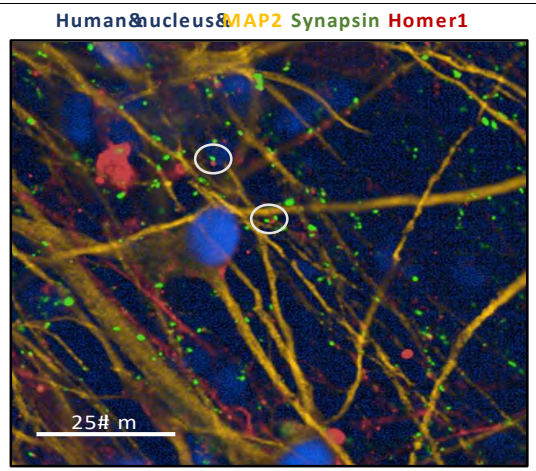
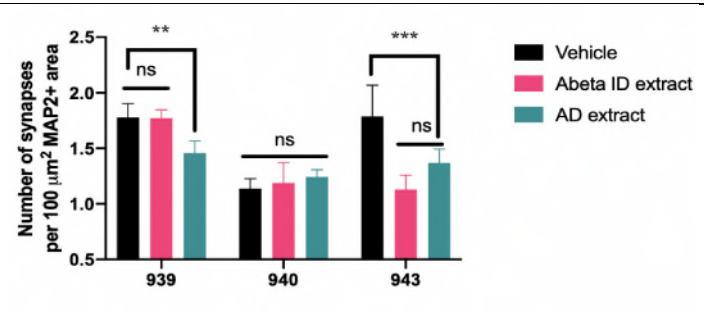
EM 2

Integration of clinical and cellular phenotypes in the DPUK Deep and Frequent Phenotype Cohort **Start date: 1 Jan 2017** **Completion date: PhD student will work on project until October 2021. DPUK end date agreed as 30 April 2020**				
Overall objective(s): This project will test the hypothesis that the cellular phenotype-induced pluripotent stem cell (iPSC-derived) neurons from patients will recapitulate the clinical phenotyping using an extensively 'deep-phenotyped' AD cohort.				
Deliverables	**Milestones**	**Milestone deadline**	**Work package dependencies**	**Person(s) responsible**
Objective 1:				
D1.1 -Lines from each of the 24 individuals will be generated and quality-controlled		M1.1.1 Completed	None	Zameel Cader
D1.2- iPSC lines from a reprogrammed ApoE allelic series from StemBANCC will be generated and differentiated into cortical neurons and brain endothelial cells using established methods		M1.2.1 Complete	None	Zameel Cader
Objective 2:				
D2.1- Protocols and assay details finalised for cortical differentiation methodology in formats required for, biochemistry, cell viability, neuronal morphology, electrophysiology and RNA-Seq.		M2.1.1 Complete	None	Richard Wade-Martins
D2.2- Measurement of neuronal morphology, neurite outgrowth and spine density using the Perkin Elmer Opera Phenix platform		M2.2 1 Complete	None	Richard Wade-Martins
D2.3 Define the pathological interaction between extracellular Abeta and intraceullar Tau using cell viability, biochemistry, electrophysiology and RNA-Seq		M2.3 Dec 2020	None	Richard Wade-Martins/Zam Cader/Simon Lovestone
Objective 3:				
D 3.1- Comparison of the levels of 3000 proteins in patient blood, CSF with that in conditioned medium harvested from cultures differentiated from patient iPSCs		M3.3.1 Jun 2020	None	Simon Lovestone
D3.4-Comparison of patient autophagy levels in peripheral PBMc with CNS levels in iPSC-derived cortical neurons		M3.2.1 Dec 2020	PBMCs required	Simon Lovestone/ Richard Wade-Martins
Updates on delivery against milestones since last report All remaining milestones on track with work underway				
Summary of plan to deliver on outstanding work (with dates) • Ongoing optimisation of high-throughput differentiation of several iPSC lines in parallel across the three collaborating laboratories, working on lines 939, 940, 943 to standardise the methods and understand the nature of experimental variability • Distribution of a common stock across the collaborating laboratories of Abeta extracted from AD brain temporal cortex and establish standard dilution conditions for its use. Healthy brain extract and Abeta immune-depleted AD brain extract selected as control conditions • Distribution of distinct phenotyping tasks across the laboratories: Wade-Martins: High content imaging of neuro-anatomy and synapses, neurite extension and axons and blebs and breaks and complexity at 80 days				

Lovestone: Somalogic on medium and cell pellet; Phospho tau and total tau by westerns and IHC; Cell viability by LDH, caspase 3 or live/dead assay Cader: MEA and transcriptomics at 80 days	
Risks 1) N/A	**Mitigation** 1)
Team members <u>funded</u> (full or part-time) by DPUK None: consumables costs only **Team members involved with the project but <u>not</u> funded by DPUK** Anne Hedegaard, Simon Lovestone, Liting Wei, Zameel Cader, Bryan Ng, Richard Wade-Martins	
Outcomes To follow	
Project narrative • The three collaborating laboratories successfully achieved differentiation of the three new DFP cohort lines (939, 940, 943) bulked up by the Lovestone laboratory and shared in the first experiment to standardize protocols. Cultures positive for cortical neuronal markers, and negative for astrocyte markers, were obtained by all laboratories. • The Cader laboratory achieved multi-electrode array (MEA) analysis of the neurons from the three lines. • The Wade-Martins laboratory successfully achieved iPSC-cortical neuron-rat astrocyte co-culture with the shared differentiation protocol involving expressing Ngn2 in iPSCs. This co-culture system is stable for at least 85 days (and counting) i.e. without other contaminant cell types, neurons appeared well-separated. These neurons expressed cortical markers and synapses on Day 40 of differentiation and achieved further maturity when aged until Day 80. • The Wade-Martins laboratory treated these neurons on Day 40 with human AD brain extract and elicited differential responses from three different patient-derived neuronal lines (as shown below, Figure 1). We are currently repeating this treatment and readout on older Day 80 neurons. • To obtain reliable & consistent source of human AD brain extract, the Wade-Martins laboratory has carried out a large-scale frontal cortical tissue extract from the same patient with the help from the Lovestone laboratory. We obtained approximately 76 ml of brain extract from 20 g of frontal cortical tissue, sufficient common stock for treating many differentiations across the laboratories.	





iPSC line	Patient ID	ApoE	MMSE	CSF Ab 42/40	CSF pTau/tau
939	3004	E3/4	29	0.038171	0.120914
940	3005	E4/4	26	0.035046	0.117411
943	6006	E3/4	24	0.077655	0.120566

Figure 1: Differentiation of the three standardising iPSC lines (939, 940, 943) into cortical neurons and treatment with AD brain extract and AD brain extract immune-depleted for Abeta.