ORAL PRESENTATIONS
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Young Investigator Award Presentation

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Modelling Therapy Resistance for the Identification of Treatment-Refractory Cell Population(s) in Human Glioblastoma

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Despite aggressive multimodal therapy, human glioblastoma (hGBM), a highly malignant grade IV astrocytic tumour, remains incurable and inevitably relapses. Recent data has implicated intratumoral heterogeneity as the driver of therapy resistance and tumour relapse in hGBM. Thus models that capture the evolving hGBM biology in response to chemoradiotherapy will allow for the identification of cellular pathways that govern GBM therapy failure. In this study, we have developed a novel model to profile the clonal evolution of treatment naïve brain tumour initiating cell (BTIC) enriched hGBMs through chemoradiotherapy using: stem cell assays, BTIC marker expression and transcriptome analysis, immunohistochemistry, and cellular DNA barcoding technology. We report that treatment of hGBM BTICs leads to increased self-renewal capacity and higher transcript expression of stem cell genes Bmi1 and Sox2. Based on global transcriptome analysis of the in vitro treated hGBM, we also identify a hyper-aggressive form of glioma. Using our therapy-adapted hGBM-mouse xenograft model, we discover that despite tumour regression and increased mouse survival post-therapy, tumour relapse remains inevitable. The treatment-refractory cells again have increased self-renewal capacity and higher expression of Bmi1 and Sox2. Furthermore, by combining cellular DNA barcoding technology, which barcodes hGBM at single cell resolution, with our novel in vitro and in vivo therapy models, we are able to determine whether a pre-existing or a therapy driven subpopulation(s) seeds hGBM tumour relapse. Profiling the dynamic nature of heterogeneous hGBM subpopulations through disease progression and treatment may lead to the identification of novel therapeutic targets for the treatment of recurrent hGBM.

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Recruitment of Immune Effector Cells against Glioblastoma-Multiforme by a MHC-Chlorotoxin Chimeric Protein

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Glioblastoma Multiforme is the most common malignant primary brain tumor, having a mean overall survival <2 years. The lack of an efficient immune response against the tumor have been attributed to its immunosuppressive capabilities and an immunosuppressing local environment. Aim: We set out to design a chimeric molecule that recognizes and binds tissue inducible metalloproteinase known to be induced in GBM cells (MMP-2) on one end. Its other end, the effector domain, mobilizes and recruits cytotoxic T-cells to mount an effective anti-tumor reaction. Methods: The targeting moiety is the small 36-amino acids Chlorotoxin, derived from the venom of the Israeli Yellow scorpion. The effector end is a single chain HLA-A2 (Human leukocyte antigen subtype A2) covalently bound to phosphoprotein-65 derived from the cytomegalovirus, to which most of the human population has developed a specific immune response. Results: The molecular construct was cloned and expressed in E.coli. The protein product was isolated, purified, and then folded in vitro. Various activity assays employed demonstrated retained activity of each domain, including flow-cytometry, intracellular staining, fluorescence immunohistochemistry, radiolabeled toxicity assays etc. Initial in-vivo studies show great promise. Conclusions: We present a proof of concept study for a new immunotherapy approach to battle GBM. A molecular construct which contains a non-antibody compact and highly specific targeting domain, combined with the ability to recruit anti-CMV T-cell lymphocyte population. The recruitment of potent memory CTL's to the tumor's milieu may prove resistant to the previously described local immunosuppressive environment brought about by the tumor.

1035 - 1105 ORAL SESSION I ~ BRAIN METASTASIS

OS1 –136

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Time-Delayed Contrast Enhanced MRI Improves Detection of Brain Metastases: A Prospective Validation of Diagnostic Yield

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The radiological detection of BMs is essential for optimizing a patient's treatment. This statement is even more valid when stereotactic radiosurgery (SRS), a non-invasive image guided treatment that can target BM as small as 1-2mm, is delivered as part of that care. The timing of image acquisition after contrast administration can influence the diagnostic sensitivity of contrast enhanced MRI for BM. Objective: Investigate the effect of time delayed acquisition after administration of intravenous Atavist® (Gadobutrol 1mmol/ml) on the detection of BM. Methods: This is a prospective IRB approved study of 50 patients with BM who underwent post-contrast MRI sequences immediately after injection of 0.1 mmol/kg Gadavist® as part of clinical care (t0), followed by axial T1 sequences after a 10 minutes (t1) and 20 minute delay (t2). MRI studies were blindly compared by 3 neuro-radiologists. Results: Single measure intraclass correlation coefficients were very high (0.914, 0.904 and 0.905 for t0, t1 and t2 respectively), corresponding to a reliable inter-observer correlation. The t2 delayed sequences showed a significant and consistently higher diagnostic sensitivity for BM by every participating neuroradiologist as well as for the

entire cohort (p=0.016, p=0.035 and 0.034 respectively). A disproportionately high representation of BM detected on the delayed studies was located within posterior circulation territories (compared to predictions based on tissue volume and blood-flow volumes). Conclusion: Considering the safe and potentially high yield nature of delayed MRI sequences, it should supplement the basic MRI sequences in all patients in need of precise delineation of their intracranial disease.

OS2 – 166

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A Novel Model of Human Lung-to-Brain Metastasis and its Application to the Identification of Essential Metastatic Regulatory Genes

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Brain Metastases (BM) represent a leading cause of cancer mortality. While metastatic lesions contain subclones derived from their primary lesion, their functional characterization has been limited by a paucity of preclinical models accurately recapitulating the stages of metastasis. This work describes the isolation of a unique subset of metastatic stem-like cells from primary human patient samples of BM, termed brain metastasis initiating cells (BMICs). Utilizing these BMICs we have established a novel patient-derived xenograft (PDX) model of BM that recapitulates the entire metastatic cascade, from primary tumor initiation to micro-metastasis and macro-metastasis formation in the brain. We then comprehensively interrogated human BM to identify genetic regulators of BMICs using in vitro and in vivo RNA interference screens, and validated hits using both our novel PDX model as well as primary clinical BM specimens. We identified SPOCK1 and TWIST2 as novel BMIC regulators, where in our model SPOCK1 regulated BMIC self-renewal and tumor initiation, and TWIST2 specifically regulated cell migration from lung to brain. A prospective cohort of primary lung cancer specimens was used to establish that SPOCK1 and TWIST2 were only expressed in patients who ultimately developed BM, thus establishing both clinical and functional utility for these gene products. This work offers the first comprehensive preclinical model of human brain metastasis for further characterization of therapeutic targets, identification of predictive biomarkers, and subsequent prophylactic treatment of patients most likely to develop BM. By blocking this process, metastatic lung cancer would effectively become a localized, more manageable disease.

OS3 – 187

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Differentiating Radionecrosis from Tumor Progression Using IVIM perfusion Fraction in Brain Metastases Treated with Radiosurgery

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Radiation necrosis occurs in 5-25% of patients who undergo stereotactic radiosurgery (SRS) for brain metastases. Intravoxel incoherent motion (IVIM) uses MRI diffusion-weighted imaging (DWI) to assess regional perfusion. We investigated the utility of IVIM to differentiate recurrent tumor from radionecrosis after SRS. Patients who had SRS and subsequent surgical resection of what was thought to be either tumor progression or necrosis were included. ROIs were contoured on the pre-operative post-Gd T1-weighted images and transferred to DWI images using automated co-registration. The perfusion fraction (f) was calculated using asymptotic fitting and the mean f (fmean), 90th percentile for f (f90), mean ADC (ADCmean) and 10th percentile for ADC (ADC10) were calculated. Pathology reports were used to identify the predominant feature (necrosis versus tumor). Nine patients with ten lesions were included. One lesion exhibited pure necrosis while the other nine were mixed; three were predominantly (>75%) tumor, three predominantly necrosis, and three were equal parts of both. The perfusion fraction was significantly higher in cases with predominantly tumor compared to those with predominantly necrosis (fmean 0.10 ± 0.01 vs 0.08 ± 0.01, p=0.02 and f90 0.22 ± 0.01 vs 0.14 ± 0.02, p<0.001). ADC did not differentiate tumor from necrosis (ADCmean 0.97 ± 0.23 vs 1.02 ± 0.36, p=0.8 and ADC10 0.53 ± 0.29 vs 0.76 ± 0.29, p=0.33). The IVIM perfusion fraction is useful in differentiating recurrent tumor from radionecrosis in brain metastases treated with SRS. This is the first study to evaluate IVIM against the gold standard (histopathology).

1215 - 1255 ORAL SESSION II ~ PEDIATRICS

OS4 – 161

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Activated Wnt Signaling for the Therapeutic Targeting of Treatment-Refractory Medulloblastoma Stem Cells

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Brain tumours represent the leading cause of childhood cancer mortality, of which medulloblastoma (MB) is the most frequent