to enhance tumour growth and proliferation, particularly within the char-
acteristic hypoxic tumour microenvironment (TME) of GBM. I hypothe-
size that the expression of ICAM1 on the surface of TAMs contributes to
GBM cell invasiveness, especially in the hypoxic TME, by enhancing the
interaction between tumour cells and macrophages, thereby facilitating
the migration and invasion of the tumour cells. METHODOLOGY: As-
sess the expression levels of ICAM1 in primary and immortalized human
and mouse macrophages under hypoxic conditions. Analyze the effect of
ICAM1 deficiency on macrophage behaviour including migration, prolif-
eration, and adhesion to tumour cells. Intracranially inject GL261 glioma
cells in ICAM1 deficient and wild type mice. RESULTS: ICAM1 is highly
expressed in different cell types within the GBM microenvironment, includ-
ing TAMs. ICAM1 expression is particularly high in primary or immortalized macrophages are treated with tumour cell-conditioned medium and is further exacerbated upon incubation of these cells in hyp-
oxia. The migration levels of bone marrow derived macrophage mouse cell type is higher in wild type cells than in ICAM1 deficient cells and higher when co-cultured with tumour cell condition media. ICAM1 deficient mice succumbed to GBM more quickly compared with wild type. CONCLUSIONS: It is evident that the hypoxic tumour microen-
vironment increases the expression of ICAM1 in macrophages. The tumour
microenvironment increases migration levels of macrophages. The expres-
sion of ICAM1 in TAMs in hypoxic TME promotes GBM cell invasiveness,
proliferation, aggressiveness and migration.

BSCI-15
INVESTIGATING CD8 T CELL EXHAUSTION STATES WITHIN
THE TME AND DRAINING LYMPH NODE OF PRIMARY BRAIN
TUMORS AND BRAIN METASTASES
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Brain metastases affect nearly 20% of all cancer patients. Likewise, glio-
blastoma (GBM) is the most common primary brain cancer in adults and
remains universally lethal. Current immunotherapeutic efficacy is hindered
by immunosuppression present in the brain tumour microenvironment (TME). T-cells, critical for tumor clearance, take on a functionally ex-
husted phenotype. Importantly, two exhaustion states, proponent (Tpe)
and terminal (Tte), have been identified in models of chronic infection and
cancer. This distinction is particularly relevant, as Tpe can remain respon-
sive to immune checkpoint blockade (ICB), while Tte cannot. To date, the
dynamics and characteristics of these exhausted populations in primary tu-
mors and brain metastases remain unclear. Using intracranially implanted
murine models of GBM (CT2A) and metastatic melanoma (B16F10), Tpe and Tte were identified by flow cytometry as PD1+SLAMF6+ and
PD1+TIM3+, respectively. Functional differences between subsets were evalu-
ed via intracranial staining of IFNγ, TNFα, IL2, CD107α, and Ki67. To determine the role of antigen, we performed adoptive lympho-
ocyte transfers of tumor-specific and non-tumor-specific transgenic T-cells
into a TRP2 or OVA overexpressing intracranial CT2A or B16 tumor, re-
spectively. Tpe displayed higher cytotoxic molecule expression than Tte,
consistent with chronic infection models. Key exhaustion-associated tran-
scription factors were identified in exhaustion subsets, including Tcx, Tcf7, Tbx21, and Bcl6 and Tpe and Tte expression levels in the
draining lymph nodes, suggesting a potential origin outside of the tumor
and the capacity for rescued function. We observed a decline in the Tpe
population over time, with an associated rise in Tte within both tumor types. Notably, the ratio of Tpe to Tte was higher at all time points in
B16F10 compared to CT2A. Tpe appeared only in tumor-specific T-cells
of the TME, further confirming the tumor-antigen dependence of T cell ex-
ahustion. Tpe may arise outside the TME in tumor-specific T-cells. Further study may reveal a means and time window for rescuing T-cell function
with ICB for brain tumors.

BSCI-17
TOPIRAMATE DECREASES RADIATION-INDUCED CYTOTOXIC
EDEMA IN HER2+ BRAIN METASTASES VIA AQUAPORIN 4
INHIBITION
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We have shown that 17β-Estradiol (E2) promotes brain metastasis (BM) of estrogen receptor negative (ER-) BC cells by inducing neuroinflammatory ERα astrocytes in the brain niche to secrete pro-metastatic factors critical for
early brain colonization. E2-depletion prevented brain colonization of human xenografts (MDA231BR/NSG) and syngeneic (E0771/C57BL6, 4T1/ Balb-c) ER+ models. Yet, whether E2-depletion can be used to decrease pro-
gression of established BM and how E2-dependent modulation of brain
immune response contributes to the pro-metastatic effects of E2 remains
unclear. To assess whether E2-depletion could decrease BM progression in a model that mimics standard of care for BM, E0771-GFP-luc cells were in-
jected intracranially in syngeneic ovariectomized (O VX)-female C57BL6 mice supplemented with E2. Seven days after injection (when micrometastases are
established), mice received a single 15Gy dose brain irradiation and were randomized to continue receiving E2, E2 withdrawal (E2WD) or E2WD
plus the aromatase-inhibitor letrozole (EWD+LET). Endpoint BM (but not systemic metastases) were significantly decreased in E2WD+Letrozole
treated mice as compared to E2-treated mice. This effect was abolished when
E0771 cells were injected in severely immunocompromised NSG mice or in
the presence of brain irradiation. Brain immune-profiling of brain irrad-
iation through boosting radiation-induced anti-tumor immunity. Accord-
gringly, there were no differences in BM progression in E2, E2 WD or E2 WD+
letrozole treated mice in a xenograft model (F2-7 TNBC cells) in NSG mice, even in
the presence of brain irradiation. Brain immune-profiling of brain irrad-
iation showed that E2WD+letrozole decrease brain metastatic burden in part through modulation of T cells. These results suggest E2-depletion therapies could be used in combination with brain irradiation to decrease progression of BMs and promote an anti-
tumoral immune response.

BSCI-19
A LENTIVIRAL CRISPR SCREEN FOR EPGENIC MODULATORS OF ANTIGENS TARGETED BY CAR T CELLS IN GLOBLASTOMA.
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Glioblastoma (GBM) is the most common adult brain tumor and is very difficult to treat. One promising treatment strategy for CAR T cell therapy,
in which T cells are used to target and kill cancer cells. However, CAR T cell therapy is not always effective, and more work is needed to realize its
potential. One strategy for improving the efficacy of CAR T cell therapy is to target the expression of the targeted antigens on glioblastoma cells.
Our goal in these studies is to identify pathways that could be modulated to increase expression of the targeted antigens. Focusing on epigenetic proteins,
Abstracts

we performed a lentiviral CRISPR screen in the human GBM cell line LN18. A lentiviral library was used to knock out various epigenetic genes in these cells in vitro. Following transduction, we use flow cytometry to examine surface expression of antigens currently being targeted in GBM clinical trials of CAR T cells, e.g. - GD2, B7-H3, etc. Cells with increased expression of the antigens of interest were selected using FACS. Genomic DNA was isolated from these cells and sequencing was performed to determine which epigenetic genes had been knocked out. Results showed multiple genes contributing to increased surface expression of targeted antigens. Future studies will determine whether small molecule inhibitors of the identified epigenetic pathways selectively induce up-regulation of these antigens in GBM cells in vivo.

BSCI-20
STING EPIGENETIC SILENCING IN GLIOMAS CAN BE RESCUED BY METHYLTRANSFERASE INHIBITION: IMPLICATIONS FOR NOVEL THERAPEUTIC APPROACHES
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The stimulator of interferons genes (STING) is a key component of the innate immune response to pathogenic cytosolic DNA, resulting in IRF3- and NF-kB-dependent transcription of type I interferons (IFN) and pro-inflammatory cytokines. STING activation primes endogenous antitumor immunity and is disrupted in a variety of cancers. Here we investigate STING signalling in glioblastoma (GBM) patient samples. STING activation and treatment of ex vivo glioma lines leads to inconsistent induction of type I IFN responses that are restricted to tumor associated myeloid cells. Indeed, single-cell transcriptome and multiplex immunofluorescence analyses demonstrate that STING expression is suppressed in neoplastic cells but not tumor-associated immune cells or stroma. Methylation analyses reveal a STING promoter region that is highly methylated in bulk tumor samples from glioma and other neuroectoderm-derived cancers, but not in most extracranial cancers. Methylation in this region strongly correlates with levels of STING RNA expression. STING epigenetic silencing is also present in normal fetal and adult brains. We demonstrate that STING expression in glioma cell lines may be rescued by decitabine, a DNA methyltransferase inhibitor (DNMTi) that is commonly used to treat hematological malignancies. However, transduction of a STING-expressing vector into these glioma cell lines is insufficient to reconstitute STING signalling, suggesting that additional decitabine-stimulated mechanisms are necessary for STING pathway rescue. Collectively, our results suggest that epigenetic silencing of STING occurs early in brain development and may provide an immunosuppressive context for the genesis of brain tumors. Furthermore, our work raises the potential of epigenetic modulation to reconstitute STING signalling as a therapeutic strategy for glioblastoma and potentially other STING-silenced, immunologically-cold cancers.

BSCI-21
COX4I1 EXPRESSION IN BRAIN METASTASES
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BACKGROUND: COX4I1 (Cytochrome C oxidase, subunit 4, isoform 1) is a mitochondrial enzyme involved in the process of switching from glycolysis to oxidative phosphorylation. A previously published prospective biomarker study in glioblastoma cells found that Cytochrome C oxidase (CoO) activity was associated with resistance to treatment with both radiation and temozolomide (TMZ). The current study was designed to retrospectively assess COX4I1 expression in brain metastases from various primary cancers. METHODS: This single-institution, blinded, retrospective biomarker study evaluated 24 patients with paired brain metastases and primary cancers including lung cancer, malignant melanoma, breast cancer, colorectal cancer, renal cell carcinoma, and urothelial carcinoma. COX4I1 immunohistochemistry expression in primary and metastatic samples was assessed using the H-score method. A paired t-test was used to assess the difference in total H-score between primary and brain metastasis tissue samples. Cox regression was used to assess the association between COX4I1 expression and overall survival (OS). For OS, time was calculated from metastatic tissue sample retrieval to death due to any cause. RESULTS: Brain metastasis tissue samples were found to have a significantly lower total H-score, on average, when compared to primary cancer tissue samples (p=0.01, mean difference 206.3 ± 33.0 vs. 157.8 ± 39.0). In addition, 58.3% of patients were on systemic treatment 6 months prior to tumor resection of brain metastases. COX4I1 expression was not associated with overall survival. CONCLUSIONS: There is significantly increased COX4I1 expression in primary cancers as compared to brain metastases. Anti-cytotoxic C oxidase therapies may be beneficial for treatment of primary tumors. No patient or treatment variables were significantly associated with overall survival in paired patients. KEYWORDS: COX4I1, cytochrome C oxidase, brain metastases

BSCI-22
DETERMINING THE EFFECT OF NOVEL SMALL MOLECULE DRUGS AGAINST THE MIGRATION OF BRAIN METASTASIS INITIATING CELLS (BMICS)
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BACKGROUND: Brain metastases are secondary tumors that predominantly arise from the spread of lung, skin, and breast cancers. The current standard of care for brain metastases is complete surgical resection, with a median survival of four months. Therefore, there is a dire need to discover new therapies that effectively target brain metastases. To do this, we have identified anti-brain metastatic drugs that specifically target brain metastasis initiating cells (BMICs), a cancer stem cell that is thought to escape standard therapies and has the ability to leave their primary tumor, seed the brain, and form a secondary brain tumor. Since the migration of the BMICs is essential to the development of brain metastases in preclinical studies, this study was designed to determine the effect of our anti-brain metastasis drugs against the migration of lung, breast, and skin BMICs. METHODS: This migration assay utilizes a bi-well silicone structure which effectively establishes a ‘wound’ healing-like migration assay. BMICs are plated in optimized equal concentrations in each silicone bi-well structure to successfully form two cellular mono-layers that are separated by a middle silicone wall. Once cells adhere to the plate the silicone structure is removed and the area between the two cell populations is imaged over time with an in vitro imaging system, RESULTS: This optimized assay has been used to screen our anti-brain metastasis drugs against the migration of lung, breast, and skin BMICs. Thus far our drugs have been tested against lung and skin BMICs which resulted in a significant decrease in BMIC migration. SIGNIFICANCE: Since brain metastasis arises from the migration of cancer cells to a secondary organ, it is crucial to discover the effect of anti-brain metastasis drugs on BMIC migration prior to the initiation of preclinical animal trials.

BSCI-23
ANEUPLOIDY PROFILING IN GliOBlastoma IDENTIFIES MACHanISMS OF DISEase PROGRESSION AND TREATMENT VULNERABILITIES
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Glioblastoma (GBM) is the most common and malignant adult brain tumor. Despite years of research, few advancements have been made in its management. One challenging area of glioblastoma research is patient stratification in clinical trials based on genomic features. Although several regions of aneuploidy have been known to drive disease progression in GBM, the degree of aneuploidy across the genome varies widely and the significance of regions of aneuploidy has not been assessed. Using whole genome sequencing profiles for matched tumor and non-tumor samples, we were able to accurately determine the degree of aneuploidy and loss of heterozygosity for a set of primary GBM tumors. Next, using machine learning techniques, distinct patterns of aneuploidy and loss of heterozygosity emerged among a set of GBM tumors, allowing us to define distinct aneuploidy subgroups. Interestingly, these aneuploidy subgroups showed distinctly different rates of patient survival, suggesting that regions of aneuploidy may be driving disease progression. Differing rates of various GBM genomic subtypes including IDH mutation, IDH-1 mutation, and tumor subtypes was also seen among the aneuploidy subgroups. We were able to derive a gene expression signature for each of these aneuploidy subgroups and revealed distinct pathways that were driving tumor growth. Furthermore, using a perturbagen-response dataset we were able to predict compounds to distinctly target each subgroup. Collectively, this suggests that aneuploidy profiling provides important clues to varying mechanisms of disease progression and is a promising approach for targeted therapy in a patient-specific manner.

BSCI-24
DETERMINATION OF THE EFFECT OF THERAPEUTIC APPROACHES ON THE TUMOR MICROENVIRONMENT IN PATIENTS WITH NOVEMBER 2021 NEURO-ONCOLOGY ADVANCES • AUGUST 2022