merging of Max and Mild Mix Motor): TMS >10 and Hk-AkR score ranges from -3 to 3. Or does not fit in any other group.

Min Mtr: TMS ≤ 10 .

Results

Group	Hyperkinetic	Akinetic Rigid	Mixed Motor	Minimal Motor
Number (%TOT)	31 (16%)	19 (10%)	121 (63%)	20 (10%)

Conclusion

This looks like the best of the various criteria, representing a fair compromise in seeking signal over noise. A difference of 4 between the Hk-AkR probably takes into account reasonably intra-rater

variability, given this is a cross-sectional study. The change from 0.8 to 0.7 results in 13 extra hyperkinetics and 5 extra AkRs.

Final Classification System

However as the actual distribution of motor phenotypes in HD had never been formally studied and was based on clinical impression, which can be inaccurate and subject to unconscious bias, an alternative final method was proposed. As an illustration, motor raters typically have better correlation for hyperkinetic scores than akinetic scores i.e. it can be easier to identify and score a patient that moves more than one that moves less. This is in itself inherent bias within the input data to our analysis.

A histogram based distribution plot of motor phenotypes scores would allow firstly visualisation of the spread of the phenotypes scores to see if they complement the clinical perception. Secondly, using centiles within that spread to segregate groups seemed ultimately a more rigorous, repeatable and objective approach. As discussed above, only participants from PADDINGTON and TRACK-HD imaging biomarker studies were included in the final analysis.

Hyperkinetic (HK) score

This is constituted of the *chorea* subscore from 7 body parts (right and left upper and lower limbs, face, mouth and trunk), giving a total score of 28.

The Akinetic Rigid (AkR) score

This comprised of the finger taps, pronation/supination and bradykinesia & rigidity (right & left upper limb) subscores, also giving a total score of 28.

A composite ratio of the $\text{HK}/(\text{HK} + \text{AkR})$ score was then calculated to determine the relative contribution of each score to the motor phenotype. The $\text{HK} + \text{AkR}$ is the phenotype motor score (pMS) and seeks to more accurately select out the hyperkinetics and Akinetic.

A histogram of these scores was then created (**Figure 24**) and based on the distribution frequency of scores seen, a percentile based division of phenotypes was carried out.

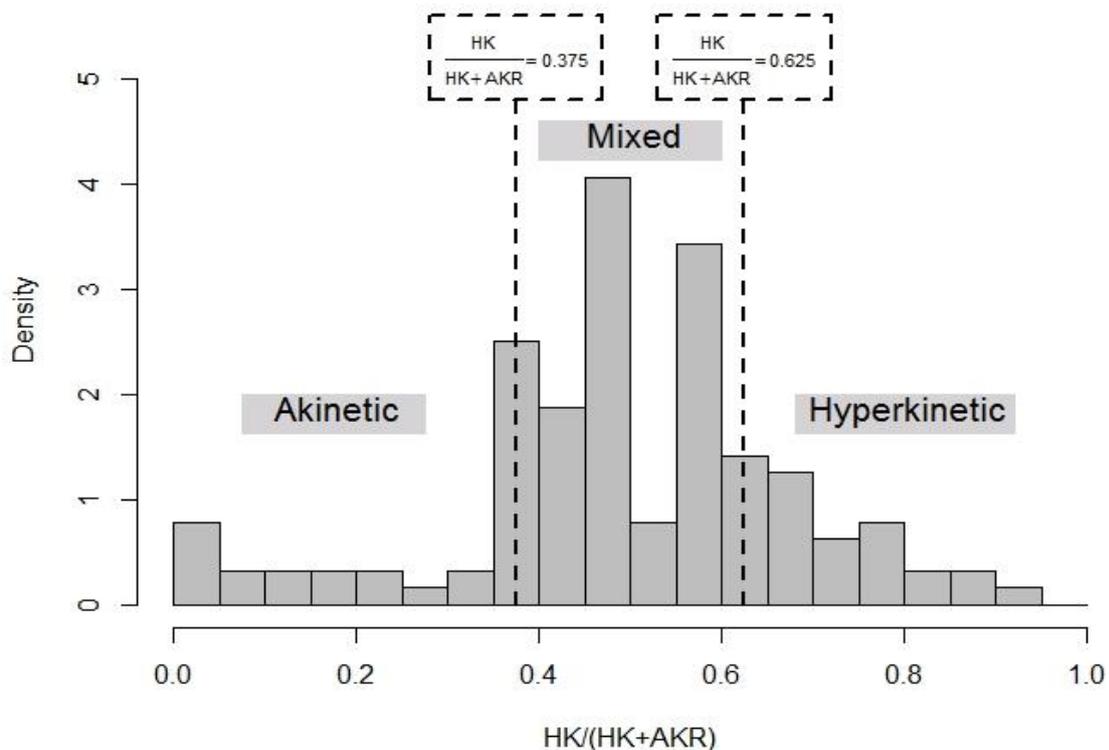


Figure 24- Histogram of hyperkinetic score/hyperkinetic + akinetic Rigid Score ($\frac{HK}{HK+AKR}$), with percentile based division into motor phenotype groupings.

All those with a total motor score of 10 or less were assigned automatically to the minimal motor group 0. Clinical convention suggests that a TMS of 10 or less is a reasonable threshold below which signs are considered fairly mild.

Those subjects within the 37.5th percentile and below constituted the akinetic motor group (1), 62.5th percentile and above, the hyperkinetic motor group 3 and those in between, the mixed motor group 2.

10.3.2 Group Characteristics

Table 37- Group characteristics of PADDINGTON and TRACK-HD cohort

Group	Age V1	Number (M:F)	CAG	Disease Burden Score	SDMT_correct	Stroop WR_correct	TMS
Akinetic		25 (17:8)					
Mean	45.9		43.5	353.4	32.9	74.7	21.8
SD	9.3		2.2	69.7	11.4	21.8	9.7
Min motor		17 (11:6)					
Mean	45		43.6	332.8	45.2	95.2	8.1
SD	11.4		3.3	50.7	12.8	9.8	1.8
Mixed motor		78 (42:36)					
Mean	48.5		44.0	382.7	31.0	73.9	26.6
SD	10.4		3.49	79.1	9.0	18.7	9.6
Hyperkinetic		25 (11:14)					
Mean	49.6		43.9	389.4	34.1	80.1	24.5
SD	10.1		2.9	63.2	8.9	17.1	9.9

There was a reasonable consistency of age & CAG size amongst the four groups, though the gender distribution was far more skewed (**Table 37**).

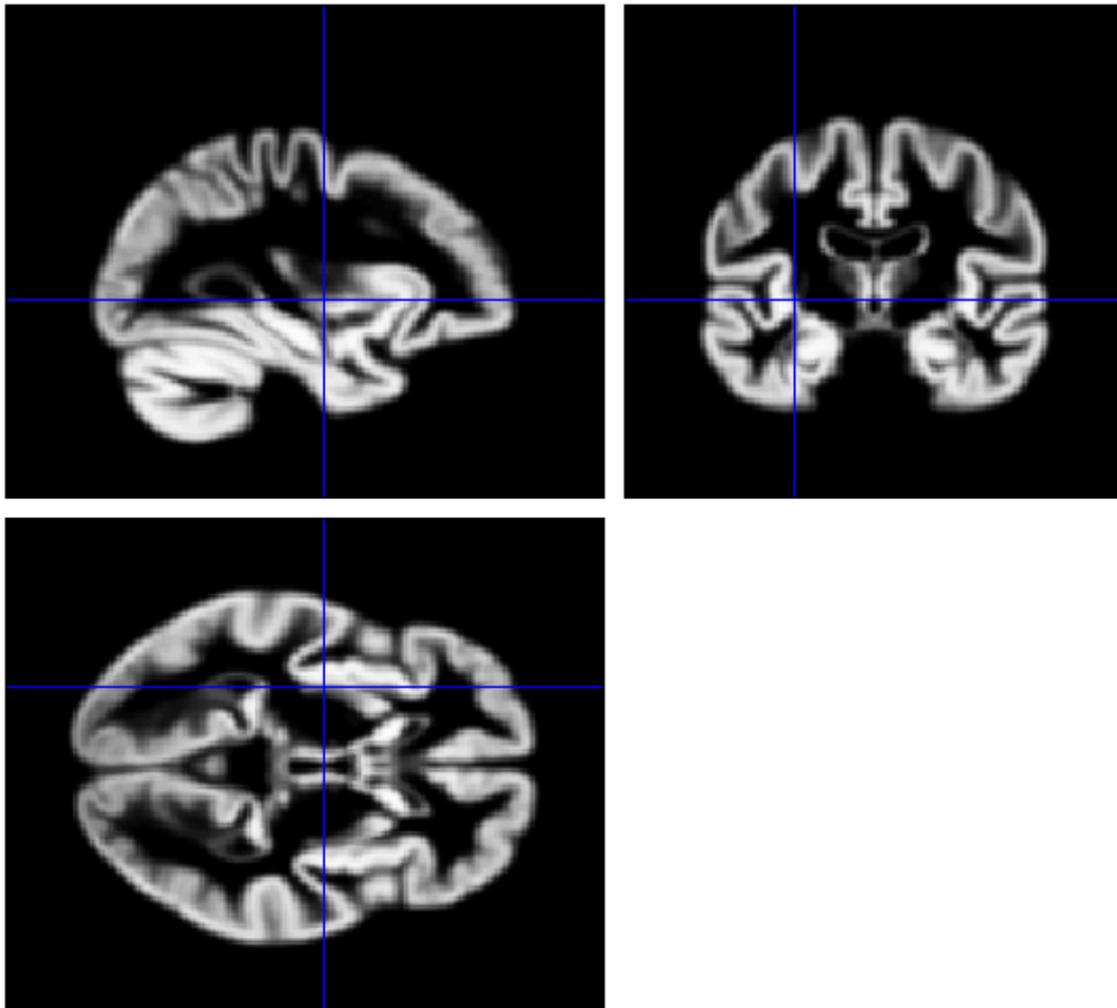
10.3.3 VBM Analysis

Given the possibility of varying disease onset amongst these groups, and cognitive dysfunction that occurs in HD, age, gender, CAG, disease burden and Stroop score were considered as co-variates in the analysis. Further co-variates included the total intracranial volume to correct for any size related confounding and the TMS (minus the phenotype motor score). The TMS was included because the pMS score is a composite of a few though not all subscores within the TMS. Thus it could be argued that any differences seen on VBM, might be explained by motor deficits in other areas, not encompassed by the segregation method.

A VBM based analysis of GM and WM covarying for age, gender, scanner, CAG, disease burden, TIV, Stroop score and TMS (minus phenotype motor score) was undertaken. One subject did not have a SDMT score so they were excluded. With adjustment for multiple comparisons (FWE and the less

stringent FDR) there were no significant between group differences in brain volumes. A second analysis was undertaken using age, gender, scanner, disease burden, total intracranial volume, Stroop score but this was also not significant (**Figure 25**).

Figure 25- Representative VBM analysis of grey matter in four different motor groups, hyperkinetic, akinetic, mixed motor and minimal motor.



10.3.4 Secondary Analysis

Previous work has explored the possibility of relationships between disease characteristics and phenotypes. Here we looked at whether there were any differences in age, gender, CAG, SDMT, stroop and disease burden scores between patients.

In the following analysis multinomial logistic regression was performed with phenotype groups as the outcome with hyperkinetic phenotype as the chosen reference level (**Table 38**).

Table 38- Multinomial Logistic regression looking for differences in clinical characteristics between akinetic, minimal and mixed motor groups.

(Hyperkinetic group used as reference.)

Coefficient	(Intercept)	AgeV1	Sex1M2F	StroopWR_ correct	SDMT_ correct	CAG	DBS
Akinetic	24.179399	-0.11615	-0.44719	-0.02121	-0.02034	-0.34877	-0.02065
Minimal	-6.409309	1.04310	7.596393	0.596729	-0.7347	7.149499	-36.5339
Mixed	10.757425	-0.05224	-0.16605	-0.00957	-0.02516	-0.13446	0.018392
p-value for:	(Intercept)	AgeV1	Sex1M2F	StroopWR_ correct	SDMT_ Correct	CAG	DBS
akinetic	0.0142769	0.0323	0.45682	0.277334	0.598513	0.04851	0.6134
minimal	0	0.3524	0	0.748547	0.407626	0	0
mixed	0.1672909	0.2459	0.72353	0.546116	0.425349	0.31290	0.5611

Prior to interpretation, it is important to note the small sample size as this limits the efficacy and generalisability of multinomial logistic regression analysis. Correction for multiple comparisons means that results must again be taken with extreme caution.

Using the hyperkinetic phenotype as the reference, in this worked example, a one-unit increase in the variable AgeV1 is associated with a decrease in the log odds of being in akinetic vs. hyperkinetic group by 0.12. This change in log off odds is significant on testing suggesting that akinetics are apparently more likely to be younger than the hyperkinetics. However, the clinical relevance of this is unclear and the actual effect is likely to be negligible, given the small sample size.

In the gender analysis and in simpler terms, being female and a higher CAG size is more of a risk factor for the minimal movement category compared to hyperkinetic. There is a hint of an effect suggesting lower CAG being seen in akinetic groups versus hyperkinetics. Neither of these findings equates to clinical experience and may reflect low numbers in the groups.

A lower DBS is apparently more likely to be found in the minimal motor group i.e. they are less afflicted than hyperkinetic groups which meets the clinical experience and is consistent with their lower TFC. There does not appear to be any correlation with cognitive scores in the form of SDMT and the various motor phenotypes.

10.4 Conclusion

A novel, potentially clinically relevant, repeatable HD phenotype segregation method has been developed here to objectively identify the well-established clinical phenotypes seen in HD, hyperkinetic, akinetic, mixed motor and minimal motor.

However it must be acknowledged that the UHDRS is an imprecise instrument, not designed for such a purpose. Bradykinesia encompasses a variety of pathologies and is not adequately assessed in the lower limbs. Dystonia is also not included and therefore the true hyperkinetic state may be underrepresented. Moreover, although the heterogeneity of motor phenotypes in HD is clinically apparent, the proposed groupings have not ever been evidenced. Thus in the development of the segregation method, criteria were adjusted to ensure enough numbers were present in each group to reflect clinical experience, which itself has never been formally ratified. In doing so, there may also be a danger of moving away from a priori hypothesis approach. A cluster based statistical analysis of motor phenotypes in HD is an alternate approach therefore, as it would not be based on what may be an inaccurate clinical presumption.

Subsequent VBM analysis of grey and white matter structures in 146 PADDINGTON and TRACK-HD patients looking for correlation with HD motor phenotypes however failed to reveal any significant differences. Correlation between minimal motor group and female gender and higher TFC scores was noted, while correspondingly lower TFC score i.e. greater functional impairment was seen in the akinetic group versus hyperkinetic or mixed motor groups but statistical significance was not reached. It is possible that functional and independence scale measures which broaden and deepen the functional performance derived from the TFC may have been a more sensitive comparator.

Multinomial logistic regression analysis confirmed the clinical impression that the minimal motor group is associated with a lower disease burden score.

These negative VBM findings could be explained by the relatively early stage of the subjects and studying moderate stage subjects and juvenile HD (pure akinetic rigid) may be helpful in this regard. The total motor score may not be sensitive enough to detect enough difference to make arbitrary phenotypic separation viable. Using quantitative measures like Q-motor testing as a representative of bradykinesia for example, would seem to be a far more reliable assessment than subjective clinical measures.

Furthermore, this data, controlled for age, cognitive scores and other co-variates, may reveal a more authentic distribution of motor phenotypes from which global and regional brain volumetric correlations can be sought. Indeed a recent regression analysis was employed to determine the association between regional atrophy and motor and cognitive measures. Diminution in Quantitative-motor performance correlated with lower grey matter volume in the left superior parietal cortex (Minkova *et al.*, 2018).

The failure to find any regional atrophy correlating with differing motor phenotypes may relate to the possibility that the origin of motor phenotypes is the net result of dysfunction across multiple motor and cognitive networks. Functional motor studies and connectivity analyses may be a more appropriate analytical tool as atrophy may be too crude and non-specific a measure.

Some clinicians would argue that the largest group are those with co-existing chorea and bradykinesia (Thompson *et al.*, 1988; Girotti *et al.*, 1988; Sanchez-Pernaute *et al.*, 2000). It may therefore be the case that identifying extremes on this distribution, the hypo and hyperkinetic, would require far higher numbers to enable a detectable difference to be appreciated. In previous work over 1800 subjects were present resulting in a significantly better powered study compared to 191 in this work (Hart *et al.*, 2013).

The effect of medication must also be carefully considered, as anti-chorea agents such as tetrabenazine and olanzapine can have significant impacts on motor scores and through dopaminergic inhibition, result in parkinsonian type symptoms. It may be that on a one-off analysis that previously hyperkinetic patients are now rendered akinetic by medications for example. There is evidence that anti-psychotic use is associated with a greater decline in TFC (Desamericq *et al.*, 2014). Additionally, long-term anti-psychotic use in mental health populations is associated with brain atrophy, cognitive impairment, apathy and the effect of this on an already degenerating brain is not known (Torres *et al.*, 2016). This was not taken into account in this work.

An open VBM based methodology was employed here. A regional atrophy based hypothesis as used by Rosas *et al.* 2008 was originally considered. This initial hypothesis stated that hyperkinetic HD phenotypes demonstrate greater caudate atrophy than Parkinsonian HD phenotypes (UHDRS 2 out of 3 of rigidity, bradykinesia and dystonia >2). A sub-analysis of total striatal and composite caudate and putaminal measures was also planned to look for further correlations. A further hypothesis was that a prominent motor HD phenotype (high balance impairment/chorea/inco-ordination scores) may correlate with greater motor cortex, striatal, cerebellum, brainstem atrophy. Conversely, a prominent non-motor HD phenotype presentation (low mood, cognitive/behavioural change) may correlate more with frontal lobe and cingulate atrophy.

11 Optical Coherence Tomography in Huntington's disease

This study was my own idea conceptually and methodologically. I recruited the patients, carried out the clinical and neurophthalmological assessments, undertook the OCT scans, tabulated and conducted initial analysis of the data. Training on and use of OCT machine was provided by Dr Rhian Raftopoulos and Dr Raj Kapoor. I formulated the hypotheses to be tested and carried out statistical tests. I received statistical advice from Zoe Scott at the ION Education Unit and Dr Mariam Shoai, UCL and where indicated, they carried out more detailed statistical analyses.

11.1 Introduction

The eye in neurological practice is often termed the 'window into the CNS.' This is because it allows direct visualisation of the retina and optic nerve through a variety of qualitative and quantitative modalities, ranging from bedside fundoscopy to electrophysiological studies and OCT. Optical coherence tomography allows the real time retinal substructures to be quantified and measured and has been in use since 1995 largely in ophthalmological practice. It is a non-ionising imaging modality of the retinal nerve fibre layer (RNFL) at micrometre resolution, with rapid, consistent data acquisition. The speed of sampling, the order of a few minutes, confers a considerable advantage over MR scanning. OCT is analogous to sonar techniques or ultrasound with light waves substituted for sound waves. Novel Spectral-domain OCT machines are able to image the retina and RNFL at extremely high resolution. Constituting part of the anterior visual pathway, the retina contains non-myelinated peripapillary retinal nerve fibre layer (RNFL), retinal ganglion cells (RGCs) and inner retinal layers amongst several other layers (**Figure 26**).

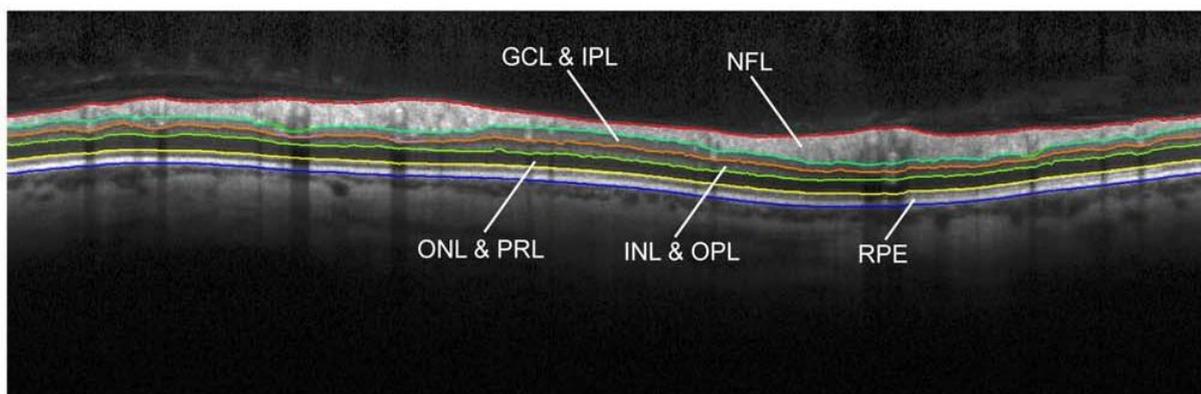


Figure 26- Retinal Architecture (NFL- Nerve Fibre Layer, GCL- Granular Cell Layer, IPL- Inner Plexiform Layer, INL- Inner Nuclear Layer, OPL- Outer Plexiform Layer Layer, ONL- Outer Nuclear Layer, PRL- Photoreceptor Layer, RPE- Retinal Pigmentary Epithelium)

Retinal ganglion cells are neurons located in the retinal ganglion cell layer and have a soma from which the originating axon runs initially in the retinal nerve fibre layer. These axons then converge turning into the optic disc, cross the lamina cribrosa at the optic nerve head and constitute the optic nerve. They are particularly sensitive to neurodegenerative damage due to defective mitochondrial dynamics and axonal transport, as well as oxidative stress and energy depletion, given the high metabolic demand and performances typical of these cells, mostly as a consequence of their patchy myelination (La *et al.*, 2017). OCT facilitates access to and quantification of these unmyelinated neuronal populations. Thus their potential as surrogate markers of intracranial neuronal pathology is highlighted and has led to their employment in clinical trials (Petzold *et al.*, 2010).

11.1.1 The Retina in Neurodegenerative Diseases

Studies in Alzheimer's disease (AD) patients have demonstrated evidence of significant loss of RNFL even in subjects with mild cognitive impairment (Valenti, 2011; Danesh-Meyer *et al.*, 2006), as well as structural and functional impairment, despite normal ophthalmological examination (Moschos *et al.*, 2012). Clear differences in RNFL thickness were also found between healthy controls and early AD (Kirbas *et al.*, 2013). In Parkinson's disease, a temporal loss of retinal nerve fibre layer thickness was identified by OCT (Kirbas *et al.*, 2013).

There also appears to be differential pattern of RNFL depending on the specific neurodegenerative disorder. The magnocellular RGCs are predominantly affected in AD and multiple system atrophy consistent with those changes found in glaucoma. In PD and Huntington's disease, the parvocellular RGCs are more affected, which is similar to the pattern seen in mitochondrial optic neuropathies (La *et al.*, 2017). Furthermore, in Multiple Sclerosis (MS), meta-analysis has shown a reliable difference in the RNFL thickness between MS subjects and controls and some prognostic potential in optic neuritis (Petzold *et al.*, 2010).

Visual impairment is a non-motor feature of PD that may be due to the loss of dopaminergic neurons located in the inner nuclear layer and inner plexiform layer of the retina. Secondary involvement of the retinal amacrine cells, which provide input to retinal ganglion cells, may also be expected.

Dopamine has been established as a major neurotransmitter in the retina, so areas that contain dopaminergic cells (such as higher visual areas, LGN, and the visual cortex) may also be responsible for visual impairment in PD. Absolute sensitivity, spatial contrast sensitivity, temporal sensitivity, and colour vision are visual functions mediated by dopamine and affected in PD (La *et al.*, 2017). Thinning of RNFL, particularly in the inferotemporal area of the optic disc, is a possible explanation for the arcuate superior visual field defect commonly seen in Parkinson's disease (Inzelberg *et al.*, 2004). Reduced RNFL, macular thickness, and volume but not in the fovea, despite normal visual function has also been found (Altintas *et al.*, 2008).

In a recent study, one hundred two eyes from 52 patients affected by PD were compared with 97 eyes from 50 age-comparable controls. In all patients, peripapillary RNFL thickness was measured by OCT. Eyes from patients with PD had a statistically significant decrease in average peripapillary RNFL thickness compared with control eyes ($P < 0.001$). This reduction was observed in every quadrant (inferior, superior, nasal ($P < 0.001$), and temporal ($P = 0.017$)) in patients with PD. As the evolution and severity of PD progress, the peripapillary RNFL layer thickness, as evaluated by OCT, gradually diminishes (Jimenez *et al.*, 2014).

11.1.2 Retina in Huntington's disease

Although visual loss has not been formally reported by patients with Huntington's disease, the evidence for retinal pathology in HD comes from a number of sources. Retinal increment thresholds were impaired to foveally directed blue light (Paulus *et al.*, 1993). In drosophila models photoreceptor degeneration is reported (Jackson *et al.*, 1998). In R6/1 and R6/2 HD mouse models loss of rod and cone function, photoreceptor degeneration, deficit in cone response on electroretinography and loss of cone opsin and transducin protein expression is seen (Batcha *et al.*, 2012; Helmlinger *et al.*, 2002). In a pre-symptomatic HD mouse model (HdhQ150), retinal intranuclear neuronal inclusions were demonstrated (Young *et al.*, 2013). In an R6/2 mouse model, retinal dysfunction and lower b-wave amplitudes as measured by electroretinogram (ERG) and pathology was noted (Ragauskas *et al.*, 2014). Later in the disease, rod dysfunction, retinal remodelling, gliosis and apoptosis occurs. Amacrine cells, which are retinal inter-neurons, are also lost as part of inner retinal degeneration (Batcha *et al.*, 2012; Helmlinger *et al.*, 2002; Johnson *et al.*, 2014).

A post-mortem histological study however, of one human HD retina, failed to reveal any significant abnormalities (Petrasch-Parwez *et al.*, 2005). From a therapeutic perspective, pre-clinical research has shown improvement of retinal dysfunction in R6/2 mice following intra-vitreous administration of a Rho-associated kinase (ROCK) via reduction in Huntingtin aggregation (Li *et al.*, 2013), implying that the presence of mutant Htt within the retina itself may be directly responsible for the retinal neurodegenerative phenotype.

In related polyglutamine triplet expansion disorders, retinal degeneration is well established clinically in Spinocerebellar Ataxia 7 patients. Similarly, a recent OCT study of nine Spinocerebellar Ataxia 1 (SCA1) patients, a polyglutamine triplet expansion disorder like HD with known cerebellar and brainstem volume loss, did demonstrate significant RNFL loss versus controls (Stricker *et al.*, 2011). Complimentary work has shown that Spinocerebellar ataxia types 1, 2, 3, and 6, also caused by polyglutamine expansions, were associated with reduced thickness of the RNFL and/or macular region (Pula *et al.*, 2011).

11.1.3 Visual Pathway in Huntington's disease

Various pathological ophthalmological signs can be found in both preclinical and clinical stages of HD. Specific retinal damage, namely, abnormal proteins formation, photoreceptor degeneration and retinal remodelling, has been studied in animal models as described above. Functional changes in occipital lobe activity and its atrophy as well as degeneration of visual pathways can already be present in the early stages of the disease.

Oculomotor symptoms of HD include disturbed visual fixation, slower tracking eye movements and saccades, and suppressed vestibulo-ocular reflex. Visual perceptual disorders, such as visuospatial difficulties, problems of stimulus identification and motion perception, along with decreased contrast sensitivity, have also been described (Svetozarskiy *et al.*, 2015).

Circadian deficits in HD are seen in both R6/2 and Q175 mouse models. Light detected by the retina by retinal ganglion cells that express the photopigment melanopsin, but also receive input from the

suprachiasmatic nuclei in the hypothalamus, which regulates circadian rhythms. In turn, they also mediate a range of non-image forming responses to light including circadian entrainment and the pupillary light response (PLR). Using an R6/2 and Q175 mouse model, the PLR was found to be attenuated in both lines and reduced levels of cone opsin and melanopsin expression were noted, implying involvement of the anterior visual pathway in HD (Ouk *et al.*, 2016).

The posterior part of the visual pathway may also be involved. Occipital volume loss is seen in both pre-manifest and symptomatic individuals (Nopoulos *et al.*, 2010; Rosas *et al.*, 2008; Tabrizi *et al.*, 2013) while in HD patients, visuomotor & visuospatial deficits in visuomotor and visuospatial functioning is established (Say *et al.*, 2011). A recent study did suggest that the executive dysfunction seen in HD may be contributed to by deficits in visual processing pathways. HD patients demonstrated occipito-temporal atrophy, impaired visuomotor and visuospatial function versus controls and association between SDMT performance and left fusiform activity on functional neuroimaging (Wolf *et al.*, 2014).

A study examining cortical thickness across four occipital regions in premanifest, early HD groups and controls, revealed that the occipital cortex in premanifest and early HD participants was reduced compared to controls. Regions associated with higher level visual processing (e.g., lateral occipital, lingual regions), highlight the potential visual deficits associated with occipital atrophy seen in multiple studies (Johnson *et al.*, 2015).

11.1.4 OCT as a biomarker in Huntington's disease

Considerable recent work has been carried out to identify candidate biomarkers in Huntington's disease, as clinical assessment alone lacks sensitivity over shorter time-spans. Amongst potential biomarkers, longitudinal MR imaging has identified atrophy of whole brain, white and grey matter as well as regional atrophy in premanifest and early HD subjects (Tabrizi *et al.*, 2012).

To the best of our knowledge, OCT had never been performed previously in HD patients (correct at time of study) and data presented below represents a pilot study investigating the potential of neuroretinal measurements as a biomarker in HD (Haider S, 2014).

11.2 Methods

The Study were carried out following the Helsinki protocol (Ethical Principles for Medical Research Involving Human Subjects), with local research ethics committee approval and clinical research organisation supervision including site initiation, monitoring and final visits. All study members were trained in good clinical practice.

11.2.1 Group Characteristics

Forty two genetically confirmed premanifest, early, moderate and juvenile HD patients, segregated based on Shoulson and Fahn total functional capacity criteria (Shoulson *et al.*, 1989) were selected for analysis. They were recruited from the multidisciplinary clinic at the National Hospital of Neurology and Neurosurgery and the PADDINGTON (Pharmacodynamic Approaches to Demonstrating Disease Modification in Huntington's disease) study. Twenty eight age matched healthy controls were also recruited. Any significant ophthalmological history of note prompted exclusion. The data underwent standard quality control measures including duplicate detection. Two were excluded as retinal registration was not possible due to difficulties with fixation and head chorea. Two subjects were excluded due to diabetes. In a further two subjects, measurements from one eye only were suitable.

11.2.2 Assessments

All participants underwent a neuro-ophthalmological assessment of colour vision (Ishihara testing), visual acuity (Snellen chart), visual field assessment by confrontation and pupillary reflexes. Optical coherence tomography (Spectralis 99165, Heidelberg Engineering) was carried out on all subjects, which involved macular volume and peri-papillary RNFL measurement of both eyes.

Inclusion Criteria

- Able to provide informed consent
- Able to tolerate OCT exam
- Gene positive Early, Moderate Stage or Juvenile HD or Controls

Exclusion Criteria

- Significant head or truncal chorea
- Diabetes, Glaucoma or other neuro-ophthalmological disease
- Advanced stage HD

11.2.3 Automated Data Analysis

Peripapillary RNFL thickness was measured and automated data readouts were produced as shown below (**Figure 27**). The RNFL is automatically compared to a normal distribution and colour coding applied to indicate this relationship.

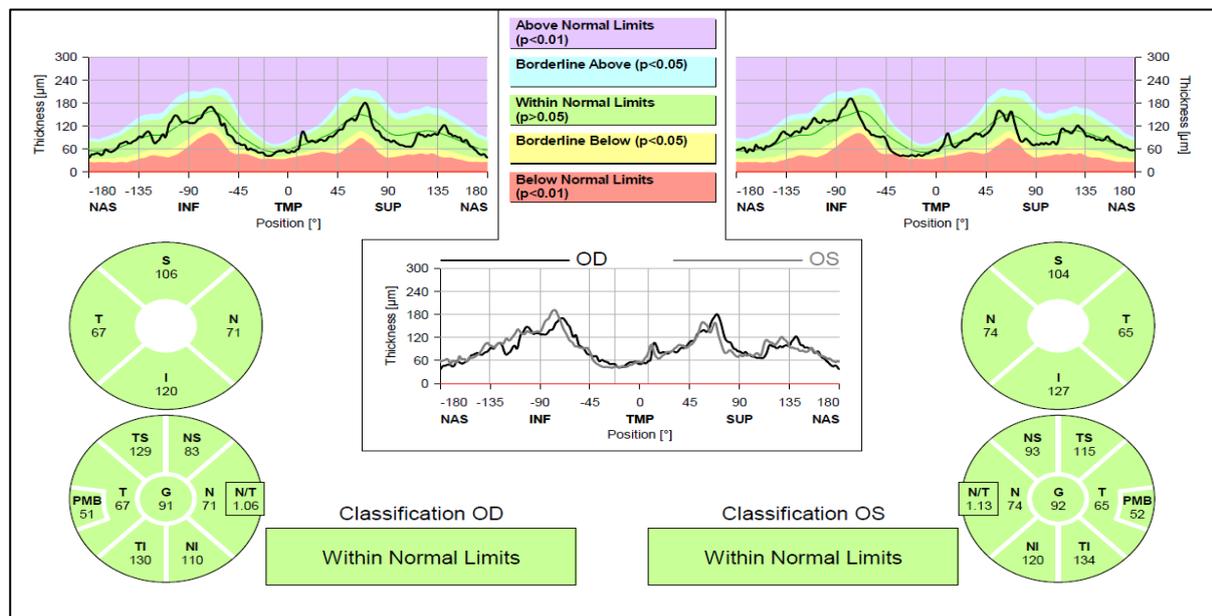


Figure 27- Example of circumferential peripapillary RNFL thickness, with colour coding representing values compared to the normal distribution. G- global; NS- nasal-superior; N- nasal; NI- nasal-inferior; N/T- nasal-temporal ratio; TI- temporal-inferior; T- temporal; TS- temporal-superior; ON- optic neuritis; PMB- papillo-macular bundle; RNFL- retinal nerve fibre layer.

The macular volume was also measured with the automated readout obtained as described in **Figure 28**.

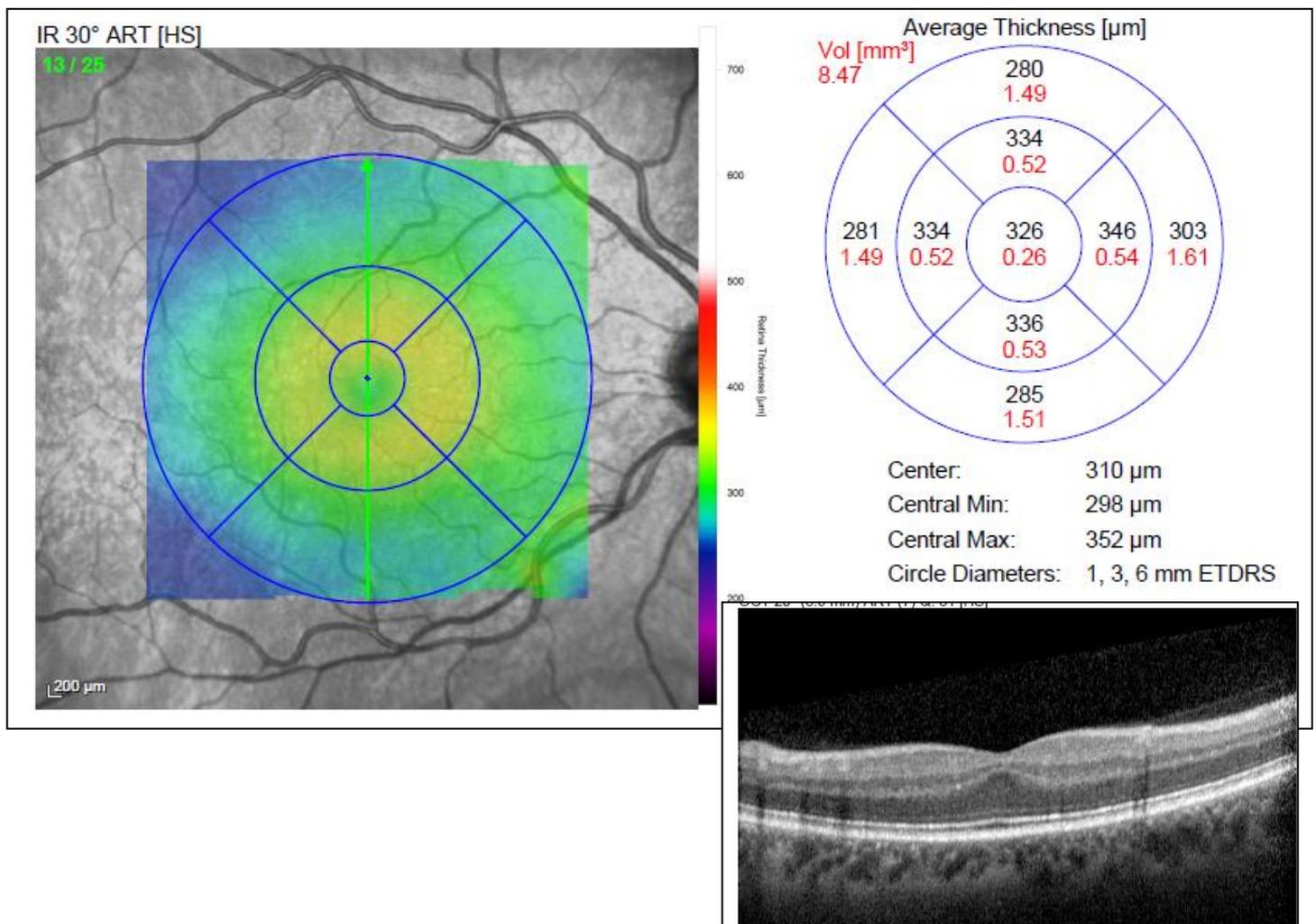


Figure 28- Example Macular Volume Readout

11.2.4 Statistical Analysis

For the purposes of analysis and given the small sample size, early and moderate stage HD patients were combined in a symptomatic HD group for some analyses. Statistical analyses were performed in R on segmented retinal data, looking at RNFL and macular volumes in order to determine if there are significant differences in the pathology of left and right eyes. RNFL and macular volumes were consistent across both eyes and thus right and left eye results were combined. However, some regions of the retina showed a trend towards difference between the eyes and thus each eye was treated as a separate independent variable.

The null hypothesis that *"the samples come from a normal distribution"* against the alternative hypothesis *"the samples do not come from a normal distribution"* was tested using the Shapiro-Wilk normality test using the *stats* package in R. The results confirm that the null hypothesis of normal distribution cannot be rejected for RNFL and macular volumes. RNFL and macular volumes between symptomatic HD groups (early, moderate and juvenile stage) were compared to suitably age-matched controls using the student's t-tests as the data fulfils the assumption of normality.

Segmented retinal data did show a trend towards non normality, and in the case of one region at least, PMBR, rejected the null hypothesis of normality. In order to allow for possible lack of normal distribution in some segmented retinal regions, samples from the aforementioned HD groups and controls were tested for deviation from the null hypothesis using the Mann-Whitney test (also known as Wilcoxon rank sum test). The null hypothesis being tested is that the true difference between HD group and control is zero.

In order to test for association whilst controlling for age and gender, logistic and ordinal logistic regressions were performed using *stats* and *MASS* packages in R respectively. Due to small sample numbers logistic regression (controls versus all symptomatic HD) was conducted on segmented retinal data, whilst ordinal logistic regression was performed with RNFL and macular volume data.

11.3 Results

11.3.1 Group Characteristics

Group demographics are shown below (**Table 39**), including CAG size. Aside from refractive errors, there were no significant abnormalities found on neurophthalmological assessment.

Table 39- Group Demographics

Characteristic	Adult Controls N=16	Premanifest HD N=9	Early HD N=14	Moderate HD N=6
Age; mean yrs (SD)	49.3 (9.1)	40.1 (6.6)	52.8 (9.5)	53.7 (6.7)
Gender; n male (%)	8 (50.0%)	5 (55.6%)	3 (21.4%)	4 (66.7%)
CAG; median (IQR)	-	44 (41.5, 44.5)	43.5 (40, 45)	44.5 (44, 45)
DBS; mean (SD)	-	307.9 (75.2)	361.1 (96.5)	461.3 (62.0)
TFC; median (IQR)	13 (13, 13)	13 (13, 13)	12 (11, 13)	6 (5, 6)

TMS; median (IQR)	0 (0, 0)	0 (0, 3)	17 (13, 24)	45.5 (37, 55)
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	Controls	Pooled HD (Early & Moderate Stage)	Juvenile HD	Juvenile Controls
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A smaller number of moderate subjects (n=6) and premanifest subjects (n=9) are noted. The average age between early and moderate stage HD subjects was relatively similar, though the control group was slightly younger. There were a larger number of female participants in the early HD group than controls. Importantly CAG size is well matched between all HD groups. DBS and functional scores (TFC) and TMS are all consistent with advancing disease stages.

Table 40- Juvenile HD and Controls group demographics

Characteristic	Juvenile HD N=4	Young Controls N=12
Age; mean yrs (SD)	29.8 (4.3)	28.7 (4.5)
Gender; n male (%)	2 (50.0%)	4 (33.3%)
CAG; median (IQR)	57.5 (53, 59)	-
DBS; mean (SD)	598.6 (82.8)	-
TFC; median (IQR)	8.5 (6, 11)	13 (13, 13)
TMS; median (IQR)	39.5 (23.5, 59.5)	0 (0, 0)

Small numbers are noted in the juvenile group due to difficulties in recruitment, though ages are well matched. A larger percentage of female subjects were noted in the control group versus males (**Table 40**).

11.3.2 Macular Volume & Retinal Nerve Fibre Layer

Macular Volume is Reduced in HD patients versus controls but not RNFL; no effect in Juvenile HD

An initial analysis looking for differences between affected (HD) populations and controls did demonstrate reduced macular volume in the symptomatic HD group (early and moderate stage combined) versus controls ($p < 0.01$). There was no effect noted with Juvenile HD subjects or with an RNFL analysis as a whole (**Table 41**).

Age	48.7	53.1	28.1	29.8
<i>SD</i>	9.2	8.9	4.35	4.34
Average Macular Volume mm³ (Combined)	8.71	8.47	8.95	8.83
<i>SD</i>	0.22	0.31	0.30	0.14
P-value versus Controls (*Young controls)	n/a	0.015	n/a	*0.41
Average RNFL μm (Combined)	94.2	92.7	102.5	103.7
<i>SD</i>	7.5	9.64	7.5	2.8
P-value versus Controls (*Young Controls)	n/a	0.47	n/a	*0.58

Table 41- Summary table of macular volume and retinal nerve fibre layer thickness in controls versus symptomatic HD groups (Student's T-test)

11.3.3 Macular Volume

Reduced macular volume in Early and Moderate Stage HD participants

There was a statistically significant difference noted between controls and symptomatic HD groups ($p= 0.015$). In the premanifest HD group, there was no clear evidence of macular volume loss versus controls, though the lack of tightly age-matched controls may confound this interpretation. Between early and moderate stage HD subjects, a difference is seen which approaches the threshold of statistical significance ($P= 0.06$ for both groups respectively). Comparison of juvenile subjects with appropriate controls failed to reveal anything of significance.

A boxplot distribution analysis of macular volumes between disease stages suggests there may be lowering of macular volume as disease advances (**Figure 29**).

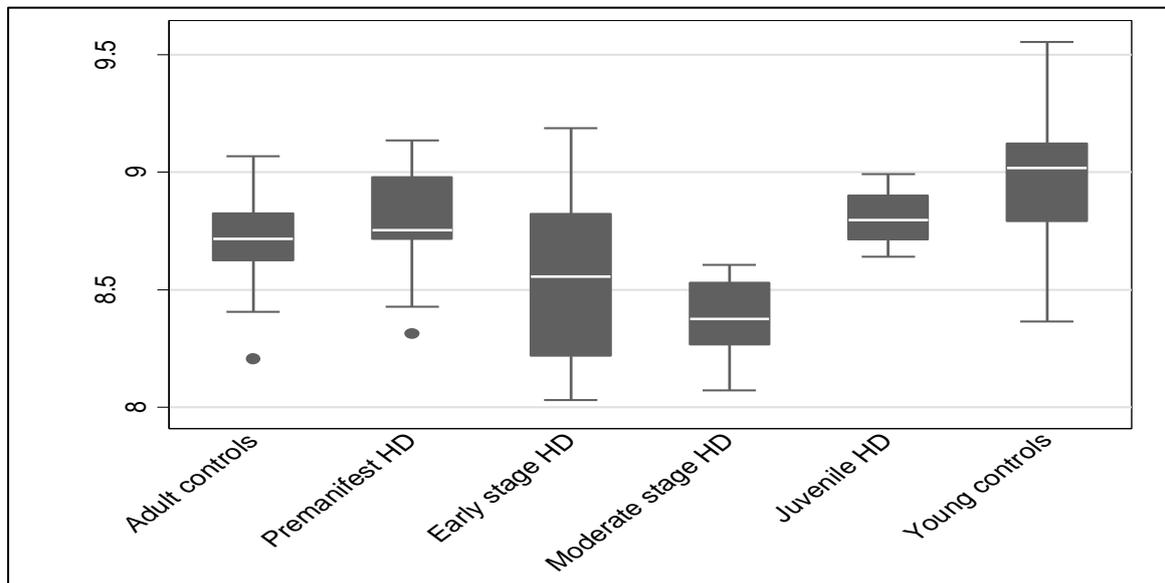


Figure 29- Boxplot Distributions of Macular Volume by Group suggesting reduced MV as disease progresses. 4 Juvenile HD subjects versus 12 age matched young controls show no clear effect.

It is noted that both age and gender are possible confounders in macular and RNFL readouts and thus the quoted p-values may be misleading. Moreover there was some asymmetry in gender distribution amongst the groups, particularly juvenile HD versus young controls. In order to address this, ordinal logistic regression was performed on symptomatic HD patients versus controls, where disease stage is ordered by moderate > early > controls and where age and gender are treated as co-variables. For the juvenile HD versus young controls comparison, a student's t-test did not suggest that gender was significantly different between the groups.

Macular Volume & Disease Stage

Table 42- Ordinal Logistic Regression of Macular Volume by Disease Stage, corrected for possible age and gender confounding, maintains statistical significance

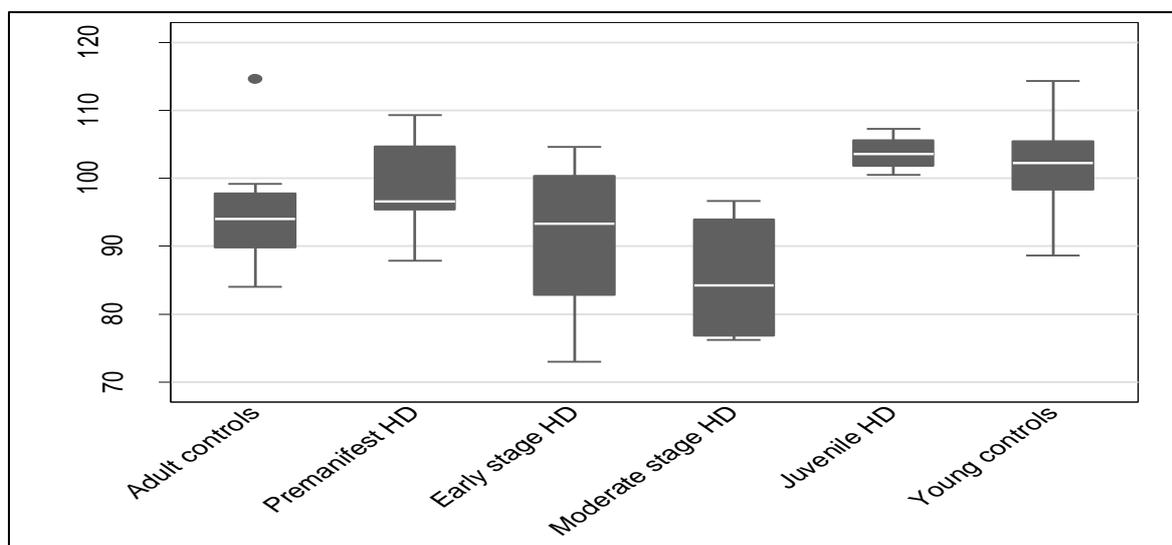
	Value	Lower 95%	Upper 95%	Std. Error	t value	p value
Macular volume	-3.043	-5.507	-0.580	1.257	-2.422	0.015
Age at Visit	0.037	-0.037	0.110	0.038	0.972	0.331
Gender	-0.409	-1.783	0.965	0.701	-0.583	0.560
Controls to Early Cut	-25.177	-	-	11.366	-2.215	0.027
Early to Moderate Cut	-22.977	-	-	11.209	-2.050	0.040

Corrected ordinal logistic regression analysis of macular volume by disease stage, suggest that with an increase in disease severity, a reduction in macular volume is seen. That is to say 1 unit increase in macular volume decreases the disease stage severity, on a log of odds scale, by 3.04. However, one notes the large confidence interval for this variable. An increase in sample size will narrow this range and give a more stable log of odds. Addition of premanifest samples, improves the p value to 0.013 with a log of odds of -2.694. Importantly, despite correction for possible effect of age at visit and gender on macular volume, significance is maintained (**Table 42**).

11.3.4 Retinal Nerve Fibre Layer

RNFL Thickness is Unaffected

Figure 30- Boxplot Distributions of RNFL by Group suggesting no clear differences between Adult Controls and Premanifest groups.



While there was a trend for lower RNFL values in affected individuals versus controls this did not reach statistical significance (**Figure 30**). This was confirmed by ordinal logistic regression models, both excluding and including premanifest group, where no association with disease stage was observed for RNFL thickness (**Table 43**).

Table 43- Ordinal Logistic Regression of RNFL by Disease Stage, corrected for possible age and gender confounding, with premanifest group excluded still shows no statistical significance

	Value	Lower 95%	Upper 95%	Std. Error	t value	p value
RNFL thickness	-0.056	-0.134	0.023	0.040	-1.381	0.167
Age at Visit	0.041	-0.033	0.116	0.038	1.088	0.277
Gender	0.299	-1.087	1.685	0.707	0.423	0.672
Controls to Early Cut	-2.678	-	-	4.364	-0.614	0.540
Early to Moderate Cut	-0.350	-	-	4.329	-0.081	0.936

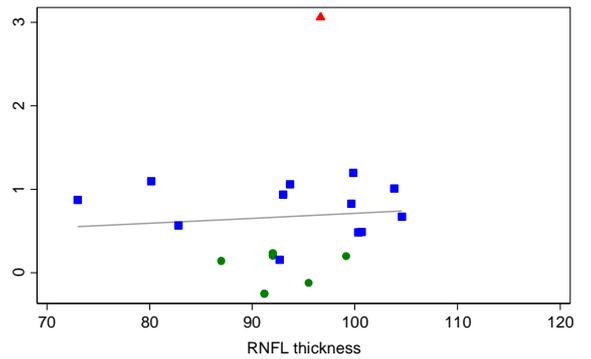
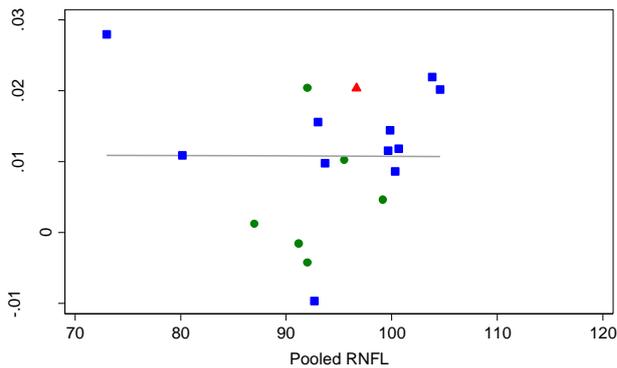
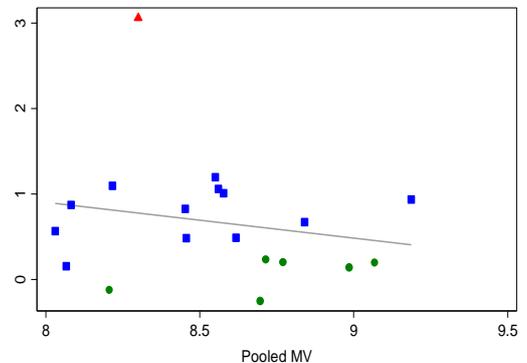
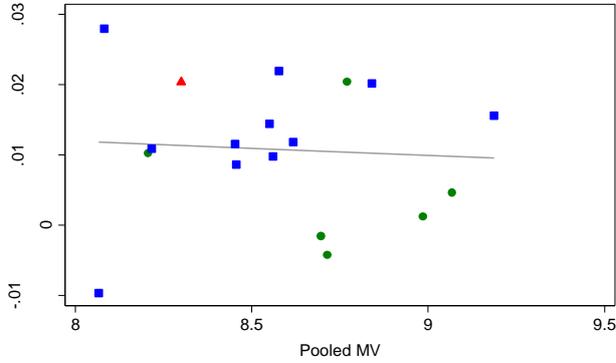
Inclusion of premanifest samples produces a worse model with p value of RNFL thickness increasing to 0.4 with log of odds reducing to -0.03.

11.3.5 Change in Whole Brain and Caudate Volume Correlation

Given the hypothesis that intra-retinal pathology could be a surrogate marker of intracranial pathology in HD, and the fact that 27 of the subjects in the OCT study had longitudinal volumetric MRI data as part of the PADDINGTON imaging biomarker project, a correlation between MV and RNFL and change in whole brain/caudate volumes over 15 months was sought. However, this analysis did not produce any results of statistical significance (**Figure 31**) (carried out by Zoe Scott, ION).

Figure 31- Regression Co-efficient of change in caudate and whole brain (over 15 months) with macular volume and RNFL thickness with no evidence of significant correlation.

	Caudate changes (N=18)		Whole Brain changes (N=19)	
Characteristic	Regression coefficient (95% CI)	P-value	Regression coefficient (95% CI)	P-value
MV; per unit higher	-0.00 (-0.02, 0.01)	0.79	-0.42 (-1.48, 0.64)	0.42
RNFL; per unit higher	-0.00 (-0.00, 0.00)	0.99	0.01 (-0.04, 0.05)	0.78



11.3.6 Retinal Segmentation

The retina can be subdivided as outlined in **Figure 27** into specific regions of interests which in different neurological as well as neurophthalmological disorders can be affected differentially. An analysis aimed at determining whether regional retinal susceptibility exists in HD subjects versus controls was initially performed using Mann-Whitney test. The results were corrected for multiple testing using Bonferoni as well as Benjamini-Hochberg corrections. However, Benjamini-Hochberg correction was deemed to be most appropriate as the variables exhibited mild correlation with one another (**Figure 32**).

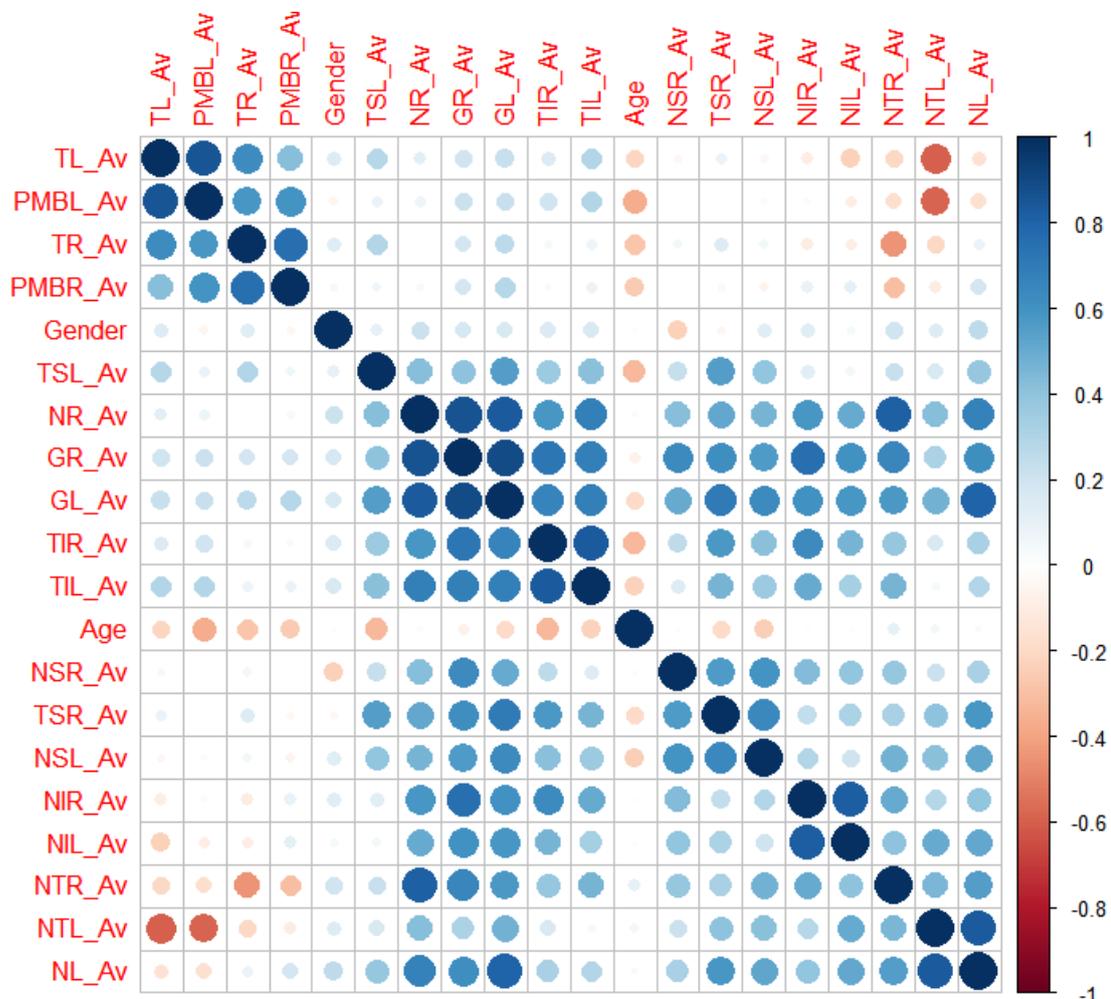


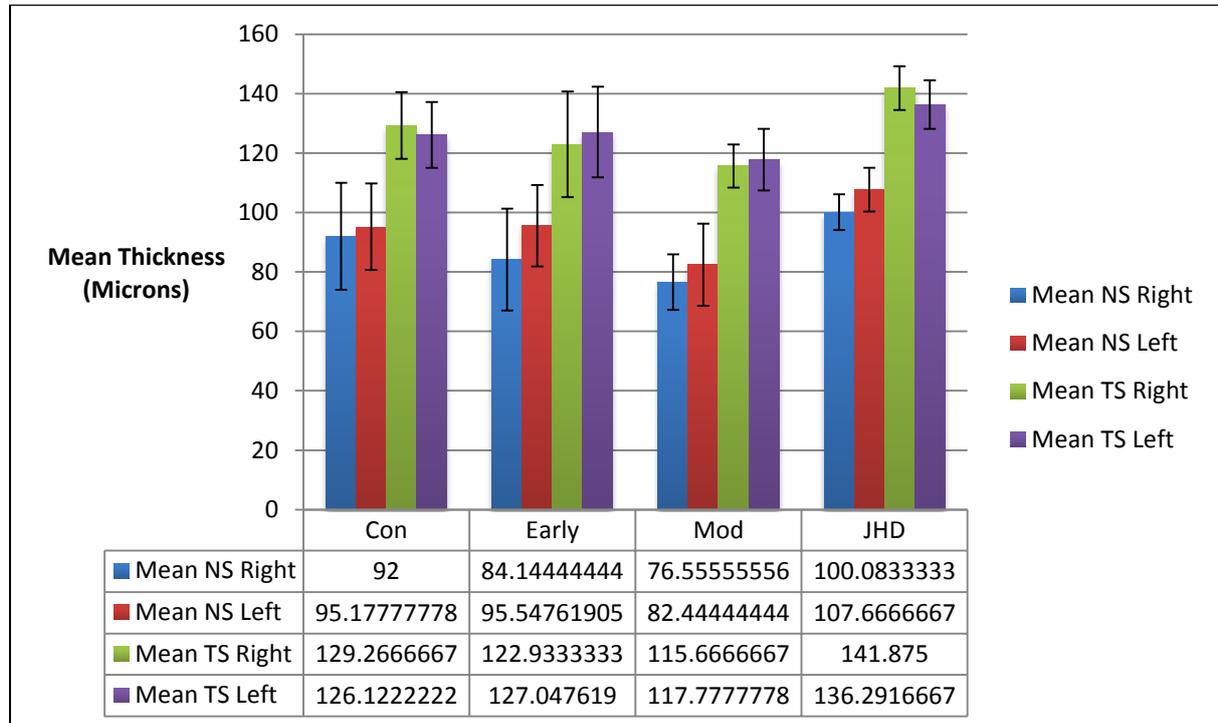
Figure 32- Rank-based measure of association correlation matrix in symptomatic HD and control groups showing mild and moderate correlation of retinal segments with one another. Blue colour indicates positive correlation; red-brown negative correlation.

Descriptive retinal data for each segment compared to controls is presented below.

RFNL Nasal Superior and Temporal Superior Regions

Figure 33- RFNL Thickness by retinal segments NS- Nasal Superior TS Temporal Superior.

Standard Deviation bars shown.

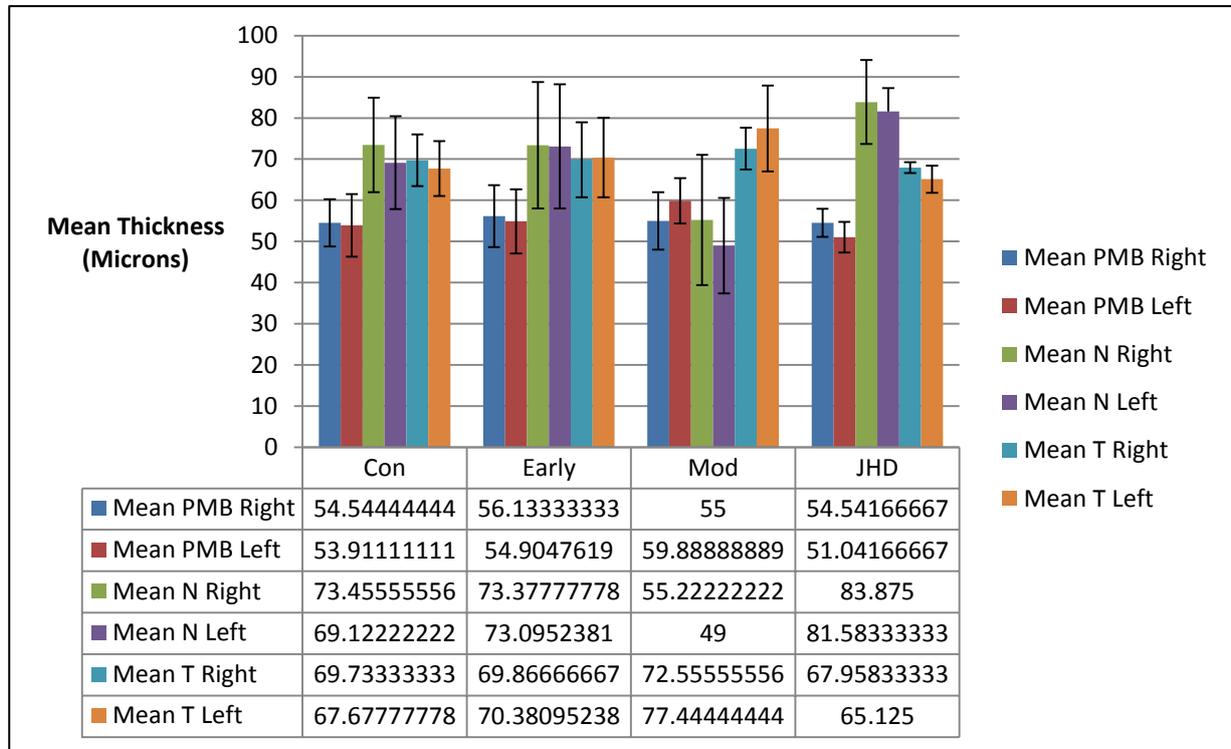


There does not appear to be any significant difference between the groups in the nasal superior and temporal retinal regions (Figure 33).

RFNL Thickness Papillomacular bundle, Nasal and Temporal Regions

Figure 34- RFNL Thickness by Retinal Segments PMB- Papillomacular bundle N- Nasal T Temporal.

Standard Deviation Bars shown.

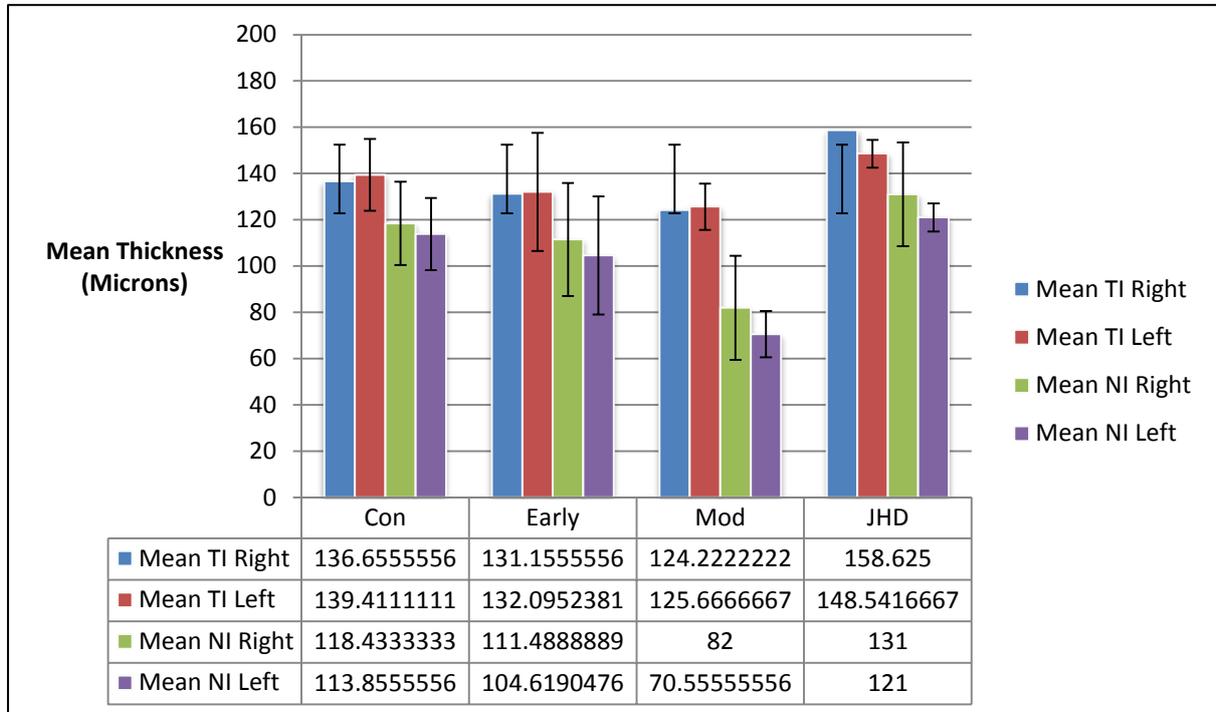


There does not appear to be any significant difference between the groups in the papillomacular bundle, nasal and temporal retinal regions (**Figure 34**).

RFNL Thickness Nasal Inferior and Temporal Inferior Regions

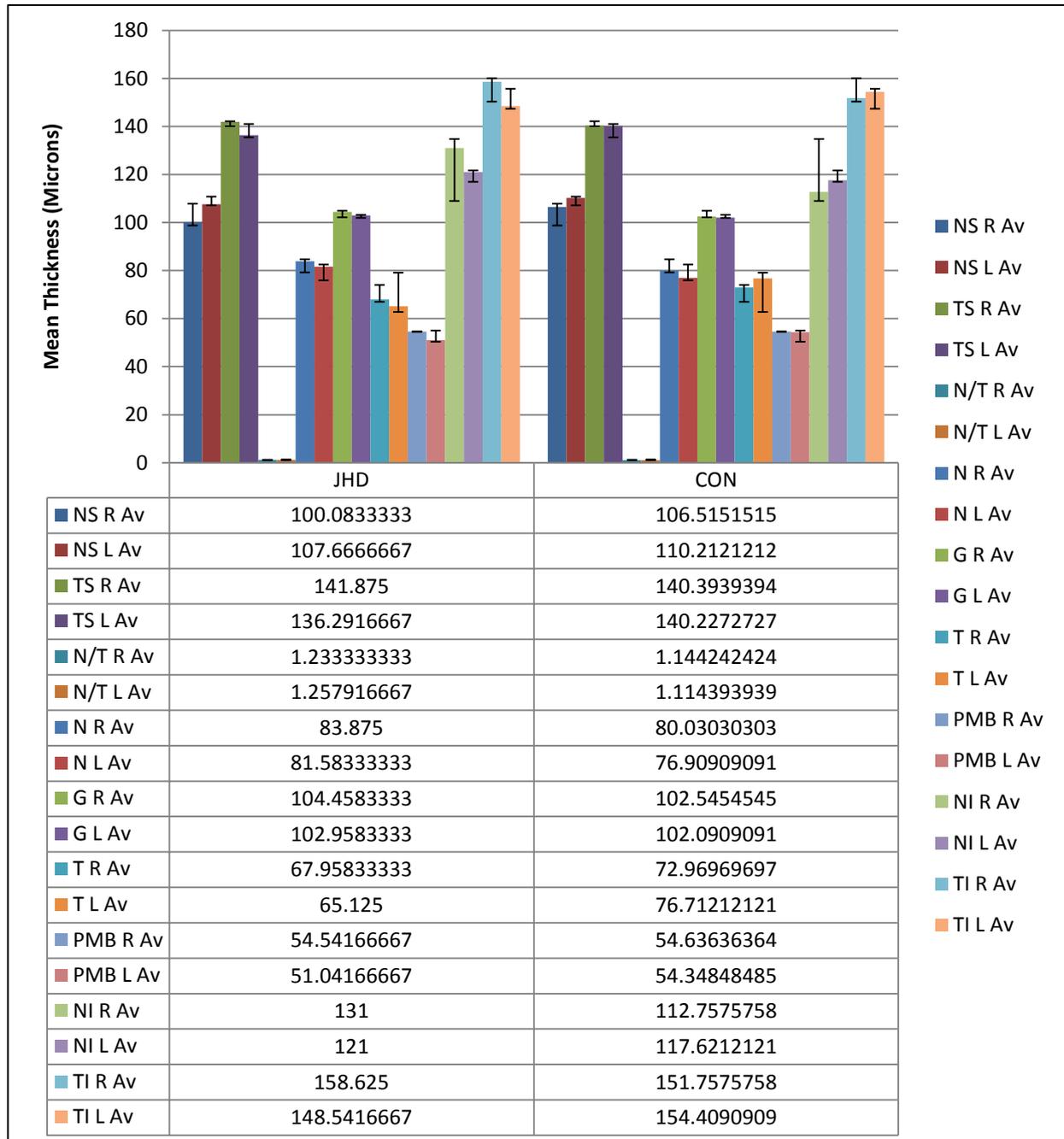
Figure 35- RFNL Thickness by retinal segments NI- Nasal Inferior TI Temporal Inferior.

Standard Deviation bars shown.



There does not appear to be any significant difference between the groups in the nasal and temporal inferior retinal regions (**Figure 35**).

Figure 36- RNFL Thickness by all retinal segments in Juvenile HD patients versus controls. Standard deviation bars shown. G- global; NS- nasal-superior; N- nasal; NI- nasal-inferior; N/T- nasal-temporal ratio; TI- temporal-inferior; T- temporal; TS- temporal-superior; ON- optic neuritis; PMB- papillo-macular bundle; RNFL- retinal nerve fibre layer.



There does not appear to be any significant difference in all retinal regions between juvenile HD and young controls (Figure 36).

In order to test the hypothesis that there is selective retinal segment loss in HD populations versus controls, a Wilcoxon rank sum test was performed with results summarised below in **Table 44**.

Table 44- Retinal segments are not significantly associated with HD subtypes on Wilcoxon rank sum test

Group	Significant after BH correction	Approaching Significance after BH correction
Premanifest	Nil	Nil
Early	Nil	Nil
Moderate	Nil	NIL, NIR, NTL, NL, GL
Juvenile	Nil	Nil
Symptomatic HD	Nil	Nil

There was no evidence of an association between retinal segment loss and HD subtypes, although in moderate HD group, several variables approached significance. Of note, the NI region approaches significance on both right and left sides, indicating a potential genuine effect that may be detectable with a larger sample size. However the correlation matrix (**Figure 32**) indicates that this cluster of variables are in fact positively correlated with each other, suggesting that there may be one true signal, which again would require larger sample sizes, to determine definitively.

11.4 Conclusion

In this pilot biomarker study, an OCT based analysis of macular and RNFL measures has been carried out for the first time in Huntington's disease patients. Macular volume appears to be reduced in symptomatic HD groups versus age-matched controls, to a statistically significant level. No statistically significant differences in RNFL thickness is noted however, between controls and symptomatic HD groups. Intriguingly, the juvenile HD population, the most aggressive HD phenotype, does not display any positive findings on macular volume and RNFL readouts versus a young control group. Retinal segmentation analysis also failed to reveal any significant findings. No clear correlation between change in whole brain and caudate volume over 15 months and either MV or RNFL is apparent.

Despite suggestive mouse model data and the macular volume loss noted here, HD patients do not complain of symptoms suggestive of colour vision loss, nor is there any suggestion anecdotally of higher rates of maculopathy for example in this patient group, although epidemiological data to answer this question accurately is not available.

The visual system in HD itself is not well studied either, though recent work using cognitive testing, structural MRI and fMRI has shown that HD patients have significantly inferior visuomotor and visual object performance compared to controls, with accompanying volumetric loss in occipitotemporal regions. Decreased activity was also noted in fusiform cortex, an area known to be involved in visual processing and facial recognition (Wolf *et al.*, 2014). Visuo-spatial memory as determined by the Visual Spatial Learning Test (VSLT), was impaired in HD patients compared to both control and premanifest populations (Pirogovsky *et al.*, 2013).

A sub-clinical level effect may be more plausible and one notes that despite full clinical recovery from optic neuritis, deficit on ERG testing can still be seen in MS sufferers (Petzold *et al.*, 2010). In the R6/1 model, ERG has demonstrated deficits present prior to histological abnormalities, indicating neural dysfunction is occurring prior to formation of intra-nuclear neuronal aggregates. An electroretinogram (ERG) study of HD patients and controls designed to gauge any correlation between ERG measurements and stage of disease revealed statistically significant increased amplitudes in HD patients compared to controls at light-adapted (photopic) 24.2 and 60.9 cd.sec/m² intensities, and dark-adapted (scotopic, red flash) 0.22 cd.sec/m² intensity. This implies that the enhanced response in HD patients is specific to the cone photoreceptor pathway dysfunction (Pearl *et al.*, 2017). However, neurophthalmological assessment on our participants was not suggestive of abnormalities in colour vision via Ishihara testing. Given the above and the cone dysfunction seen both in terms of opsin expression and ERG abnormalities in previous studies (Ouk *et al.*, 2016; Batcha *et al.*, 2012), more sensitive testing of colour vision using FM100 Hue test could be undertaken in follow-up studies. It should be noted that the major limiting factor of this study, in particular with respect to brain volume and retina segmentation analysis is the small sample numbers.

The macula is made up of large numbers of densely packed retinal ganglion cells which are intimately associated with cones. Thus it may be that the ganglion cells themselves are affected more so than cones. Direct testing of cone function to explore this hypothesis could be attempted in

the future as well as comparative analysis of the contribution of each of the nerve fibre layers to the overall volume loss noted. Indeed, in Parkinson's disease, there is evidence that examination of the pre-ganglionic layers is a better indicator of retinal pathology (Shrier *et al.*, 2012; Spund *et al.*, 2013). Furthermore, the absence of a clear RNFL thinning may also be explained by a relative increased susceptibility of cones to the effect of mutant *Huntingtin*.

Complimentary work carried out independently in 26 HD patients and 29 controls who underwent spectral domain OCT scans showed Temporal RNFL thickness was significantly reduced in the HD group (62.3 vs. 69.8 μm , $p = 0.005$), and significant negative correlation between temporal RNFL thickness and disease duration ($R^2 = -0.51$, $p = 0.04$). There was a significant negative correlation between macular volume and disease duration ($R^2 = -0.71$, $p = 0.002$), and motor scores ($R^2 = -0.56$, $p = 0.01$). Interestingly and in contrast to other work, colour vision was significantly poorer in the HD group. They propose that the preferential temporal RNFL loss in HD patients may implicate mitochondrial dysfunction as the temporal RNFL is reduced similarly in patients with some mitochondrial disorders, including Leber's hereditary optic neuropathy (Kersten *et al.*, 2015).

In contrast to work presented here, no difference between absolute values of macular volume between cases and controls was observed, macular volume did significantly correlate with UHDRS motor scores and disease duration. The sample size between both studies is comparable (22 HD eyes to 24) and small, limiting interpretation. Additionally, there were non-contemporaneous/absent ocular and motor assessments in this external work.

In a later study using spectral domain OCT, a significant reduction in macular choroidal thickness was found in HD patients when compared with controls. In addition, several retinal macular thickness measurements were inversely correlated with TMS-UHDRS, suggesting that the macular choroid may be altered early in the progression of the disease and that retinal macular thickness may decrease with increasing motor severity of the disease. Hence, both the choroidal and retinal macula may be affected in HD pathology (Andrade *et al.*, 2016). As a major vascular structure, choroidal thickness may change in volume either because of structural changes or abnormal perfusion status. Recent studies have highlighted neurovascular dysfunction and blood-brain barrier dysfunction in the brains of mice and humans with HD may contribute to the pathophysiology of the disease (Drouin-Ouellet *et al.*, 2015; Lin *et al.*, 2013).

The relatively small numbers of both juvenile and moderate HD subjects (4), the gender difference between juvenile and control subjects and the overall small numbers in the study must be considered. Both macular volume and RNFL can be influenced by age and sex which could impact on both positive results in the case of macular volume, and negative findings in the case of absence of a readout in the juvenile HD group. However, there was no evidence of a statistically significant difference in gender between the juvenile HD and young control groups. Additionally, it may be that there is relative greater durability of neuro-retinal neurones than elsewhere in brain but this is importantly not obviously seen in the equivalent mouse models. Paradoxically this is supported by the lack of correlation noted between macular volume and change in whole brain and caudate volume data.

Technical limitations with OCT must also be considered. Retinal layers are not evenly distributed in the macula with inner retinal layers absent in the foveola (macular centre) and gradually emerging toward the macular periphery. Therefore, radial distance to the foveola may be a more relevant measure than macular segmentation. Indeed this was evident in PD with analysis of an annular zone surrounding the foveola improving readout between patients and controls (Andrade *et al.*, 2016; Spund *et al.*, 2013).

The JHD phenotype is noted to be the most aggressive form and the lack of a clear effect, despite age matched controls is unusual. Firstly, the low number of JHD subjects significantly reduces the ability of study to detect a difference. However, it is clear that retinal nerve fibre layer thickness and macular volume diminish with age, as do corresponding neuronal populations do in the brain. It is therefore possible that because of a combination of their relatively young age combined with relatively resistant neuro-retinal neurones versus those intracranially, no clear effect is seen. Testing and comparing relative expression of mutant Htt in retinal tissues versus the brain could provide valuable supplementary data to better answer this question.

In support of this theory, one notes the absence of an effect in the premanifest population, despite evidence of active brain neurodegeneration from imaging and clinical studies, showing volumetric loss and cognitive deficits respectively. This could indicate that retinal neurodegeneration at the

macula and peripapillary RNFL, may occur later on in the evolution of HD pathology. The peripheral retina however has not been explored in this study and may therefore be worth investigating.

However, neuropathological, clinical and imaging studies in JHD indicate far more severe neuropathological changes than in adult onset disease. Thus it may be that in contrast to affected neuronal populations in the brain, retinal neurones may be more resistant to the early cellular insults of pathogenic mutant *Huntingtin*. With further ageing, susceptibility of this population may increase, rendering adult onset HD subjects more liable to show detectable change within the retina. Longitudinal follow-up of this juvenile HD cohort as well as increasing its numbers would be helpful in resolving this question. Finally, juvenile HD does represent a clinically distinct phenotype to adult onset HD and it may be therefore that a retinal phenotype is not seen in this grouping and indeed may never be so, despite the larger CAG size.

Interpretation of OCT can also be influenced by pre-existing ophthalmological disease, particularly glaucoma, cataracts, retinal disease and high myopia (Leung *et al.*, 2006). All patients had a carefully taken ophthalmic history but did not undergo pressure testing to exclude glaucoma. As a relatively novel imaging modality, OCT may also lack from large cohort normative data to discern any clear effects of gender, ethnicity and age on readouts. All participants were Caucasian in origin and the group demographics indicate reasonable age-matching. A higher proportion of female subjects are noted in the early stage HD groups and literature does suggest that OCT is influenced by gender, body mass, height or weight. Recent work has shown statistically significant differences in retinal thickness between subjects of different race, gender, and age. When compared to Caucasians and Hispanics, African-American race is a predictor of decreased mean foveal thickness while male sex regardless of race, is a significant predictor of increased mean foveal thickness (Kashani *et al.*, 2010). A sub-analysis was undertaken to determine the extent of influence of age and gender in this work which did not reveal it to be a factor in the correlation of macular volume with the presence of HD phenotype.

Given the chorea and eye movement abnormalities that occur in HD, including difficulties with fixation, at the outset of study it was not clear how patients of different stages would tolerate the procedure. Dual beam eye tracking technology helped to correct for microsaccadic unintended deviations. FoDi- fovea-to-disc alignment also automatically orients the anatomy for PMB measurement accuracy. In general only 2 subjects out of 65 found the procedure too difficult to

complete. One notes with great interest also the development of hand held OCT devices for paediatric populations though in this study children were sedated (Avery *et al.*, 2014b; Avery *et al.*, 2014a) meaning assessments could be carried out at home.

Data presented here suggests that macular volume is reduced in symptomatic HD groups compared to controls and to the best of our knowledge, is the first such demonstration of an ocular neurodegenerative phenotype in human HD retina. If longitudinal change as measured by OCT can be proven, its value as a biomarker would rise significantly and further study is warranted.

Finally, statistical measurements in small sample size cohorts, are extremely sensitive to small changes in sample numbers. Thus, we note that definitive results cannot be obtained without collaborative studies to incorporate larger data sets thus raising the possibility of meta-analysis of published data as future work.

Acknowledgements

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12 Conclusions

12.1 Disease Modification and Clinical Trial Design

The need for a truly disease modifying strategy for neurodegenerative disorders in general and HD specifically is readily apparent. Thus far no therapeutic approach that was successful in animals has translated to success in human trials (Mestre *et al.*, 2009). Trials have utilised re-purposed compounds without established mode of action. Despite several promising approaches found in animal models, and over twenty five years since the discovery of the responsible gene, even a truly promising potential therapy remained elusive, until the recent Ionis-HTTRx trial.

In part this reflects, the lack of commutability of animal model research to its human counterpart, but also the inherent complexity of targeting a primarily neurodegenerative disease process (Mestas & Hughes, 2004). The validity of preclinical research has also been questioned with many studies unable to be replicated independently, suggesting that methodological considerations, publication bias, statistical concerns may be impeding translation of therapies (Munafo *et al.*, 2014; Menalled & Brunner, 2014). Furthermore, in spite of recent advances in molecular genetics and shared pathogenic mechanisms with other more common neurodegenerative diseases, specific Huntington's disease research arms within pharmaceutical companies were not prioritised until recently, largely due to its rarity compared to other neurological diseases e.g. Alzheimer's and cerebrovascular disease, heart disease and cancer. Takeda, Roche and TEVA now have established programmes in Huntington's disease.

Indeed, tetrabenazine is still currently the only medicine licensed within the European Union (EU) for symptomatic control of chorea. Other therapies in use range from anti-psychotics, for irritability, behavioural and chorea management, selective serotonin re-uptake inhibitors (SSRIs) for depression and anxiety, mood stabilisers, are in routine use by HD specialists but have not been ratified formally in specific studies (Reilmann *et al.*, 2013).

Selisistat demonstrated impressive pre-clinical data with two postulated mechanisms of action, acetylation of mutant HTT to drive autophagy and amelioration of transcriptional dysregulation. It

was safe in healthy volunteers. Phase 1 and 2B data demonstrated reasonable safety and tolerability with no clear change in clinical efficacy parameters. However significant concern over liver function disturbance requires further clarification which unfortunately due to financial constraints will not be forthcoming. The putative mode of action has not been definitively evaluated nor published to be subjected to scrutiny from the scientific community.

Modern pharmaceutical development especially for neurological diseases, despite considerable investment and evident need, yields a relatively low number of novel compounds. Even when they do reach Phase III trials, they often fail to demonstrate efficacy. For example, over the last twenty years, 99 clinical trials have been undertaken in HD utilising 41 different therapeutic compounds but only 3.5% have transitioned to the next stage of drug development (McColgan & Tabrizi, 2018). To ensure that the transition to proof of concept trials is well founded, a valid pharmacodynamic interaction must be demonstrated. This is elaborated further into the “three pillars of survival,” as outlined (Morgan *et al.*, 2012):

1. Exposure at the target site of action over a desired period—required concentration of drug in the right compartment. If substituted by plasma levels, there needs to be adequate pharmacokinetic (PK) modelling and evidence that barriers such as the blood–brain barrier (BBB) are crossed.
2. Binding to the pharmacological target in line with proposed mode of action—PET studies, namely, occupancy studies, are helpful here.
3. Expression of pharmacological activity commensurate with the demonstrated target exposure and target binding- pharmacokinetic/pharmacodynamic (PD) studies with biomarkers that reflect the expression of primary pharmacology at the site of action. The phase II trial should produce sufficient evidence that the drug is safe and has efficacy in the target population. Defining what constitutes sufficient evidence to support a “Go” decision to phase III is difficult to articulate, but there have been several attempts to quantify the process.

12.2 Disease Modification

A consensus of what constitutes disease modification is also lacking. It is reasonable to propose that to demonstrate disease modification an intervention must:

1. Impede the underlying pathological processes of disease that lead to cell death and dysfunction.
2. Lead to an improvement in the phenotype.

It can be argued that symptomatic therapies *affect* the pathology of disease, or indeed may access compensatory pathways, that underpin a clinical improvement. Therefore, for a given therapeutic strategy to be termed truly disease modifying, it becomes fundamental to connect retardation of pathology and functional amelioration/dysfunctional slowing, with retardation of disease. Neither of the therapeutic studies presented here meet the criteria above and this acid test, and how best to carry it out must be kept at the forefront from bench to bedside in future drug trials.

It may prove important to distinguish disease modification from a *curative therapy*, which might be defined as an intervention which removes the underlying the disease process entire from the organism; *restorative therapy* e.g. stem cell transplantation, where the underlying disease pathology is not *directly* impacted, rather supplanted by healthy tissue; *rescue therapy*, aimed at salvaging at risk populations of neurones and *compensatory therapy*, to boost compensatory networks, which is currently being studied via a novel brain-training neurofeedback and fMRI paradigm (Papoutsis *et al.*, 2018). Combinations of these approaches may in fact be the most effective in the treatment of complex, progressive neurogenetic and degenerative disease

Mechanistically, recovery may be underpinned by activation of compensatory pathways such as those well-known following cerebrovascular events for example, plasticity and recovery of neuronal

circuits that were salvageable. In an fMRI study of premanifest HD subjects, as atrophy increased performance-related activity was enhanced in the right parietal cortex during a working memory task. Increased functional coupling between the right dorsolateral prefrontal cortex and a left hemisphere network predicted better cognitive performance as atrophy increased (Kloppel *et al.*, 2015). In the future one can envisage the simultaneous administration of a disease arresting treatment with therapy designed to boost compensatory pathways.

12.3 Biomarker Development

Alongside biologically sound therapies, sensitive, reliable, objective readouts or genuine biomarkers to demonstrate the potential for disease modification were lacking until recent studies. These biomarkers have focussed on neuroimaging, clinical assessments and quantitative motor strategies. However in neurologically impaired subjects long, arduous and mentally taxing assessments are far from ideal. The innate immune system is postulated as a modifier in Huntington's disease while Sirtuins are known to modulate the immune system. A proposed mechanism for selisistat might be via an interaction with the immune system in HD patients but unfortunately no evidence of this effect was seen. However it is unclear what proportion, indeed if any, of the dysfunction of the innate immune system is driving disease progression.

Optical coherence tomography represents a relatively novel retinal nerve fibre layer imaging modality that is well tolerated, with scan times of the order of a few minutes and an automated analysis generated within a few seconds. Furthermore for multi-centre studies, as long as the same machine is employed, inter-site consistency is far easier to achieve compared to MR based techniques.

12.4 Future Work

It is clear that further validation of the mechanism of action of selisistat in terms of modulation of peripheral and central HTT levels and its role in the innate immune system is required. With the

availability of novel methods of accurately quantifying HTT species both in blood and CSF potentially, there is an opportunity to demonstrate true abrogation of the pathogenic process. Restoration of normal transcriptional function in HD patients must also be demonstrated. Given the hepatotoxicity observed in selected subjects with selisistat, a wider dosing range and period, enzymatic, pharmacodynamic and susceptibility studies may help to clarify the source of this disturbance. If the drug is shown to prevent pathobiology in HD, then liver function monitoring regimens can be introduced. Finally, if and when true target engagement with HTT species is demonstrated, phase III studies can be conducted to look for changes in imaging, clinical (TMS & quantitative motor) as well as functional outcomes. However with the completion from HTT lowering therapies drugs such as selisistat are ultimately no longer attractive options.

Although the VBM analysis failed to show anything of significance, developing an easy, quantifiable and repeatable method to define motor phenotypes in HD or indeed wider phenotypes beyond pure motor symptomatology, captures the true heterogeneity of disease. The UHDRS assessment tool is not suited to this. In turn this would allow research to determine what underpins these differences with the potential to target more focussed therapies if and when they become available. By way of example, this technique of histogram based segregation if applied to dystonia or oculomotor function, or more widely to gait and balance using tandem gait and retropulsion test measures, could facilitate creation of motor, cognitive and psychiatric domains to better define and refine disease burden. Alternatively, a cluster analysis could be employed to determine if HD patients segregate easily into phenotypic groups.

Alternatively, more direct neuro-anatomically based hypotheses could be tested using volumetric and DTI based modalities expressing functional connectivity measures, looking for more caudate/putaminal volume loss for example in the hyperkinetic group phenotype versus more corticospinal/sub-cortical and motor and supplementary motor area loss in the akinetic grouping. This would mirror the clinical observation that bilateral lesions of the putamen such as stroke (particularly subthalamic nucleus), space-occupying lesions or indeed in hyperglycaemic states result in chorea; similarly that those patients with vascular dementia have significant subcortical hypertensive and atherosclerotic small vessel disease.

Optical coherence tomography would benefit from longitudinal assessments as well as increasing of sample size. For future work, in order to detect a difference in MV between early stage HD and moderate stage HD, using estimates from current work in order to detect a difference with 80% power and a two-sided significance level of 5% we would need to obtain macular volume data on 55 patients in both groups. Similarly, in order to detect a difference in RNFL between adult controls and early stage HD /moderate stage HD combined with 80% power and a two-sided significance level of 5%, we would need to obtain RNFL data on 102 patients in both groups.

In the case of the macular volume loss, it would be important to determine the contribution the choroidal macula as this has been implicated in subsequent work. A sub-segmental analysis would be essential to confirm more recent work and determine the origin of the neurodegeneration occurring. Indeed a recent cross-sectional study used spectral domain optical coherence tomography with automatic segmentation to measure peripapillary retinal nerve fibre layer (pRNFL) thickness and retinal layers in foveal scans was carried out in 15 patients with HD and 15 age- and sex-matched controls. In complement to results presented above, Temporal pRNFL, macular RNFL (mRNFL), ganglion cell layer (GCL), inner plexiform layer (IPL), inner nuclear layer and outer plexiform layer thicknesses and IPL, retinal pigment epithelium and outer macular volume were found lower in HD compared to controls, while outer nuclear layer and outer retinal layer thickness were increased ($p < 0.05$) (Gulmez *et al.*, 2018). Correlation with occipital lobe and visual tracts via volumetric techniques and tractography (DTI) could also shed light on the anatomical substrate for this neurodegeneration. Clinical correlation with more dedicated ERG based methods of assessing cone function as well as better bedside testing of colour vision would seem sensible.

13 Appendix

13.1 Unified Huntington's Disease Rating Scale '99 (Motor) (Huntington Study Group 1999)

General

Motor score⁽ⁱ⁾:

Motor Assessment

Ocular pursuit⁽ⁱ⁾:

- 0 = complete (normal)
- 1 = jerky movement
- 2 = interrupted pursuits/full range
- 3 = incomplete range
- 4 = cannot pursue

Saccade initiation⁽ⁱ⁾:

- 0 = normal
- 1 = increased latency only
- 2 = suppressible blinks or head movements to initiate
- 3 = unsuppressible head movements
- 4 = cannot initiate saccades

Saccade velocity⁽ⁱ⁾:

- 0 = normal
- 1 = mild slowing
- 2 = moderate slowing
- 3 = severely slow, full range
- 4 = incomplete range

Dysarthria:

- 0 = normal
- 1 = unclear, no need to repeat
- 2 = must repeat to be understood
- 3 = mostly incomprehensible
- 4 = anarthria

Tongue protrusion⁽ⁱ⁾:

- 0 = can hold tongue fully protruded for 10 sec
- 1 = cannot keep fully protruded for 10 sec
- 2 = cannot keep fully protruded for 5 sec
- 3 = cannot fully protrude tongue
- 4 = cannot protrude tongue beyond lips

Finger taps⁽ⁱ⁾:

- 0 = normal ($\geq 15/5$ sec.)
- 1 = mild slowing, reduction in amplitude (11-14/5 sec.)
- 2 = moderately impaired (7-10/5 sec.)
- 3 = severely impaired (3-6/5 sec.)
- 4 = can barely perform task (0-2/5 sec.)

Pronate/supinate-hands⁽ⁱ⁾:

- 0 = normal
- 1 = mild slowing and/or irregular
- 2 = moderate slowing and irregular
- 3 = severe slowing and irregular
- 4 = cannot perform

Adopted from 1999 Huntington Study Group.

Tandem walking⁽ⁱ⁾:

- 0 = normal for 10 steps
- 1 = 1 to 3 deviations from straight line
- 2 = >3 deviations
- 3 = cannot complete
- 4 = cannot attempt

Retropulsion pull test⁽ⁱ⁾:

- 0 = normal
- 1 = recovers spontaneously
- 2 = would fall if not caught
- 3 = tends to fall spontaneously
- 4 = cannot stand

Diagnostic Confidence

Diagnostic confidence level:

- 0 = Normal (no abnormalities)
- 1 = non-specific motor abnormalities (less than 50 % confidence)
- 2 = motor abnormalities that may be signs of HD (50 - 89 % confidence)
- 3 = motor abnormalities that are likely signs of HD (90 - 98 % confidence)
- 4 = motor abnormalities that are unequivocal signs of HD ≥ 99 % confidence)

Luria⁽ⁱ⁾:

- 0 = ≥ 4 in 10 sec, no cue
- 1 = <4 in 10 sec, no cue
- 2 = ≥ 4 in 10 sec with cues
- 3 = <4 in 10 sec with cues
- 4 = cannot perform

Rigidity-arms⁽ⁱ⁾:

- 0 = absent
- 1 = slight or present only with activation
- 2 = mild to moderate
- 3 = severe, full range of motion
- 4 = severe with limited range

Bradykinesia-body⁽ⁱ⁾:

- 0 = normal
- 1 = minimally slow (?normal)
- 2 = mildly but clearly slow
- 3 = moderately slow, some hesitation
- 4 = markedly slow, long delays in initiation

Maximal dystonia⁽ⁱ⁾:

- 0 = absent
- 1 = slight/intermittent
- 2 = mild/common or moderate/intermittent
- 3 = moderate/common
- 4 = marked/prolonged

Maximal chorea⁽ⁱ⁾:

- 0 = absent
- 1 = slight/intermittent
- 2 = mild/common or moderate/intermittent
- 3 = moderate/common
- 4 = marked/prolonged

Gait⁽ⁱ⁾:

- 0 = normal gait, narrow base
- 1 = wide base and/or slow
- 2 = wide base and walks with difficulty
- 3 = walks only with assistance
- 4 = cannot attempt

Adopted from 1999 Huntington Study Group.

13.2 Total Motor Score Tabulated Data

Visit Statistic	Group A- 10mg (N=17)		Group B- 100mg (N=19)		Group C- Placebo (N=19)	
	Observed	Change from Baseline	Observed	Change from Baseline	Observed	Change from Baseline
Baseline						
Mean (SD)	29.5 (14.50)		32.6 (10.3)		32.1 (11.55)	
Median (Min, Max)	28.0 (8, 64)		32.5 (17, 56)		33.0 (14, 53)	
Day 14						
Mean (SD)	26.1 (14.68)	-3.4	32.7 (10.91)	0.1	31.6 (12.07)	-0.5
Median (Min, Max)	25.0 (8, 62)	-3.0	33.5 (16, 56)	1.0	32.0 (10, 52)	-1.0
Day 28						
Mean (SD)	27.1 (16.42)	-2.4	31.4 (10.78)	-1.2	32.1 (12.87)	-0.1
Median (Min, Max)	25.0 (8, 71)	-3.0	29.5 (19, 58)	-3.0	32.0 (10, 62)	-2.0

13.3 Chorea Score Tabulated Data

Dose	Group A- 10mg			Group B- 100mg			Group C- Placebo		
Time	Baseline	Day 7	Day 14	Baseline	Day 7	Day 14	Baseline	Day 7	Day 14
Mean	9.35	8.94	8.7	8.38	8.38	8.44	9.31	9.31	9.21
SD	5.13	4.95	4.42	4.84	4.23	4.59	3.93	3.74	3.64

13.4 Total Functional Capacity Tabulated Data

Visit Statistic	Group A (N=17)		Group B (N=19)		Group C (N=19)		All Patients (N=55)	
	Observed	Change from Baseline	Observed	Change from Baseline	Observed	Change from Baseline	Observed	Change from Baseline
Baseline								
Mean (SD)	10.6 (2.06)		9.9 (2.23)		10.0 (2.13)		10.2 (2.13)	
Median (Min, Max)	11.0 (7, 13)		10.0 (7, 13)		10.0 (7, 13)		10.0 (7, 13)	
Day 14								
Mean (SD)	10.6 (2.06)	0	9.8 (2.24)	-0.1 (0.52)	9.9 (2.20)	-0.1 (0.40)	10.1 (2.16)	0.0 (0.38)
Median (Min, Max)	11.0 (7, 13)	0	9.0 (6, 13)	0.0 (-1, 1)	10.0 (6, 13)	0.0 (-1, 1)	10.0 (6, 13)	0.0 (-1, 1)
Day 28								
Mean (SD)	10.6 (2.03)	0.0 (0.35)	9.8 (2.41)	-0.1 (0.62)	9.9 (2.35)	-0.1 (0.94)	10.1 (2.27)	-0.1 (0.68)
Median (Min, Max)	11.0 (7, 13)	0.0 (-1, 1)	9.0 (5, 13)	0.0 (-2, 1)	10.0 (6, 13)	0.0 (-3, 2)	10.0 (5, 13)	0.0 (-3, 2)

Max = maximum; Min = minimum; SD = standard deviation; TFC = total functional capacity; UHDRS = Unified Huntington's Disease Rating Scale

13.5 Functional Assessment Score & Independence Scale



PADDINGTON
UHDRS '99 - FUNCTIONAL ASSESSMENT

Subject:

Date:
D D M M Y Y Y Y

Examiner:

Signature:

All items must be completed. Use U if information is unavailable. Use N if information is not applicable.

General

Functional Assessment Score⁽ⁱ⁾:

--	--

Functional Assessment

For the next 25 questions, please use:

1 = yes
0 = no

Could subject engage in gainful employment in his/her accustomed work⁽ⁱ⁾:

--

Could subject engage in any kind of gainful employment⁽ⁱ⁾?

--

Could subject engage in any kind of volunteer or non-gainful work⁽ⁱ⁾?

--

Could subject manage his/her finances (monthly) without any help⁽ⁱ⁾?

--

Could subject shop for groceries without help⁽ⁱ⁾?

--

Could subject handle money as a purchaser in a simple cash (shop) transaction⁽ⁱ⁾?

--

Could subject supervise children without help⁽ⁱ⁾?

--

Could subject operate an automobile safely and independently⁽ⁱ⁾?

--

Could subject do his/her own housework without help⁽ⁱ⁾?

--

Could subject do his/her own laundry (wash/dry) without help⁽ⁱ⁾?

--

Could participant prepare his/her own meals without help⁽ⁱ⁾?

--

Could subject use the telephone without help⁽ⁱ⁾?

--

Could subject take his/her own medications without help⁽ⁱ⁾?

--

Could subject feed himself/herself without help⁽ⁱ⁾?

--

Could subject dress himself/herself without help⁽ⁱ⁾?

--

Could subject bathe himself/herself without help⁽ⁱ⁾?

--

Could subject use public transportation to get places without help⁽ⁱ⁾?

--

Could subject walk to places in his/her neighbourhood without help⁽ⁱ⁾?

--

Could subject walk without falling⁽ⁱ⁾?

--

Could subject walk without help⁽ⁱ⁾?

--

Could subject comb hair without help?

--

Could subject transfer between chairs without help?

--

Could subject get in and out of bed without help?

--

Could subject use toilet/commode without help?

--

Could subject's care still be provided at home⁽ⁱ⁾?

--

Adopted from 1999 Huntington Study Group.



PADDINGTON
UHDRS '99 - FUNCTIONAL ASSESSMENT

Subject:

Date:
D D M M Y Y Y Y

Examiner:

Signature:

All items must be completed. Use U if information is unavailable. Use N if information is not applicable.

Information sources:

Was the functional assessment information obtained from:

- 1 = subject only
- 2 = subject and family/companion

Independence Scale

Subject's independence in %⁽⁴⁾:

- 100 = no special care needed
- 95
- 90 = no physical care needed if difficult tasks are avoided
- 85
- 80 = pre-disease level of employment changes or ends; cannot perform household chores to pre-disease level, may need help with finances
- 75
- 70 = self-care maintained for bathing, limited household duties, e.g. cooking and use of knives, driving terminates; unable to manage finances
- 65
- 60 = needs minor assistance in dressing, toileting, bathing; food must be cut for subject
- 55
- 50 = 24-hour supervision appropriate; assistance required for bathing, eating, toileting
- 45
- 40 = chronic care facility needed; limited self feeding, liquified diet
- 35
- 30 = subject provides minimal assistance in own feeding, bathing, toileting
- 25
- 20 = no speech, must be fed
- 15
- 10 = tube fed, total bed care
- 5

13.6 MMSE

PADDINGTON

**PADDINGTON
MINI-MENTAL STATE EXAM**

Subject:

Date:

Examiner:

Signature:

All items must be completed. Use U if information is unavailable. Use N if information is not applicable.

General

MMSE score:

Mini-Mental State Exam

Orientation to time:

What is the ... ?

For the next 5 questions, please use:

0 = incorrect
1 = correct

Year:

Season:

Month of the year:

Day of the week:

Date:

Attention and calculation (Serial 7s):

What is 100 take away 7?

For the next 5 questions, please use:

0 = incorrect
1 = correct

[93]:

[86]:

[79]:

[72]:

[65]:

Orientation to place⁽¹⁾:

Where are you now?

For the next 5 questions, please use:

0 = incorrect
1 = correct

Country:

County:

City/town:

Building:

Floor:

Registration:

For the next 3 questions, please use:

0 = incorrect
1 = correct

Word 1:

Word 2:

Word 3:

Put on floor (or table):

Reading:

For the next question, please use:

0 = incorrect
1 = correct

Close your eyes:

Writing:

For the next question, please use:

0 = incorrect
1 = correct

Sentence:

Drawing:

For the next question, please use:

0 = incorrect
1 = correct

Figure:

Recall:

For the next 3 questions, please use:

0 = incorrect
1 = correct

Word 1:

Word 2:

Word 3:

Naming⁽¹⁾:

What is this?

For the next 2 questions, please use:

0 = incorrect
1 = correct

1 [Pencil or pen]:

2 [Watch]:

Repetition:

For the next question, please use:

0 = incorrect
1 = correct

NO IFS, ANDS, OR BUTS:

Comprehension:

For the next 3 questions, please use:

0 = incorrect
1 = correct

Take in right hand:

Fold in half:

13.9 Symbol Digit Modalities Data

Visit Statistic	Group A (N = 17)		Group B (N = 19)		Group C (N = 19)		All Patients (N = 55)	
	Observed	Change from Baseline	Observed	Change from Baseline	Observed	Change from Baseline	Observed	Change from Baseline
Total Corrects								
Baseline								
n	17		19		18		54	
Mean (SD)	28.8 (15.61)		21.3 (9.18)		19.6 (7.77)		23.1 (11.72)	
Median (Min, Max)	26.0 (12, 70)		21.0 (2, 36)		17.5 (5, 32)		21.5 (2, 70)	
Missing	0		0		1		1	
Day 1								
n	17	17	19	19	18	18	54	54
Mean (SD)	31.4 (15.78)	2.6 (6.44)	22.0 (9.08)	0.7 (3.74)	21.1 (9.66)	1.4 (4.38)	24.6 (12.44)	1.6 (4.90)
Median (Min, Max)	25.0 (10, 77)	4.0 (-12, 11)	22.0 (2, 37)	0.0 (-6, 8)	19.0 (5, 38)	1.5 (-6, 9)	23.0 (2, 77)	2.0 (-12, 11)
Missing	0	0	0	0	1	1	1	1
Day 14								
n	17	17	19	19	17	17	53	53
Mean (SD)	32.3 (13.75)	3.5 (7.12)	22.8 (10.76)	1.5 (4.53)	21.8 (10.18)	2.3 (4.62)	25.5 (12.34)	2.4 (5.47)
Median (Min, Max)	30.0 (13, 72)	3.0 (-16, 16)	22.0 (1, 42)	1.0 (-7, 12)	21.0 (6, 43)	1.0 (-5, 14)	25.0 (1, 72)	2.0 (-16, 16)
Missing	0	0	0	0	2	2	2	2
Total Errors								
Baseline								
n	17		19		18		54	
Mean (SD)	1.3 (1.26)		1.2 (1.96)		0.9 (1.11)		1.1 (1.48)	
Median (Min, Max)	1.0 (0, 4)		0.0 (0, 8)		1.0 (0, 4)		1.0 (0, 8)	
Missing	0		0		1		1	
Day 1								
n	17	17	19	19	18	18	54	54
Mean (SD)	1.1 (1.34)	-0.2 (1.39)	0.9 (1.43)	-0.3 (2.08)	1.2 (1.25)	0.2 (1.31)	1.1 (1.32)	-0.1 (1.63)
Median (Min, Max)	1.0 (0, 4)	0.0 (-3, 3)	0.0 (0, 4)	0.0 (-5, 4)	1.0 (0, 4)	0.0 (-2, 3)	0.5 (0, 4)	0.0 (-5, 4)
Missing	0	0	0	0	1	1	1	1
Day 14								
n	17	17	19	19	17	17	53	53
Mean (SD)	0.9 (1.05)	-0.4 (1.54)	0.4 (1.17)	-0.8 (1.18)	0.7 (1.05)	-0.2 (1.56)	0.7 (1.09)	-0.5 (1.42)

13.10 Phase 2 Study- Serious Adverse Events Description (Reilmann *et al.*, 2013)

Patient 3030003 (*liver function tests abnormality*) was a 49-year-old white female with a body weight of 56 kg and a height of 162 cm (BMI 21.6 kg/m²). She had no relevant medical history, and no history of alcohol abuse or viral hepatitis. No concomitant medications were reported. The patient commenced study treatment with selisistat 50 mg once daily on 22nd February 2012. Elevation of LFTs was noted on 21st March 2012 (Study Day 29) after four weeks of treatment, with AST 234 U/L (ULN 37) and ALT 428 U/L (ULN 47). The patient was without symptoms; the event was assessed as mild, possibly related and classified as a serious adverse event based on medical importance of the event. The study drug was discontinued on 23rd March 2012 (Study Day 31).

During follow-up, LFTs improved and were normal at the latest visit on 24th May 2012, approximately 9 weeks after termination of study treatment. Bilirubin and eosinophils were normal throughout the study and follow-up visits. The event resolved on 24th May 2012 (Study Day 93), and was considered to be possibly related to the study drug.

Patient 3040006 (*hepatitis*) was a 48-year-old white male with a body weight of 87 kg and a height of 184 cm (BMI 25.6 kg/m²). Besides minor surgery, medical history included episodes of depression but no alcohol abuse or viral hepatitis. No concomitant medications were reported. The patient commenced study treatment with selisistat 50 mg once daily on 3rd April 2012. At Baseline, AST (41 U/L; ULN 37) and ALT (67 U/L; ULN 47) were slightly elevated. On 19th April 2012 (Study Day 17) the patient felt anxious, feverish, and had a temperature. Laboratory results demonstrated markedly elevated LFTs with eosinophilia. The patient's symptoms continued on the subsequent days and he felt tired and experienced nausea. On 22nd April 2012 (Study Day 20), the patient experienced nausea all day with vomiting in the evening. The patient discontinued the study drug on this day. Repeat laboratory assessment on 24th April 2012, confirmed markedly elevated LFTs with ALT 776 U/L (ULN 47), AST 190 U/L (ULN 37), GGT 81 (ULN 51) and eosinophils 7.5% (ULN 7.0%), with normal bilirubin. The patient also had diarrhoea and was hospitalised. He was diagnosed with severe hepatitis. Further laboratory abnormalities included low potassium, reduced phosphate, elevated glucose, urine ketones and bacteria. During hospitalisation, the patient was treated with 500 mg paracetamol orally every 4 to 6 hours for temperature and the patient was feeling better with no nausea on the following day. The patient improved rapidly and was discharged from hospital on 27th April 2012 (Study Day 25). Investigations from the admission did not reveal viral hepatitis and no evidence of autoimmune disease. Hepatitis A IgM and hepatitis B surface antigen were reported as normal. On 12th June 2012 (Study Day 71), all values were within normal range and the event was considered resolved. The event was considered to be related to the study drug.

Patient 3050002 (liver function test abnormal) was a 46-year-old white female with a body weight of 51 kg and a height of 163 cm (BMI: 19.2 kg/m²). The patient had a medical history of hysterectomy and oophorectomy, fracture of right wrist, irritability in relation to Huntington's disease and pelvic inflammatory disease. There was no reported medical history of alcohol abuse or viral hepatitis. Her concomitant medication included carbamazepine 300 mg daily and Ellese solo (hormonal replacement). The patient commenced study treatment with selisistat (selisistat) 200 mg once daily on 08th December 2011. At Baseline, all LFTs were normal with GGT close to the ULN (screening GGT 34 U/L; baseline 32 U/L; ULN 33). After 4 weeks of treatment, the patient did not report any

emerging, new symptoms but LFTs were slightly elevated with AST 49 U/L (ULN 47) and GGT 41 U/L (ULN 33), with all other values normal. The patient was therefore seen at an extra visit 9 days later (on 13th January 2012). The patient was still without symptoms but LFT were now elevated with AST 216 U/L (ULN 37), ALT 321 (ULN 47) and GGT 99 U/L (ULN 33). Bilirubin and eosinophils were normal. On 16th January 2012 (Study Day 40), the patient was diagnosed with the mild SAE of liver function tests abnormal, which was also considered to be medically important. Study treatment was discontinued on this day and the patient was followed until normalisation of LFTs. On 08th February 2012, ALT was still elevated to 93 U/L (local lab ULN 41) while on 10th April 2012, all values were back to normal with GGT 45 U/L (local lab ULN 45) and ALT 14 U/L. Viral titres for hepatitis B and C were negative. The event of liver function tests abnormal was ongoing at the time of last follow up and was considered to be possibly related to the study drug.

Adverse events assessed as non-serious

Patient 1020004 was a 64-year-old white female with a body weight of 72 kg and height of 158 cm (BMI: 28.6 kg/m²). She had a medical history including erythema nodosum and partial thyroidectomy. Her concomitant medication included olanzapine 5 mg once daily, Tiaprid 600 mg 3+2+1+0 daily, Venlafaxine 150 mg daily, ASS 100 mg daily and L-thyroxine 75 mg daily. The patient commenced study treatment with selisistat (selisistat) 200 mg once daily on 24th February 2012. At Baseline, ALP was elevated (148 U/L; ULN 135) and GGT was at the ULN (33 U/L; 43 U/L at screening; ULN 33). After 1 week of treatment, all values were normal. At the Week 4 visit, LFTs were elevated with AST 60 U/L (ULN 37), ALT 101 U/L (ULN 47) and GGT 50 U/L (ULN 33). The patient was continued on study treatment. On 18th April 2012 (Study Day 55), the patient's LFTs were slightly more elevated and the patient was diagnosed with the mild AE of hepatic enzyme increased. Study treatment was discontinued on 20th April 2012 (Study Day 57) and the patient was discontinued from further treatment. At this visit, ALP was 176 U/L (ULN 135), AST 72 U/L (ULN 37), ALT 158 U/L (ULN 47) and GGT 194 U/L (ULN 33); bilirubin and eosinophils were normal throughout. The patient was asymptomatic and did not receive any treatment for the elevated LFTs. During subsequent follow-up visits values normalised to the same level as before the study with slightly elevated GGT (55 and 47 U/L; ULN 33 on 16th May 2012 and 14th June 2012, respectively). The AE was considered to be resolved on 16th May 2012 (Study Day 83) and was considered to be related to the study drug.

Patient 1020013 was a 49-year-old white male with a body weight of 83 kg and height of 190 cm (BMI: 22.7 kg/m²). He had a medical history including facial dermatitis and episodes of depression;

there were no reported concomitant medications. The patient commenced study treatment with selisistat (selisistat) 200 mg once daily on 1st March 2012. At Baseline and at the Week 1 visit, all laboratory values were within normal range. On 29th March 2012 (Study Day 29), the patient experienced the mild AEs of alanine aminotransferase increased and aspartate aminotransferase increased (AST 89 U/L [ULN 37] and ALT 175 U/L [ULN 47]). Study treatment was discontinued on 9th April 2012 (Study Day 40). Bilirubin and eosinophils were normal throughout and the patient was asymptomatic and did not receive any treatment for the elevated LFTs. During subsequent follow-up visits one and two weeks after discontinuation, LFTs were still elevated but were then normalised 3 weeks after study drug termination. The AEs were considered resolved on 26th April 2012 (Study Day 57). Both AEs were considered to be related to the study drug.

Patient 1020018 was a 54-year-old white male with a body weight of 96 kg and a height of 180 cm (BMI 29.5 kg/m²). His medical history included glaucoma. No concomitant medications were reported. The patient commenced study treatment with selisistat (selisistat) 50 mg once daily on 20 March 2012. At both Baseline and Screening, ALT was slightly elevated (55 and 63 U/L; ULN 47), but other values were normal. After one and four weeks of treatment, values were unchanged with ALT 48 and 61 U/L, with all other normal. At the Week 8 visit, elevated LFTs were noted with AST 100 U/L (ULN 37), ALT 241 U/L (ULN 47), GGT 63 U/L (ULN 51) and eosinophils 8.1% (ULN 7.0%). On 18th May 2012 (Study Day 60), the patient was diagnosed with the mild AE of hepatic enzyme increased. Study treatment was withdrawn on 22nd May 2012 (Study Day 64). The patient was asymptomatic and did not receive any treatment for the elevated LFTs. At a follow-up, on May 30th, LFT values had improved but remained slightly elevated with AST 43 U/L (ULN 37), ALT 93 U/L (ULN 47) and GGT 69 U/L (ULN 51). At the end-of-treatment visit and one week later, all values except ALT, which had returned to baseline levels (57 U/L and 57 U/L, ULN 47), were normalised. At the time of last follow-up, the AE was ongoing, and was considered to be related to the study drug.

Patient 1030007 was a 39-year-old white male with a body weight of 83 kg and a height of 185 cm (BMI 24.4 kg/m²). The patient had no relevant medical history and his concomitant medication included Lyrica 75 mg daily. The patient commenced study treatment with selisistat (selisistat) 200 mg once daily on 07 December 2011. At Baseline, bilirubin was at the ULN (19 µmol/L); other values were normal. On 14 December 2011 (Study day 8), the patient experienced the moderate AE of hepatic enzyme increased: elevated LFTs were noted with AST 99 U/L (ULN 37), ALT 215 U/L (ULN 47) and GGT 59 U/L (ULN 51). Bilirubin and eosinophils were normal. The patient was asymptomatic and did not receive any treatment for the elevated LFTs. Study treatment was continued. After four

weeks of study treatment, LFTs were improved with normal AST, ALT 54 U/L (ULN 47) and GGT 63 U/L. At Week 8, ALT remained slightly elevated to 58 U/L, with all other values normal. At Week 12, after completion of the full duration of study treatment, ALT was further elevated (178 U/L), AST 78 (ULN 37), GGT 90 U/L (ULN 51); other values normal. At the follow-up visit, four weeks later ALT and AST were again slightly elevated (69 and 39 U/L, respectively). The patient completed the full duration of study treatment and LFTs were normal at Week 12/EoT visit. At the time of last follow-up, the AE of hepatic enzyme increased was ongoing. The AE was considered to be possibly related to the study drug.

Patient 1030027 was a 45-year-old white female with a body weight of 81 kg a height of 162 cm (BMI 30.7 kg/m²), without notable relevant medical history. Her concomitant medication included amisulpride 150 mg daily and various dietary supplements. The patient commenced study treatment with placebo once daily on 3rd May 2012. At Screening and Baseline, slightly elevated GGT values were noted (40 and 33 U/L; ULN 33) and on 04 June 2012 (Study Day 33), a mild AE of hepatic enzyme increased with GGT 54 was recorded. The patient continued on study treatment and GGT values were 49 and 48 U/L at the Week 8 and Week 12/EoT visits. Other LFTs, bilirubin and eosinophil were normal. At the time of last follow-up, the AE was ongoing, and considered to be possibly related to the study drug.

Patient 1040003 was a 49-year-old white female with a body weight of 51 kg and a height of 162 cm (BMI: 10.5 kg/m²). Her medical history included hypertension, allergic rhinitis, episodes of depression and elevated GGT values without known cause. Her concomitant medication included citalopram 20 mg daily, metoprolol 50 mg daily, amitriptyline 50 mg daily and Valoron 40 mg daily. The patient commenced study treatment with selisistat 50 mg once daily on 31 January 2012. At Baseline and screening, GGT was elevated to 121 and 133 U/L (ULN 33). On 07 February 2012 (Study Day 8) GGT was somewhat higher (181 U/L) and AST was just above ULN (48 U/L; ULN 47); other values were normal. The patient was diagnosed with the mild AEs of alanine aminotransferase increased and gamma glutamyl transferase increased. Study treatment was continued. The patient completed the full study duration and GGT value ranged from 108 to 209 U/L (ULN 33) with all other values normal. At the follow-up visit, 4 weeks after the last treatment visit, GGT was at the same level as at baseline (110 U/L). All other values including eosinophils were normal throughout the study; the patient was asymptomatic. At the time of last follow-up, the AEs of alanine aminotransferase increased and gamma glutamyl transferase increased were ongoing. The AEs were considered possibly related to the study drug.

Patient 1040014 was a 48-year-old white male with a body weight of 97 kg and height of 193 cm (BMI: 26.1 kg/m²). His medical history included hypertension, electrocardiographic signs of left ventricular hypertrophy and episodes of depression. His concomitant medication included atenolol 50 mg daily, lercanidipine 10 mg daily, valsartan 80 mg daily, hydrochlorothiazide 12.5 mg daily, nortriptyline 200 mg daily and green tea capsules 340 mg TID. The patient commenced study treatment with selisistat (selisistat) 200 mg once daily on 28 February 2012. All values were normal at Baseline and at Screening. On 23 April 2012 (Study Day 56) elevated LFTs were noted with AST 163 U/L (ULN 37) and ALT 287 U/L (ULN 47) and normal GGT, bilirubin and eosinophils. The patient was diagnosed with the moderate AEs of alanine transferase increased and aspartate transferase increased. The patient was asymptomatic throughout the episode. Study treatment was discontinued on 25th April 2012 (Study Day 58). After discontinuation of study treatment, LFT values quickly normalised and all values were within normal range at the latest follow-up visit, 1 month after withdrawal of study treatment. The AEs were considered to be resolved on 25th May 2012 (Study Day 88). The events were considered to be possibly related to the study treatment.

Patient 1060007 was a 58-year-old white female with a body weight of 64 kg and height of 163 cm (BMI: 24.0 kg/m²), without relevant medical history and with no reported concomitant medication. The patient commenced study treatment with selisistat (selisistat) 200 mg once daily on 30th January 2012. At Baseline and Screening, all values were normal and remained within normal ranges after one week of treatment. At the Week 4 visit, markedly elevated LFTs were noted with AST 334 U/L (ULN 37), ALT 289 U/L (ULN 47), GGT 63 U/L (ULN 33) and slightly elevated ALP to 139 U/L (ULN 135). The patient was diagnosed on 27th February 2012 (Study Day 29) with the mild AEs of alanine aminotransferase increased, aspartate aminotransferase increased and gamma glutamyl transferase increased. Bilirubin and eosinophils were normal. The patient did not experience any symptoms suggestive of liver disease during the study. Study treatment was discontinued on 28th February 2012 (Study Day 30). Follow-up samples one week after discontinuation showed essentially unchanged values, whereas all values were within normal with the exception of a slightly elevated GGT to 51 U/L (ULN 33) range 4 weeks after withdrawal of study treatment. The AEs of alanine aminotransferase and aspartate aminotransferase abnormalities resolved on 29th March 2012 (Study Day 30) and the AE of gamma glutamyl transferase increase resolved on 19 June 2012 (Study Day 142). All three AEs were considered possibly related to the study drug.

Patient 2040013 was a 40-year-old white male with a body weight of 65 kg and a height of 176 cm (BMI 21.0 kg/m²), with no relevant medical history. His concomitant medication included Zyprexa 5 mg daily, Pasaden 10 drops daily, Laroxyl 5 drops daily and dietary supplement. A mild AE of elevated LFTs was noted at the screening visit (Day -20) and remained ongoing. The patient commenced study treatment with placebo once daily on 10th January 2012. At Screening and at Baseline, ALT was increased to 113 and 111 U/L (ULN 47) and AST to 58 and 49 U/L (ULN 37). Study treatment was initiated and LFT values remained essentially unchanged, with ALT and AST values somewhat lower on treatment. All other values were normal. On 1st March 2012 (Day 52), the patient experienced a mild AE of hepatic steatosis, which was ongoing at the time of last follow-up. The AE was not considered to be related to the study drug.

Patient 2040016 was a 30-year-old white female with a body weight of 51 kg and height of 165 cm (BMI: 24.6 kg/m²). She had no relevant medical history. Her concomitant medication included citalopram 20 mg daily, Pasaden 7 drops twice daily and dietary supplement. The patient commenced study treatment with placebo once daily on 12th March 2012. At Baseline and Screening, all LFT values normal and remained normal until the last study visit (Week 12) on 6th June 2012 (Study Day 87), at which an elevated GGT of 62 U/L (ULN 33) was noted as a mild AE. All other LFTs, including bilirubin and eosinophils were normal. The AE resolved on 27th June 2012 (Study Day 108) and was considered to be possibly related to the study drug.

Patient 2040021 was a 54-year-old white male with a body weight of 78 kg and height of 178 cm (BMI: 24.6 kg/m²). The patient had no relevant medical history and his concomitant medication included Pasaden 10 drops twice daily and dietary supplement. The patient commenced selisistat 200 mg once daily on 19th March 2012. At Baseline, all values were within normal range. At Week 4, GGT of 52 U/L (ULN 51) was noted. On 16th May 2012 (Study Day 59), at the Week 8 visit, mild adverse events of alanine aminotransferase increased and aspartate aminotransferase increased were recorded with ALT 144 (ULN 47), AST 65 (ULN 37) and GGT 52 U/L (ULN 51). Dosing with study treatment was continued and values remained at similar levels 2 weeks later and at the Week 12 visit. At follow-up 5 weeks later, all values were back to normal, except GGT (60 U/L). The AEs were considered resolved on 16th June 2012 (Study Day 120) and were considered to be possibly related to the study drug.

Patient 2040024 was a 37-year-old white male with a body weight of 92 kg and a height of 172 cm (BMI 31.1kg/m²). He had no relevant medical history. His concomitant medications included

paroxetine 20 mg daily, valproate 400 mg daily, clozapine 50 mg daily, clonazepam 20 mg daily and vitamins. The patient commenced study treatment with selisistat (selisistat) 50 mg once daily on 11th April 2012. At Baseline, all values were within normal range. At Week 4, and 8, values remained normal. On 20th June 2012 (Study Day 71), at the 10 week visit, mild AEs of alanine aminotransferase increased and aspartate aminotransferase increased were noted: AST 38 U/L (ULN 37) and ALT 53 U/L (ULN 47). Study treatment was continued with essentially unchanged LFT values: at week 12 (last treatment visit) AST was 46 U/L (ULN 37) and ALT 60 U/L (ULN 47). At follow-up, three weeks later, a higher ALT value of 114 U/L (ULN 47) was however observed and values remained elevated at an additional follow up at the local laboratory in September with AST 65 U/L (ULN 37) and ALT 118 U/L (ULN 47). The site will continue to follow the patient with repeat samples. The AE of alanine aminotransferase increase was considered to be not related to the study drug and the AE of aspartate aminotransferase increase was considered to be possibly related to the study drug.

Patient 2040025 was a 56-year-old white female with a body weight of 64 kg and a height of 160 cm (BMI: 25.0 kg/m²). Her medical history included bronchitis and urinary tract infection one month before start of study treatment. Her concomitant medication included citalopram 7 mg daily, Pasaden 7 drops daily. The patient commenced study treatment with selisistat 200 mg once daily on 10 May 2012. LFT values were normal at Screening and at Baseline. On 7th June 2012 (Study Day 29), after 4 weeks of study treatment, mild adverse events of alanine aminotransferase increase and aspartate aminotransferase increase were noted with ALT 69 U/L (ULN 47) and AST 44 U/L (ULN 37). Study treatment was continued and LFT values remained on the same level with ALT 55 U/L at Week 12/EoT. Bilirubin, GGT and eosinophils were normal throughout the study. The AEs were ongoing at the time of last follow-up and were considered to be related to the study drug.

Patient 3020005 was a 68-year-old white male with a body weight of 69 kg and a height of 177 cm (BMI 21.9 kg/m²). His medical history included recurrent erysipelas and mildly reduced vibration sense in lower extremities. His concomitant medication included paracetamol 500 mg as needed. The patient commenced study treatment with selisistat (selisistat) 50 mg once daily on 21st February 2012. At Baseline and after 4 weeks of treatment, all values were normal. After 8 weeks of treatment, elevated LFTs were seen with AST 113 U/L (ULN 37), ALT 271 U/L (ULN 47) and GGT 84 U/L (ULN 51); other values including bilirubin and eosinophils were normal. The patient was diagnosed with the mild AE of hepatic enzymes increased on 15th April 2012 (Study Day 53). The event was assessed as a non-serious adverse event of mild intensity and as unlikely related and study treatment was withdrawn on 20 April 2012 (Study Day 60). The patient was asymptomatic and

did not experience any symptoms suggestive of liver disease during the study. Two weeks later (on April 27th), values were somewhat improved and at the latest follow-up, on May 16th, five weeks after discontinuation of study treatment, all values except GGT (117 U/L; ULN 51) were within normal range. The AE remained ongoing at the time of last follow-up and was considered to be possibly related to the study drug.

Patient 3020008 was a 44-year-old white male with a body weight of 83 kg and a height of 178 cm (BMI 26.2 kg/m²). His medical history included asthma and hepatitis five years ago (2007). His concomitant medication included citalopram 20 mg daily. The patient commenced study treatment with selisistat 50 mg once daily on 30th March 2012. LFT values at Screening and at Baseline were elevated with GGT 114 and 63 U/L (ULN 51 U/L), ALT 67 and 37 U/L (ULN 47 U/L) and AST 39 U/L (screen; ULN 37 U/L). After 4 weeks of treatment, GGT remained slightly elevated to 64 and elevated eosinophils were also seen; 7.9% (ULN 7.0%). On 01 June 2012 (Study Day 64), after 10 weeks of study treatment, an adverse event of acute rise in liver enzymes was noted (hepatic enzymes increased) with GGT 72, ALT 89 and AST 98 and further study treatment was discontinued on 6th June 2012 (Study Day 69). At follow-up visits, values were normalising with GGT remaining elevated to 107 U/L 4 weeks after discontinuation of study treatment. On August 14th, 9 weeks after discontinuation of study treatment, a clearly elevated ALT was noted by the local laboratory (253 U/L; ULN 47) and the patient was referred to a gastroenterologist. Repeat blood sampling at the local laboratory in October showed normalisation of all LFT values. At the time of last follow-up, the AE was ongoing, and was considered to be possibly related to the study drug.

Patient 3030004 was a 51-year-old white male with a body weight of 82 kg and height of 106.4 cm (BMI: 23.7 kg/m²), without relevant medical history. His concomitant medication included St. John's Wort 1 tablet daily, and paracetamol 2 tablets as needed. The patient commenced study treatment with selisistat 50 mg once daily on 29th February 2012. GGT was elevated at Screening and Baseline (72 and 140 U/L; ULN 51), ALT was 92 U/L (ULN 47 U/L) and AST 53 U/L (ULN 37 U/L) at baseline. After 4 weeks of study treatment, ALT, AST and GGT remained slightly elevated and after 8 weeks, on 25th April 2012 (Study Day 57) a mild AE of alanine aminotransferase increased was noted with GGT 106 U/L, ALT 103 U/L, AST 54 and other values normal. Study treatment was continued and values remained essentially unchanged. At the follow-up visit, four weeks later, GGT was elevated to 64 U/L but all other values were normal. Bilirubin and eosinophils were normal throughout. The AE resolved on 20th June 2012 (Study Day 113), and was considered to be possibly related to the study drug.

Patient 3030006 was a 59-year-old white female with a body weight of 82 kg and a height of 173 cm (BMI 27.3 kg/cm²), and a relevant medical history including eczema and lower back pain. Her concomitant medication included Fexofenadine 180 mg daily, co-codamol 1 to 2 tablets as needed, Naproxen 250 mg as needed, Paracetamol 1 g as needed, nasal mometasone, skin ointments. The patient commenced study treatment with selisistat 200 mg once daily on 14th March 2012. GGT was slightly elevated to 37 and 35 U/L (ULN 33 U/L) at Screening and Baseline. During study treatment, GGT values ranged between 37 and 50 U/L. After 8 weeks treatment, on 9th May 2012 (Study Day 57) a mild AE of elevated GGT (47 U/L) was noted. Study treatment was continued without further elevations and at follow-up, 4 weeks after completion of study treatment (04 July 2012); GGT was at the same level (49 U/L). A later follow-up (October 3rd 2012) performed at the local laboratory, also showed GGT slightly above the ULN. At the time of last follow-up, the AE was ongoing, and was considered to be possibly related to the study drug.

Patient 3040004 was a 40-year-old white male with a body weight of 87 kg and a height of 180 cm (BMI 26.7 kg/m²) with a medical history of depression and anxiety and colonoscopy for a rectal bleeding 2 years earlier. The patient was on concomitant medication with sertraline hydrochloride 350 mg once daily, zopiclone 7.5 mg daily, propranolol 150 mg daily, quetiapine 350 mg daily, tadalafil 10 mg as needed, lamotrigine 250 mg daily and clonazepam 1 mg daily. He was enrolled and started on study treatment with selisistat 200 mg once daily on March 1st 2012. At Baseline and after 4 weeks of treatment, all values were normal. The patient experienced loose bowel movement 2 to 5 times a day that started approximately 4 weeks after initiation of study treatment and study treatment was interrupted on April 20th 2012 (Study Day 51). On 24th April 2012 (Study Day 55), at the Week 8 visit, a moderate AE of alanine aminotransferase increased was noted (62 U/L; ULN 47) and study treatment was withdrawn; all other values were normal. Two weeks later and at follow-up, all values were normal. The AE of alanine aminotransferase increase was considered to be resolved on 10th May 2012 (Study Day 71), and was considered to be possibly related to the study drug. According to the investigator, the patient had previously also experienced diarrhoea in episodes and the relation between the symptoms and the discrete ALT elevation is therefore not fully clear.

Patient 3050003 was a 59-year-old white female with a body weight of 57 kg and height of 154 cm (BMI 24.8 kg/m²). She had no relevant medical history and no reported concomitant medication. The patient commenced study treatment with selisistat 200 mg once daily on 12th January 2012. At

Baseline and after 4 weeks of treatment, all values were normal. After 8 weeks of treatment, slightly elevated LFTs were noted with AST 40 U/L (ULN 37) and GGT 90 U/L (ULN 33). The patient was diagnosed with the mild AE of liver function test abnormality on 07th March 2012 (Study Day 56). Study treatment was continued and the patient was asymptomatic throughout the course of the study. The patient completed the full duration of the study (12 weeks) and LFTs were slightly but not further elevated during continued study treatment with AST 42 U/L (ULN 37) and GGT 38 U/L (ULN 33) after 12 weeks. The AE resolved on 2nd May 2012 (Study Day 112) and was considered to be possibly related to the study drug.

Patient 3050007 was a 45-year-old white female with a body weight of 70 kg and height of 154 cm (BMI 29.4 kg/m²). Her medical history included endometriosis, headache and depression and her concomitant medications included paroxetine 40 mg daily, ibuprofen 800 mg daily. During the study, the patient also received trimethoprim 400 mg daily (from 29th April 2012 to 1st May 2012) and paracetamol in a total dose of 8 g daily (from 27th April 2012 to 7th May 2012). The patient commenced study treatment with selisistat 50 mg once daily on 13th March 2012. At Baseline, at screening and after 4 weeks of treatment, GGT was slightly elevated (42, 45 and 49 U/L; ULN 33), other values were normal. After 8 weeks of treatment, a moderate AE of liver function test abnormal was noted with ALP 275 U/L (ULN 135), AST 74 U/L (ULN 37), ALT 110 U/L (ULN 47) and GGT 184 U/L (ULN 33). Study treatment was withdrawn on 14 May 2012 (Study Day 63). During follow-up, LFT values quickly normalised and were normal 2 weeks after cessation of study treatment, with the exception of a remaining, slightly elevated GGT (63 U/L; ULN 33). At the latest follow-up visit, on 11th June 2012, values were essentially unchanged with GGT 43 U/L (ULN 33), i.e. back to the same level as before the study. The patient was asymptomatic throughout the study. The AE resolved on 11th June 2012 (Study Day 91), and was considered to be unlikely related to the study drug.

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15 Published Articles from this and related work

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