recurrence-associated changes in genetics and the microenvironment that can be targeted to shape disease progression following initial diagnosis.

OMRT-5. THERAPY-INDUCED REPROGRAMMING DRIVES GLIOMA VASCULAR TRANSDIFFERENTIATION AND RECURRENCE

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Therapy-resistant glioma cells elicit remarkable phenotypic plasticity leading to aggressive tumor recurrence. Here, we used single-cell and whole transcriptomic sequencing to uncover that radiation treatment induces a dynamic shift in functional states of glioma cells allowing for acquisition of either stem-like, mesenchymal-like or vascular-like phenotypes. The predominant phenotype switch induced by radiation in surviving tumor cells is the vascular-like cell state, resulting in transdifferentiation to endothelial-like and pericyte-like cells in distinct cell clusters. The transdifferentiated endothelial-like and pericyte-like cells secrete trophic factors to support proliferation of tumor cells, and their selective ablation results in reduced tumor growth and recurrence post-treatment. Mechanistically, the acquisition of vascular-like phenotype is driven by increased acetylation and chromatin accessibility in vascular genes and in regions for binding of vascular specification transcription factors. Blocking histone acetylation using a small molecule inhibitor targeting P300 histone acetyltransferase activity prior to radiation treatment inhibits the vascular-like transdifferentiation of glioma cells and tumor growth. Our findings indicate that radiation therapy-induces rewiring of glioma cells that promotes vascular cell-like transdifferentiation, tumor growth and recurrence.

OMRT-6. OPTIMIZING MDM2 INHIBITION FOR THE TREATMENT OF HIGH-GRADE GLIOMA

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Over 80% of high-grade gliomas have alterations in members of the p53 pathway, a central regulator of cell cycle progression and apoptosis that becomes activated in response to cellular stress and DNA damage. For tumors that retain wild-type p53, pathway deregulation frequently occurs through the amplification of negative regulators of p53, including the E3 ubiquitin ligase MDM2. The p53/MDM2 interaction axis has served as basis for the development of several classes of MDM2 inhibitors, with AMG232 being the most potent molecule currently undergoing clinical evaluation. As the effects of MDM2 inhibition (MDM2i) remain poorly understood in high-grade glioma, we performed genomic and transcriptomic analyses in patient-derived models to better characterize sensitive tumors and identify putative biomarkers of drug response. Treatment with AMG232 impaired the growth of cell lines with wild-type p53 status, particularly in tumors with additional amplification of MDM4 or PPM1D activating mutations. Treatment with AMG232 upregulated both cell cycle arrest and apoptotic cellular responses, as measured by annexin V/PI staining and immunoblotting. Interestingly, the dynamics of these two downstream p53 signaling axis were dependent on treatment duration across models. In addition to p53 pathway activation and apoptotic induction, RNA-sequencing revealed MDM2i to be associated with the activation of oncogenic MAPK and KRAS signaling as well as epithelial to mesenchymal transition markers. In most solid tumors, resistance to MDM2i is mainly mediated by acquisition of p53 inactivating mutations. We hypothesized that resistance mechanisms in glioma may be partially driven by transcriptional changes, as these tumors consist of subpopulations with diverse cell differentiation states. By chronic AMG232 treatment, we have developed in vitro and in vivo models of acquired MDM2i resistance that are not mediated by p53 inactivation. Ongoing work is focused on characterizing the transcriptional profile of these cells to identify transcriptional changes leading to decreased drug response.

OMRT-7. ANGIOGENESIS INHIBITORS STRONGLY SYNERGIZE WITH THERAPEUTICS TARGETING TUMOR METABOLISM

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Angiogenesis inhibition has become a mainstay of oncology despite having fallen short of its early promise. As originally envisioned, angiogenesis inhibition would cut off the blood supply, deprive tumor cells of key nutrients, leading to their death. In practice, while there is evidence that tumors under angiogenesis treatment do in fact exhibit some degree of metabolic stress, this is stress is not sufficient to induce significant cancer cell death. We posit that the full potential of angiogenesis inhibition can be realized by the combination of angiogenesis inhibition with emerging tumor metabolism targeting therapies. Because tumors under angiogenesis inhibition are already in a state of nutrient stress, the effects of metabolically targeted therapies such as amino acid depletion (e.g. asparginase, methionine restriction), inhibitors of stress adaption (AMPK and GCN2 inhibitors) or energy metabolism (e.g. IACS-010759, Metformin, POMHEX) stand to dramatically increase in potency whilst remaining selective for (angiogenic) tumor versus (non-angiogenic) normal tissue. Here, we provide proof-of-principal for this thesis. First, we performed metabolomic profiling of angiogenesis-inhibited tumors, which corroborates a state of nutrient stress in angiogenesis-inhibited tumors. Second, we demonstrate dramatic anti-neoplastic synergy (effectively curing of xenografted tumor-bearing mice, irrespective of initial tumor size), without enhanced adverse toxicities, between the OxPhos inhibitor IACS-010759 and the angiogenesis tyrosine kinase inhibitor, Tivozanib. The same results were recapitulated with the anti-VEGFA antibody, Avastin, and the OxPhos inhibitor could be substituted with the Enolase inhibitor HEX, with similar effects. The synergy was observed in a broad range of tumor types, even those without clear genetic susceptibilities. Together, these results suggest that angiogenesis inhibitors synergize broadly with cancer therapies targeting metabolism, allowing the realization of the full potential of these previously disappointing drugs. Our results warrant systematic combination clinical trials between angiogenesis inhibitors and established, as well as emerging anti-metabolic cancer therapies.

OMRT-8. PRECISION TARGETING OF CELLULAR PATHWAYS WITH COMPLEMENTARY DIAGNOSTICS

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Precision medicine tailors treatment for each patient by identifying the molecular drivers of their disease. This can allow more effective tumour targeting, avoid harmful standard chemotherapeutic side-effects, and offer savings to the healthcare system through not treating patients who are unlikely to respond to a specific agent. Treatment regimes are usually designed by identifying DNA-level alterations and selecting drugs tailored to that mutation. However, cancer is not a one-pathway disease and not all patients with particular mutations will respond to treatment, while patients without canonical pathway-activating mutations are excluded from potentially life-saving treatment. To address this, we have developed a NanoString assay combining proteomic and transcriptomic profiles of 4 key actionable, cancer-related pathways (MAPK, PI3K, NFκB and JAK/STAT). We used RNA-Seq data from gold standard cell lines with defined pathway changes to identify minimal gene sets indicative of pathway activation, and integrated them with phospho protein measurements to generate a pathway activation score. The combined panel was run on isogenic cell lines as well as glioma samples with both known and unknown driving alterations. We found pathway activation to be more variable than expected based on DNA alterations alone, implying that consideration of proteomic and/or transcriptomic-level information is important for future therapeutic decision-making.

OMRT-9. EFFECT OF PRE-OPERATIVE STEREOTACTIC RADIOSURGERY ON BRAIN METASTASIS: ANALYSIS OF DNA AND RNA GENOMIC PROFILES FROM PHASE-II CLINICAL TRIAL NCT03398694

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BACKGROUND: With improved systemic therapy that has limited impact on the intracranial compartment, the incidence of brain metastasis (BM) from solid cancers is rising and negatively impacting patient's overall survival (OS). Treatment varies based on presentation, however, for patients with <4 symptomatic BMs current clinical practice involves surgical resection followed by stereotactic radiosurgery (SRS) to the resection cavity. Post-operative SRS is associated with increased risk of leptomeningeal disease (LMD) and local recurrence in the follow-up period. We hypothesize that pre-operative SRS will decrease the incidence of LMD as well as local recurrence and increase patient's OS by delivering a lethal dose of radiation to tumor cells before they are disturbed by surgical resection. In a Phase II clinical trial (NCT03398694) we are treating patients with 1–4 symptomatic BMs with pre-operative SRS while collecting DNA and RNA sequencing data from core and peripheral edges of the resected tumor to examine the genomic effects of SRS on tumor. METHODS: Post-SRS resected tumor specimens were divided into two groups: 'center' and

'periphery' with respect to the center of SRS treatment with periphery within 50% isodose line. Previously resected untreated BMs were used as control. DNA and RNA were isolated from all samples for sequencing. CONCLUSIONS: Our initial analyses show that pre-treatment with SRS, results in significant genomic changes at DNA and RNA levels throughout the tumor, in both center as well as periphery. Furthermore, significant transcriptomic differences were noted among matched samples between the central and peripheral SRS locations implicating differential effect of SRS dosing within a tumor. Initial gene ontological analysis on non-small cell lung cancer samples demonstrated an overexpression of WNT and BMP signaling pathways ($p < .001$, $p < .01$). These pathways are typically involved in neuronal development, hinting that adaptation to the brain microenvironment was occurring post SRS treatment.

OMRT-11. THE EFFECT OF MICROENVIRONMENT ON GLIOBLASTOMA STEM CELLS THERAPEUTIC RESISTANCE

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Epithelial-to-mesenchymal transition (EMT) is an essential molecular and cellular process in physiologic processes and invasion of various types of carcinoma and glioblastoma (GBM) cells. EMT is activated and regulated by specific endogenous triggers in complex network of intercellular interactions and signaling pathways. The hallmark of cancer-linked EMT are intermediate states that show notable cell plasticity, characteristic of cancer stem cells (CSCs), including glioblastoma stem cells – GSCs. GSCs resistance to irradiation (IR) and temozolomide (TMZ) chemotherapy is responsible for early relapses, even at distant brain sites. As GSCs are mostly homing to their "niches" as slowly-dividing GSC-subtype, mimicking a proneural-like non- invasive phenotype PN-genotype, we assume that this, by undergoing an EMT-like transition, GSCs are-reprogrammed to an invasive mesenchymal (MES) GBs/GSCs phenotype in a processes, called PMT (1). However, it is not known, if and by which environmental cues within the niche, this transition of GSCs is induced in vivo. In this work, we are presenting the transriptome data obtained when we exposed GSC spheroids to irradiation alone, TMZ alone and to the combined treatment *in vitro* and compared their differential genetic fingerprints related to EMT/PMT transition to the GSCs PMT transition, when embedded in their natural microenvironment in the GBM organoid model. The differential gene expression upon GSCs therapeutic perturbation (when alone and *vs* in the tumoroid microenvironment) will reveal the effects of the major candidate genes, associated with micronevironmendt stromal cells and matrix are contributing their observed EMT/PMT transition of GSCs in vivo.

•1. Majc, B., Sever, T., Zarić, M, Breznik, B., Turk, B, Lah Turnšek, T. Epithelial- to-mesenchymal transition as the driver of changing carcinoma and glioblastoma microenvironment. DOI: 10.1016/j.bbamcr.2020.118782

OMRT-12. NANOPARTICLE-BASED CRISPR-CAS9 DELIVERY FOR ANTI-GLIOBLASTOMA IMMUNOTHERAPY

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Anti-glioblastoma GBM) immunotherapy poses a great challenge due to immunosuppressive brain tumor environments and the blood brain barrier (BBB). Programmed death ligand 1 (PD-L1) is an immune checkpoint that mediated the immune resistance. Inhibition of PD-L1 by antibodies was widely studied to treat many type of cancers. However, the inefficient therapeutic immune response became a significant barrier for treatment of GBM. CRISPR/Cas9 gene editing can be used to knockout both membrane and cytoplasmic PD-L1, leading to an enhanced immunotherapeutic strategy. It is extremely difficulty to deliver CRISPR/Cas9 containing plasmid for translational and clinic applications. We have been developed a core-shell nanoparticle (NP) to carry CRISPR/Cas9 plasmid for PD-L1 knockout. The different NP formulations were made and optimized to deliver CRISPR/Cas9 plasmid. NPs were prepared by modifying the water temperature, sonication power and time and formulation time. The obtained NPs had a size of 115-160nm and a charge of 40-50mV. The size and charge were significantly altered after CRISPR/Cas9 plasmids were loaded into NPs (Cas9-NPs). Agarose gel electrophoresis showed that CRISPR/Cas9 plasmids were fully encapsulated by NPs with 1 and 2 ug. The positive DNA bands occurred with 4ng, indicating the overloaded CRISPR/Cas9 plasmid. Fluorescence microscopy determined Cas9-NPs uptake by U87 cells under a time-dependent manner. GFP tagged Cas9-NPs were treated to U87 cells for transfection evaluation. The obtained different NPs delivery of CRISPR/Cas9 exhibited various transfection efficiencies in U87 cells. Visualization of intracellular Cas9-NPs showed increases in uptake by U87 cells from 0.5, 1, 2, and 4 hours. The greater nuclear accumulation of Cas9-NPs was seen at 24 hours. A western blot assay determined the success of PD-L1 deletion by Cas9-NPs in human GBM U87 cells. NPs-based CRISPR/Cas9 gene-editing system has great potential as an immunotherapeutic platform to treat GBM.

OMRT-13. DELIVERY OF UBIDECARENONE (BPM 31510) TO MITOCHONDRIA EFFECTUATES METABOLIC REPROGRAMMING AND REDOX ACTIVATED APOPTOSIS IN GLIOBLASTOMA

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GBM is a highly metabolic cancer phenotype that confers sustained growth and evasion of cell death mechanism via mitochondrial dysregulation. Efforts to re-engage mitochondrial metabolism via anti-cancer therapeutics has not been successful. BPM 31510 is a CoQ10-lipid conjugate nanodispersion for delivery of CoQ10 preferentially to mitochondria of human cells. BPM has demonstrated anti-cancer effects across multiple cancers, without adversely affecting normal tissue. The anti-cancer mechanism of CoQ10 was elucidated by Interrogative Biology, a data-driven approach to understand disease biology, identify targets and biomarkers of disease. Specifically, oncogenic and corresponding non-disease normal cell-based models (e.g. breast, liver, prostate, kidney) were subjected to cancer specific perturbations (e.g. hypoxia, metabolic stress). Comprehensive multi-omic (genome, proteome, lipidome, metabolome) and functional endpoints data were profiled. A Bayesian artificial intelligence analytics was used to generate network models in a data driven manner to identify BPM 31510 mechanism (i.e. shift in oxygen and glucose utilization, increase in oxidative stress and apoptosis in cancer cells). BPM 31510 re-capitulated its anti-cancer effect in GBM models, including LN-229 xenograft and C6 glioma allograft, both as monotherapy and in combination with temozolomide (TMZ)/radiation. The platform generated network maps from longitudinal pharmacodynamic samples (20 samples/28 days) collected from GBM patient refractory to TMZ/radiation/bevacizumab (Phase 1, NCT03020602, Stanford) identified alterations in intermediary metabolism as drivers of Progression Free Survival (PFS) and Overall Survival (OS) in response to BPM 31510 treatment. The platform supports the ongoing Phase 2 trial of adjuvant BPM 31510 plus TMZ/radiation in newly diagnosed GBM patients and potential accelerated approval.

OMRT-14. SMALL MOLECULE CIRCADIAN CLOCK COMPOUNDS EXHIBIT POTENTIAL AS A NOVEL THERAPY PARADIGM FOR GLIOBLASTOMA

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Glioblastoma multiforme (GBM) is the most prevalent and aggressive primary brain tumor type, claiming the lives of patients within 2 years of diagnosis. The major challenges in treating GBM are largely due to the biological characteristics of the tumor and the brain and pharmacokinetics of many drugs approved for other cancers. These tumors are located in areas that make it difficult to surgically resect without posing major issues and exposure to many drugs and therapies are limited due to the blood-brain barrier (BBB). GBMs also contain cancer stem cells, called GSCs, that have self-renewal and tumor initiating abilities, can secrete angiogenic factors, invade into the normal brain, and are chemoresistant and radioresistant. We found that GSCs have an exclusive dependence on core circadian clock transcription factors, Brain and Muscle ARNT-Like 1 (BMAL1) and Circadian Locomotor Output Cycles Kaput (CLOCK). These results suggest the potential for small molecule modulators of the circadian clock as a novel therapy paradigm for GBM treatment following surgical resection to prevent GSC infiltration and reoccurrence of the primary tumor. Here we found that multiple classes of clock compounds (Cryptochrome (CRY) stabilizers, REV-ERB agonists, Casein Kinase 1 (CK1) inhibitors, and Casein Kinase 2 (CK2) inhibitors) have the ability to elongate circadian periods in a clock reporter cell line. They also selectively and potently target patient-derived GSCs that range in sensitivity to temozolomide (TMZ) chemotherapy treatment while having limited effects on control cells both as single agents and in combination with each other. This data provides a platform for further exploration of synergistic effects of combining clock compounds with each other or with current GBM therapies, such as chemotherapy and radiation, with the ultimate goal of developing a clinical model of treatment.