a bioavailable anti-migratory drug for glioblastoma treatment potentially improving disease outcome in patients.

CHROMATIN REMODELLING TO FACILITATE TREATMENT RESISTANCE IN GLIOBLASTOMA

Alexander-F Bruns[1,2], Nora Rippaus[1], Alastair Droop[3], Muna Al-Jabri[1], Matthew Care[3], Michael Jenkinson[4,5], Andrew Brodbelt[6], Aruna Chakrabarty[6], Azzam Ismail[6], Susan Short[1,6], Lucy F Stead[1]; [1]Leeds Institute of Medical Research at St James's, University of Leeds, Leeds, United Kingdom, [2]Leeds Institute of Cardiovascular and Metabolic Medicine, University of Leeds, Leeds, United Kingdom, [3]Leeds Institute of Data Analytics, University of Leeds, Leeds, United Kingdom, [4]Walton Centre NHS Trust, Liverpool, United Kingdom, [5]Institute of Translational Medicine, University of Liverpool, Liverpool, United Kingdom, [6]Leeds Teaching Hospitals NHS Trust, St James's University Hospital, Leeds, United Kingdom

Recent findings from our group, and the wider community, show that standard treatment does not impose an apparent bottleneck on the clonal evolution of adult glioblastoma (GBM), implying a lack of direct therapeutic opportunity. This does not negate the possibility that multiple treatment-resistance mechanisms co-exist in tumours, repeated across patients, making a combination of targeted therapies a potentially effective approach. We investigated whether treatment resistance may be driven by selection of cellular properties conferred above the level of the genome. Differential expression analysis was performed on 23 pairs of primary and recurrent tumours from patients who received standard treatment and had a local recurrence treated by surgery and second line chemotherapy. This revealed a treatment-induced shift in cell states linked to normal neurodevelopment. The latter is orchestrated by cascades of transcription factors. We, therefore, applied a bespoke gene set enrichment analysis to our paired expression data to investigate whether any factors were implicated in co-regulation of the genes that were altered through therapy. This identified a specific chromatin remodelling machinery, instrumental in normal neurogenesis. We validated our results in an independent cohort of 22 paired GBM samples. Our results suggest that the chromatin remodelling machinery is responsible for determining transcriptional hierarchies in GBM, shown elsewhere to have different treatment sensitivities such that their relative abundances are altered through treatment.

CHARACTERISATION OF THE INVASIVE TUMOUR NICHE USING ASTROCYTE-GLIOBLASTOMA ORGANOIDS AND DECELLULARISED HUMAN BRAIN

Mohammed Diksin[1], Jonathan Rowlinson[1], Alexandar Kondrashov[2], Chris Denning[2], Jamie Hughes[3], Tim Constantin-Teodo[4], Wei Cui[5], Chris Gell[3], David Onion[3], Nicola Croxall[3], Stuart Smith[1], Ruman Rahman[1]; [1]Children's Brain Tumour Research Centre, University of Nottingham, Nottingham, United Kingdom, [2]Wolfson Centre for Stem cells, Tissue Engineering and Modelling (STEM), Centre for Biomolecular Sciences, University of Nottingham, Nottingham, United Kingdom, [3]School of Life Sciences Medical School, University of Nottingham, Nottingham, United Kingdom, [4]MRC/Arthritis Research UK Centre for Musculoskeletal Ageing Research, Division of Physiology, Pharmacology and Neuroscience, Medical School, University of Nottingham, Nottingham, United Kingdom, [5]Faculty of Medicine, Department of Surgery & Cancer, Imperial College of London, London, United Kingdom

Glioblastoma therapeutic challenges are in considerable part due to myriad survival adaptations and mechanisms, which allow malignant cells to repurpose signalling pathways within discreet microenvironments. These Darwinian adaptations facilitate invasion into brain parenchyma and perivascular space or promote evasion from repressive factors that represent anti-cancer defence mechanisms.

We hypothesised that pre-clinical modelling of glioma invasion by recapitulating early events occurring immediately after surgery at the glioblastoma invasive margin, could reveal the cross-talk between malignant cells and the surrounding healthy astrocytes, which facilitates tumour recurrence.

We first generated transgenic H1-derived neural stem cells using CRISPR/Cas9-mediated knock-in of the YFP reporter gene under the control of the GFAP promoter. Reproducible ultrahigh-throughput AggreWells™ (19,200 micro-wells per 24-well plate) were used to create astrocyte-glioblastoma organoids, which we term 'Gliomasphere Matrices'. YFP-labelled astrocytes were co-cultured with 10 treatment-naïve patient-derived cell lines isolated from the 5-aminolevulinic (5ALA)-determined glioblastoma invasive margin. Co-cultures were seeded upon on a sequentially constructed, time-of-flight secondary ion mass spectrometry (ToF-SIMS)-characterised 3D scaffold, composed of decellularised human brain extract with defined PEGDA hydrogel.

YFP-astrocytes were purified from each of the 10 Gliomasphere Matrices using fluorescence-activated cell sorting (FACS) after 6- and 10-days co-culture. RNAseq profiling to address both putative astrocytic reprogramming by invasive glioblastoma cells and gene expression changes intrinsic to tumour cells will be discussed in relation to RNAseq data from patient-derived 5ALA FACS-purified glioblastoma invasive margin tissue.

This novel multi-faceted model offers a unique opportunity to recapitulate early molecular cross-talk which facilitates glioblastoma recurrence and may be utilised for high-throughput drug screening.

CRISPR/CAS9 GENE EDITING OF BRAIN CANCER STEM CELLS USING LIPID-BASED NANO-DELIVERY

Nadia Rouatbi[1], Yau Mun Lim[1], Vivien Grant[2], Pedro Miguel Costa[1], Steven M. Pollard[2], Julie Tzu-Wen Wang[1], Khuloud T Al-Jamal[1]; [1]School of Cancer and Pharmaceutical Sciences, King's College London, London, United Kingdom, [2]MRC Centre for Regenerative Medicine, University of Edinburgh, Edinburgh, United Kingdom

Despite advances in cancer therapy glioblastoma (GBM) remains one of the deadliest brain tumours. Effective therapy is restricted by the presence of multiple resistance mechanisms. Physical barriers such as the blood-brain barrier limit the brain delivery of therapeutic compounds. In addition, the presence of a subset of GBM-stem-like cells (GSCs), characterized by radio/chemoresistance, and the intratumor heterogeneity impede standard therapies from being effective. New therapeutic approaches are urgently needed. Given its high specificity, CRISPR/Cas9-mediated genome editing provides new prospects for novel therapeutic targets. While promising, *in vivo* application of CRISPR/Cas9 is currently hampered by poor pharmacokinetics and limited ability to cross biological membranes.

The present research is designed to utilise stable nucleic acid lipid nanoparticles (SNALPs) for *in vivo* delivery of CRISPR/Cas9 to GSC by disrupting the epidermal growth factor receptor variant III (EGFRvIII), a GBM associated mutation, responsible for tumour cell proliferation, angiogenesis and invasion. Near Infrared fluorescence labelling and live optical imaging confirmed SNALPs uptake in GSC tumours implanted intracranially in mice after intravenous injection. Higher uptake of SNALPs in tumourous tissues compared to healthy brain tissues was further confirmed by *ex vivo* imaging and flow cytometry. EGFRvIII-specific sgRNA has been designed and validated in GSCs using commercial transfection regents. Studies are underway to load CRISPR/Cas9 mRNA/gRNA into SNALPs to test their *in vitro* gene editing efficacy. Successful *in vivo* delivery of CRISPR/Cas9 will represent a promising approach for identifying GBM therapeutic targets *in vivo* which in the long-run can be applied for GBM treatment.

CHOLESTEROL PROTECTS GLIOBLASTOMA CELLS AGAINST PREMATURE MITOSIS

Natividad Gomez-roman[1], Mark Jackson[1], Anthony J Chalmers[1]; [1]University of Glasgow, Glasgow, United Kingdom

Glioblastoma is the most common malignant primary tumour with a dismal prognosis. So far, no inhibitors targeting frequently altered pathways in GBM have improved patient survival. Premature entry to mitosis by small molecules that promote cancer cells to bypass the cell-cycle checkpoints have shown potent anti-tumour activity in vitro in a variety of cancers including GBM. We have reproduced these cytotoxic effects in our patient-derived GBM cell lines with the small molecule ME-344 and the Wee1-specific inhibitor AZ1775 both by by clonogenic survival and cell viability assays (EC_{50} values ranging from 0.003–0.02 μM and 0.2–0.4 μM, respectively). ME-344 and AZ1775 triggered profound morphological and cell cycle effects including mitotic induction, arrest and mitotic catastrophe. Bioinformatic analysis of global mRNA expression of our GBM cell lines stratified by ME-344 sensitivity showed a correlation between high ABCA1 and low cholesterol pathway gene expression with high sensitivity, and vice-versa. Cholesterol is a main component of membranes and is critical for cell growth and mitosis progression. GBM cells rely on cholesterol for survival. Due to the unique metabolic environment of the brain where a nearly unlimited supply of cholesterol is provided by astrocytes, targeting cellular activities regulated by cholesterol might lose their anti-tumour activity. Here we report that cholesterol confers cytoprotection to AZ1775 and ME344 in all GBM cell lines tested. These results suggest that cholesterol can override premature mitotic anti-tumour activity, indicating that mitotic induction and cholesterol inhibition might be a better therapeutic strategy for GBM than either treatment alone.

USING NANOBIOPSY OF SINGLE BRAIN TUMOUR CELLS TO INVESTIGATE TRANSCRIPTIONAL REPROGRAMMING DURING STANDARD TREATMENT.

Marilena Elpidorou[1], Paolo Actis[1], Lucy Stead[1]; [1]University of Leeds, Leeds, United Kingdom

Glioblastoma (GBM) is an incurable brain cancer because, despite aggressive standard treatment (consisting of surgery, radiation and