B Experimental therapeutics: preclinical

B.1 MAGNETIC RESONANCE IMAGING: PRE-MANIFEST HUMAN HUNTINGTON'S DISEASE AND POTENTIAL FOR PRECLINICAL THERAPEUTIC TRIALS IN HUNTINGTON'S DISEASE MOUSE MODELS

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Background: Magnetic resonance imaging (MRI) measures may have potential as biomarkers for clinical trials in both premanifest and manifest Huntington's disease (HD).
Methods and Results: Structural MRI imaging studies at Johns Hopkins in HD indicate that striatal volumes begin to atrophy at least 11–12 years before expected onset and then continue to shrink predictably with disease course—and may prove a marker with greater power than motor ratings to detect change. Data from the PREDICT–HD study are corroborating and extending these findings. We have also found changes in functional MRI and DTI measures, which may begin even before detectable volumetric changes. Validation as outcome measures for clinical trials will require a demonstration that they change with treatment. We have therefore begun detailed MRI studies in several HD mouse models in which we are conducting treatment trials. Using T2-weighted MRI scans combined with large deformation diffeomorphic mapping, significant brain atrophy was present as early as 4 weeks of age in R6/2 mice and then continued in parallel with motor behavioural deficits. Striatum and cortex changes correlate best with motor changes. These studies suggest that R6/2 mice develop brain atrophy and behavioural changes very early, so that it may be difficult to study alterations in disease onset in this model. Preliminary studies in N171-82Q mice indicate that there is little or no significant brain regional atrophy at 6 weeks, but progressive atrophy after that. Our data suggest that N171-82Q mice might make it possible to track both the onset and progression of disease by MRI. Therapeutic trials in both R6/2 and N171-82Q mice are in progress.
Conclusions: MRI measures may thus have potential as biomarkers for preclinical mouse and both premanifest and manifest human clinical trials for HD.

B.2 COQ10 ANALOGUES TARGETING MITOCHONDRIAL IMPAIRMENT IN HUNTINGTON'S DISEASE

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Background: Edison's drug development programme in Huntington's disease (HD) is focused on developing a CoQ10 analogue targeting the purported mitochondrial impairment in HD. The observations underpinning the logic for the programme include: (1) positive correlation of CAG-repeat length with impairment in mitochondrial oxidative phosphorylation; (2) the presence of systemic markers of oxidative stress 8-OH 2d-guanosine; (3) reduction in central nervous system lactate, a biomarker of anaerobiosis, upon treatment with CoQ10, and subsequent return to baseline on withdrawal of therapy.
Aims: The development of a high-throughput assay reflective of the mitochondrial impairment component of HD. The development of analogues of CoQ10 with improved intrinsic efficacy and increased systemic and central nervous system bioavailability. The development of physiological, metabolic and biochemical indices to guide phase II endpoints.
Methods/Techniques: A primary HD cell line was developed modelling the energy defect observed in HD. Correlations were made with regard to Q-length, used to screen CoQ10 analogues. A library of more than 100 analogues of quinone-based CoQ10 analogues were designed, synthesised and characterised with regards to cLogP, redox potential, and biological activity. The measurement of physiological, metabolic and biochemical energy parameters at rest and under gated workloads are underway in HD versus control subjects.
Results/Outcome: A patient-derived primary cell assay has been established that reflects the energy defect in HD and correlates with Q-length. This assay is being deployed to screen and optimise CoQ10 analogues. Over 100 CoQ10 analogues have been screened. EPI-808,583 and EPI-442,983 have been selected as leads with EC_{50} values of 6 nmol and 15 nmol, respectively. A redox silent CoQ10 analogue is absent in biological activity, demonstrating redox dependency on drug action. The mitochondrial stress test is underway.
Conclusions: EPI-808,583 and EPI-442,983 are being readied for clinical development. Phase II design is pending completion of the mitochondrial stress test, anticipated in Q1, 2009.
Acknowledgement: Edison would like to thank the CHDI Foundation for their support of this work.

B.3 TRANSPLANTATION OF HUMAN OLFACTORY NEUROEPITHELIAL CELLS IN R6/2 MODEL OF HUNTINGTON'S DISEASE

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Background: The olfactory neuroepithelium contains neural stem cells that undergo continual neurogenesis and tissue regeneration. We investigated whether intrastriatal transplantation of human olfactory neuroepithelial cells (ONEC) can rescue the striatal pathology in a transgenic model of Huntington's disease (HD).
Methods: Human ONEC were cultured from nasal endoscopic biopsy specimens of healthy volunteers and induced into neurosphere-forming cells. In R6/2 transgenic mice, ONEC (0.5 million cells, R6/2-ONEC) or saline (R6/2-control) were transplanted into each bilateral striatum at the age of 5 or 8 weeks. We measured Rotarod performance, body weights, and limb clasping score twice every week and checked the survivals of the mice. Striatal atrophy and ubiquitin-positive nuclear aggregates, as well as the differentiations of transplanted ONEC were measured by stereological methods at the age of 12 weeks.
Results: ONEC transplantation improved Rotarod performance and attenuated the limb clasping phenomenon of R6/2 mice from 8 to 12 weeks of age. The R6/2-ONEC mice showed expanded survival lengths compared with R6/2-control mice. Striatal neuronal loss and ubiquitin-positive huntingtin aggregation were all decreased in the R6/2-ONEC mice. Transplantation of ONEC at the earlier age (5 weeks) showed no difference in efficacy compared with transplantation at 8 weeks. Transplanted cells expressed Nestin, Tuj-1, GABA and GAD, although they showed limited migratory patterns and low cell survival.
Conclusion: Our data suggest that ONEC is a feasible cell source for future cell transplantation therapy for HD patients.

B.4 CASPASES-2 AND 6 AS DRUG TARGETS IN HUNTINGTON'S DISEASE

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Background and Aim: Caspase cleavage of huntingtin (htt) is a crucial event in Huntington's disease (HD). Mutant htt resistant to cleavage by caspase-6 (C6) does not cause HD in a mouse model (Graham *et al*, 2006). Other lines of evidence support the role of C6 and also a role for caspase-2 (C2) in HD (Hermel *et al*, 2004). C2 and C6 are therefore validated drug targets for HD, although no specific small molecule inhibitors currently exist. We have undertaken two different approaches to inhibit caspases relevant to HD.
Methods and Results: First, we have utilised antisense oligonucleotides to reduce C2 and C6 levels in vitro and in vivo. Antisense

oligonucleotides (ASO) provide specificity for individual caspases due to their sequence targeting. In primary neurons ASO reduce C2 (64% reduction, one-way analysis of variance (ANOVA) $p = 0.0012$) and C6 (56% reduction, one-way ANOVA $p = 0.0166$) messenger RNA levels. In vivo caspase ASO are effective centrally when delivered intracerebroventricularly with osmotic pumps. After 4 weeks of intracerebroventricular delivery (25 μg/day), C2 mRNA was reduced 52% (one-way ANOVA $p = 0.0018$) and C6 mRNA was reduced 43% (one-way ANOVA $p = 0.0007$) in the cortex. This knockdown is stable or improved after 4 weeks of drug washout. The knockdown is specific, allowing us to examine the role played by individual caspases in HD pathology both in vitro and in vivo. C2 and C6 ASO are currently being tested in the YAC128 model of HD to determine their efficacy in reducing or eliminating previously validated signs and symptoms of HD.

In parallel we have developed a method suitable for screening for caspase-6 inhibitors. The assay relies on a luminescent signal generated by luciferase after caspase-6 cleavage, which is highly specific for caspase-6, with minimal cleavage by caspase-3 (6%). The method exhibits excellent linearity, signal-to-noise ratios (>300) and Z′ values (>0.7) for a wide range of caspase concentrations in a 384-well format and will be used for high-throughput screening of compound libraries.

B.5 GENETIC KNOCK-DOWN OF HDAC 4 IMPROVES MOTOR IMPAIRMENT IN THE R6/2 MOUSE MODEL OF HUNTINGTON'S DISEASE

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Background: Transcriptional dysregulation is a central mechanism underlying Huntington's disease (HD) pathogenesis. We have previously shown that administration of the histone deacetylase (HDAC) inhibitor suberoylanilide hydroxamic acid (SAHA) improves the motor performance of R6/2 mice. However, SAHA is a pan HDAC inhibitor that can inhibit 11 HDAC enzymes, namely: HDAC 1, 2, 3 and 8 (class I), HDAC 4, 5, 7 and 9 (class 2a), HDAC 6 and 10 (class 2b) and HDAC 11 (class IV). The SAHA target important for therapeutic development for HD requires delineation.

Aims: To identify the HDAC inhibitor target(s) relevant to HD therapeutic intervention.

Methods: We are in the process of conducting a series of genetic crosses to determine whether the genetic knock-down of specific HDAC improves HD-related phenotypes in the R6/2 mouse.

Results: We examined the effect of individually knocking down the expression level of HDAC 4, HDAC 5, HDAC 7 and HDAC 9 on the phenotype of the R6/2 mouse. Genetic knock-down of HDAC 5, HDAC 7 and HDAC 9 had no effect on a range of behavioural and molecular outcome measures. In contrast, reduction in HDAC 4 levels led to a pronounced improvement in R6/2 motor impairment as assessed by RotaRod performance. These beneficial effects occurred in the absence of the profound weight loss that occurred upon chronic SAHA administration. Identification of the molecular correlates of the phenotypic improvements is in progress.

Conclusions: Genetic reduction of HDAC 4 improves motor performance in the absence of detrimental effects, indicating that it is possible to dissociate the beneficial and toxic effects of SAHA administration.

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