PS1 – 186

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Needs Assessment for a Pediatric Proton Therapy Program in Canada

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Proton therapy enables normal tissue sparing for curative-intent treatment of children with cancer who require radiation therapy. In the USA and elsewhere, proton therapy is being rapidly adopted, and many new proton centres are being established. Without a proton centre in Canada, children and their families must travel abroad for treatment at high cost and has raised the question whether a Canadian proton therapy facility is needed. METHODS: Canadian Pediatric Oncology centres were surveyed to assess current and future clinical practices. Needs were modeled by screening the Alberta Cancer Registry, ascertaining the number of children eligible for proton RT and comparing to the number who actually received this therapy. RESULTS: Most centres (63%) referred children, and 49 children were referred abroad between 2008 and 2013. Referrals were estimated to increase to 36 cases per annum across Canada. Most respondents (75%) supported that proton therapy will reduce late effects in most or selected cases compared to photon therapy. The registry search revealed 37,170 patients irradiated of which 379 children (1.0%) were potentially eligible for proton therapy, accounting for 15.9% of the new cases of childhood cancers diagnosed in Alberta over the interval. CONCLUSIONS: A strong perceived need for a pediatric proton therapy in Canada was identified. Proton therapy utilization was lower than modeled needs. Future referrals are anticipated to increase, with annual estimated cost of approximately $60 million spent outside of Canada that could be invested within the Canadian health care economy. These issues are worthy of further national discussion.

PS1 – 218

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Characterization of Ganglioglioma as a Neurodevelopmental Disorder: A Cast of Arrested Development?

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Gangliogliomas (GG) are low grade neuroepithelial tumours of the central nervous system (CNS) comprised of neoplastic glial and neuronal cells. There are no animal models or cell lines to study. Microarray data of a panel of low grade gliomas including GG revealed overexpression of the DLX2 homeobox gene required for tangential interneuronal migration and differentiation in the embryonic forebrain. We hypothesized that DLX2 regulates neural versus glial cell fate decisions in CNS progenitors and that GG are arrested in development. METHODS: DLX2 expression was examined along with glial fibrillary acidic protein (GFAP; glial marker) and synaptophysin and/or NeuN (neuronal markers) expression in a cohort of GG tumours using immunohistochemistry and dual immunofluorescence labelling of formalin fixed paraffin embedded (FFPE) tissue sections. BRAF mutational status was also assessed. RESULTS: In our discovery cohort (Genoa), 10/30 samples (33%) expressed DLX2. In our validation cohort (Edmonton), 22/36 (61%) expressed nuclear DLX2 and 12/22 DLX2+ cases had BRAF mutations (55%; 11 V600E, 1 V600G). 12/18 cases with BRAF mutations were DLX2+ (67%). One heavily pre-treated child with progressive cervicomedullary GG has had a very good partial response to BRAF inhibitor therapy. All 22 DLX2+ tumours co-expressed GFAP (100%) and 21/22 (95%) also expressed synaptophysin or NeuN. CONCLUSIONS: Our results support GG as neurodevelopmental tumours arising from bipotential CNS progenitors that are arrested at the neural-glial cell fate "decision" point. Biological or differentiation-based treatments could be considered +/- BRAF inhibitors for those GG with/without the V600E mutation, respectively.

SCIENTIFIC POSTER SESSION II
10 JUNE 2016 ~ 1730 - 1830

GLIOMA BASIC SCIENCE

PS2 – 153

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Dianhydrogalactitol (VAL-083) Treatment Causes Irreparable DNA Double-Strand Breaks, S/G2 Phase Cell-Cycle Arrest and Cell Death in Cancer Cells

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Dianhydrogalactitol (VAL-083) is a unique bi-functional alkylating agent causing N7-guanine-methylation and inter-strand DNA crosslinks. VAL-083 readily crosses the blood-brain barrier, accumulates in brain tumor tissue and has shown activity in prior NCI-sponsored clinical trials against various cancers, including glioblastoma (GBM) and medulloblastoma. VAL-083 is also active against GBM cancer stem cells and acts as a radiosensitizer independent of O6-methylguanine-DNA methyltransferase activity (in contrast to e.g. temozolomide and BCNU). Here we report new insights into VAL-083 mechanism of action by showing that VAL-083 induces irreversible cell-cycle arrest and cell death caused by replication-dependent DNA damage. In lung (H2122, H1792, H23, A549) and prostate (PC3, LNCaP) cancer cell lines VAL-083 treatment caused irreversible S/G2 cell-cycle arrest and cell death (IC50 range 3.06-25.7 µM). VAL-083 pulse-treatment led to persistent phosphorylation of DNA double-strand breaks (DSB) sensors ATM, single-strand DNA-binding Replication Protein A (RPA32), and histone variant H2A.X, suggesting persistent DNA lesions. After 10 months in culture with increasing VAL-083 concentrations, H1792 and LNCaP cells survive at concentrations up to 9.4 µM and 7.4 µM, respectively, suggesting that efficient resistance mechanisms are not easily acquired by the cancer cells. Taken together with previous results showing that VAL-083

circumvents cisplatin-resistance and is less dependent on p53 activity than cisplatin, these results suggest a molecular mechanism for VAL-083 that differs from both TMZ, BCNU and cisplatin. They further suggest that irreparable DNA damage induced by VAL-083 is impervious to common strategies employed by cancer cells to escape effects of alkylating drugs used in GBM treatment.

PS2 – 167

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CIC Deficiency is Associated with Dysregulation of Genes Involved in Cell Adhesion and Developmental Processes

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Somatic mutations in the Capicua (*CIC*) gene were first identified in Type I low-grade gliomas (LGGs), which are characterized by 1p/19q co-deletions and *IDH* mutations. They are found at frequencies of ~50-70% in this glioma subtype, and have since been identified in ~40% of stomach adenocarcinomas (STADs) of the microsatellite instability (MSI) subtype; however, the role of these somatic mutations in malignancy has yet to be established. In *Drosophila*, CIC functions as a transcriptional repressor whose activity is inhibited upon activation of the mitogen-activated protein kinase (MAPK) signalling pathway. Though mammalian CIC appears to retain these functions, only three of its target genes have been established in human cells: *ETV1*, *ETV4*, and *ETV5* (*ETV1/4/5*). To further probe CIC's transcriptional network, we developed *CIC* knockout cell lines and performed transcriptomic and proteiomic analyses in these and in control cell lines expressing wild type *CIC*, identifying a total of 582 differentially expressed genes. We also used RNA-seq data from The Cancer Genome Atlas (TCGA) for Type I LGGs and STADs to perform additional differential expression analyses between CIC-deficient and CIC-expressing samples. Though gene-level overlap was limited between the three contexts, we found that CIC appears to regulate the expression of genes involved in cell-cell adhesion, metabolism, and developmental processes in all three contexts. These results shed light on the pathological role of *CIC* mutations and may help explain why these have been associated with poorer outcome within Type I LGGs.

PS2 – 171

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Bmi1 Identifies Treatment-Refractory Stem Cells in Human Glioblastoma

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Glioblastoma (GBM) is an aggressive brain tumor that is resistant to conventional radiation and cytotoxic chemotherapies. We hypothesize that brain tumor initiating cells (BTICs), a subpopulation of treatment-resistant cells with stem cell properties cause tumor relapse and a subset of neural stem cell genes regulate BTIC self-renewal, driving GBM recurrence. We adapted the existing treatment protocol for adults with primary GBM for in vivo treatment of immunocompromised mice engrafted with GBMs. Post-chemoradiotherapy, the recovered GFP+ GBMs were profiled for self-renewal and expression of critical stem cell genes. Using invitro and invivo gain-of-function/loss-of-function experiments, we investigated the regulatory functions of Bmi1 in primary neural stem & progenitor cells (NSPCs) and GBM tumor formation. Finally, global RNA-Seq profiling was performed to understand the consequences of Bmi1 dysregulation on target gene expression. GBM cells showed an increase in Bmi1 levels post-chemoradiotherapy, suggesting the presence of a treatment-refractory BTICs. GFP+ cells extracted from treated xenografts had higher self-renewal and BTIC marker expression. Although treated mice responded to therapy, we observed tumor relapse with increased Bmi1 expression. Knockdown of Bmi1 diminished self-renewal and proliferation of GBM cells and delayed tumorigenesis, highlighting a critical role for Bmi1 in tumor maintenance. Conversely, over-expressing Bmi1 in NSPCs failed to initiate tumor formation in vivo. Using high-throughput sequencing data, we generated a map of signaling pathways dysregulated in GBM that may lead to tumor recurrence. Our data confirms the existence of a rare treatment-refractory BTIC population with enhanced self-renewal capacity that escapes therapy and drives tumor relapse.

PS2 – 172

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Preclinical Validation of a Novel CD33/CD3 Bispecific T-Cell Engager (BiTE) Antibody to Target Patient-Derived Glioblastoma Cells

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Glioblastoma (GBM), an aggressive primary adult brain tumor, is feared for its near uniformly fatal prognosis. Despite the use of aggressive treatment including surgical resection, radiotherapy and chemotherapy, the outcome of patients with GBM has failed to improve significantly. Numerous studies have implicated CD133+ GBM subpopulation as driver of chemo- and radio-resistance. CD133 expression correlates with disease progression, recurrence, and poor overall survival of GBM patients. Here, we describe the preclinical evaluation of a recombinant CD133xCD3 bispecific T-cell engager (BiTE) antibody that redirects human polyclonal T cells to CD133+ GBM cells, inducing very potent anti-tumor response. CD133-specific BiTE was constructed; with one arm recognizing the tumor antigen (CD133) while the second is specific to CD3 antigen. Using CD133high and CD133low primary GBM lines, we validated the binding of BiTEs to CD133+ GBMs and CD3+ T cells. In order to test the ability of BiTEs to functionally elicit CD133-specific cytotoxic responses in vitro, we performed killing assays. We observed CD133-specific BiTE mediated T cell activation and redirection to kill CD133-expressing GBM cells in a co-culture of T cells and GBM cells. The killing was more efficient in CD133high GBMs compared to CD133low GBMs, validating its specificity to target CD133+ BTICs. Treatment with BiTEs yielded significant reductions in