BSCI-26. COMPARATIVE METHYLATION PROFILING OF EGFR MUTANT LUNG ADENOCARCINOMA AND PAIRED BRAIN METASTASIS

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BACKGROUND: Adenocarcinomas (ADC) are the most common lung tumours and EGFR-mutant lesions have a higher risk of brain metastasis development, which confers poorer survival. Genetic and epigenetic signatures of brain metastasis have not been comprehensively identified. The aim of this study is to compare the methylome of EGFR-mutant primary lung tumor and matched brain metastasis to identify mechanisms of brain metastasis and new treatment targets. METHOD: Seven matched primary to brain metastasis tumours were profiled using the Illumina Infinium MethylationEPIC BeadChip array. Hierarchical clustering and principal component analyses (PCA) were performed using most variable CpG sites. Supervised analyses were performed between lung and brain tumour samples. Copy number variation (CNV) plots identified alterations between pairs along with Leukocytes unmethylation for purity (LUMP) score and prediction lymphocyte proportion analyses to measure immune infiltration. RESULTS: Unsupervised clustering showed that the fourteen tumours clustered according to patient with similar methylation profiles between each of seven matched pairs. On the supervised analysis using 83K significant CpG sites, the fourteen samples clustered into two groups based on tumour site being lung or brain. Of these 83K CpG sites, 2.4K were either hypermethylated or hypomethylated in all lung samples. One quarter of these 2.4K CpG sites were located in promoter regions. CNV analyses showed losses of FGFR1, C19MC, CDKN2A, PTCH1, and MYCN genes with higher deep deletions in brain versus lung primary samples. Immune infiltration measures were similar between lung and brain metastasis pairs (LUMP-score=0.64) consistent with high immune cell infiltration. CONCLUSION: In this EGFR-mutant lung adenocarcinomas and matched brain metastases, differentially methylated CpG sites and CNV alterations are identified that distinguish lung from brain samples. Further work with additional matched samples may further elucidate signatures specific to brain metastasis and aid in our understanding of the mechanisms of brain metastasis.

BSCI-27. MELATONIN REDUCES MALIGNANCY OF BREAST CANCER BRAIN METASTATIC CELLS

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Around fifteen to thirty percent of stage IV breast cancer metastasizes to the brain, severely decreasing the quality of life of these patients by causing neurological decline and eventually death. In metastatic cancers there is a small subset of cells in the primary tumor bulk called Metastatic Tumor Initiating Cells (MTICs) which are able to escape and produce a niche establishment at distal sites where they can quickly become resistant to surgery and radiation. Melatonin has shown an inhibitory role in the viability and invasiveness of breast cancer and in modulating the expression of proteins related to Breast Cancer Stem Cells (BCSCs). These findings suggest its potential anti-metastatic role in different breast cancer cell lines. In this study we aimed to evaluate the effects of melatonin treatment *in vitro* for breast cancer brain metastasis. The cell line MDA-BT was originally obtained from MDA-MB-231, passed through the rat's heart and then isolated once engrafted as a tumor in the brain. After a dose response assay, cells were treated with melatonin at doses of 1500 and 3000 µM for 48hrs. Clonogenic assay, MTT, as well as a stem cell signature through RT-qPCR, including CD44, CD24 and ALDH1 markers, were performed to evaluate the malignancy of the MTICs. The results showed that melatonin at high doses impacts morphology, declines viability, reduces colony formation ability, and decreases stemness in MDA-BT cells. Therefore, our findings highlight melatonin as a relevant therapeutic candidate to target breast cancer brain metastases.

LEPTOMENINGEAL DISEASE

LPTO-01. LEPTOMENINGEAL METASTASIS FROM OVARIAN CARCINOMA TREATED WITH SYSTEMIC HIGH-DOSE METHOTREXATE

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BACKGROUND: Central nervous system metastasis in ovarian cancer is reported in approximately 1% of cases and typically localizes to the parenchyma. Leptomeningeal disease (LMD) is exceedingly rare. Prognosis is extremely poor with median survival of 60 days. Given the rarity of LMD in ovarian cancers, there is no consensus on the optimal treatment approach. High-dose systemic methotrexate (HD-MTX) has been studied in LMD. CASE REPORT: A 41-year-old Caucasian woman with a history of BRCA-positive stage IIIC ovarian carcinoma who was treated with cytoreduction and multiple rounds of intravenous and intraperitoneal chemotherapy presented to the emergency room with progressive headaches, vomiting and neck pain. Contrasted MRI of the brain showed nodular enhancement along the cerebellar folia, concerning for leptomeningeal disease. Cerebrospinal fluid cytology analysis was positive for malignancy and she was diagnosed with leptomeningeal metastasis 2.5 years after her diagnosis with ovarian carcinoma. Induction therapy with systemic HD-MTX was given every other week and complete response was achieved after two induction cycles. Serum CA-125 level decreased from 565.7 U/mL (prior to treatment) to 50.4 U/ML after the sixth induction cycle and she was transitioned to maintenance HD-MTX. Approximately 6 months after initiation of HD-MTX, contrasted MRI demonstrated LMD recurrence with corresponding rise in CA-125 to 395 U/mL. Whole body CT imaging did not show local disease recurrence. Temozolomide was added to the HD-MTX regimen and craniospinal radiation therapy was planned but her neurologic condition continued to decline. She died 8 months after her diagnosis of LMD. CONCLUSION: LMD has a dismal prognosis. HD-MTX may prolong the overall survival in patients with LMD secondary to ovarian carcinoma. In these patients, serum CA-125 level may serve as a biomarker for monitoring CNS disease burden. Due to the rarity of LMD, multi-institutional prospective studies are needed to explore novel therapeutics in LMD.

LPTO-02. INTRATHECAL (IT) TRASTUZUMAB (T) FOR THE TREATMENT OF LEPTOMENINGEAL DISEASE (LM) IN PATIENTS (PTS) WITH HUMAN EPIDERMAL RECEPTOR-2 POSITIVE (HER2+) CANCER: A MULTICENTER PHASE 1/2 STUDY

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Patients with HER2+ breast cancer have frequent LM. A multi center phase 1/2 study assessing safety and efficacy of IT T in LM patients was conducted. The primary endpoint in Phase 2 was response rate (RR). Complete response (CR) required cytologic CR (CCR) + radiographic CR (RCR) + stable clinical function. Partial response (PR) required either CCR with stable/improved imaging or RCR with stable cytology + stable/improved clinical symptoms. Pts received IT T via intraventricular Ommaya reservoir. Phase 1 dosing started at 10 mg, then increasing by 20 mg up to 80 mg. Each cycle (C) was 4 weeks with 2x treatment/week in C1, weekly in C2, and every two weeks after C2. Pts were allowed to continue on hormonal agents if systemic disease was controlled at the time of LM development. Concurrent radiation therapy was not allowed unless exceptionally needed locally for pain control. 34 pts were enrolled with 26 its in the phase 2. The median age was 51 (25–69). IT T was well tolerated with no DLTs seen throughout; determined MTD was 80 mg for phase 2. All patients treated in the Phase 2 had HER2+ breast cancer, 2 patients in the Phase 1 had non-breast histologies. Median cycles completed was 2 (1–22). Median follow up was 9.1 months (0.4- 28.9). In Phase 2, 5 pts (19.2%) had PR, 13 (50%) had SD, and 8 (30.8%) had PD. For Phase 2 pts, median PFS was 2.4 months (CI 1.0–5.5) and median OS was 12.1 months (CI 4.3–19.6) IT T was well tolerated up to a dose of 80 mg. Primary endpoint of 25% RR was not met, however 69% had clinical benefit (stable disease or better). Median OS exceeded historical controls. Future studies are warranted to evaluate IT T in HER2+ LM.

LPTO-03. IN-VITRO & IN-VIVO CULTURE OF PATIENT (PT) DERIVED CSF-CTCS IN LEPTOMENINGEAL DISEASE (LMDZ) FROM MELANOMA TO IDENTIFY NOVEL TREATMENT STRATEGIES

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BACKGROUND: Approximately 5% of melanoma pts develop LMDz. There are essentially no models of LMDz available for therapeutic development. Here we report, the *in-vitro* & *in-vivo* culturing of CSF-CTCs. METHODS: CSF-CTCs were detected by the Veridex CellSearch® System. Cell-free DNA and cell-associated DNA were extracted, sequenced and profiled. Expanded *ex-vivo* CSF-CTCs were grown *in-vitro* and tested for drug sensitivity. CSF-CTCs were grown successfully *in-vivo* from 1 pt; labeled human Braf V600E WM164 cells were injected IT in as a control. RESULTS: *CSF-CTCs:* 12 LMDz

pts and 8 melanoma pts without LMDz were studied. All but 1 LMDz pts (92%) had CSF-CTCs (avg: 2148.60; range 23 - 3055 CTCs/ml). In contrast, 3/8 (37%) melanoma Brain Mets pts without LMDz had CSF-CTCs but fewer of them (avg: 0.31; range 0.13 - 0.6 CTCs/ml CSF). *CSF-CTCs Profile:* These had BrafV600E (83%), and GNAQ Q209P & NRAS Q61R in 1 pt each. *Ex-vivo culture of CSF-CTCs and PDX model:* After lengthy optimization of conditions we successfully expanded CSF-CTCs *in-vitro* (~25% of pts), and *in-vivo* in immunodeficient mice from 1 pt (~10% of samples). Ceritinib, used as a FAK inhibitor, with MEKi was effective *in-vitro* ($p=3.17e^{-6}$) and prolonged survival *in-vivo* in LMDz (median survival: >32 days vs control: 18 days; $p=7.81^{e-5}$). CONCLUSIONS: Though the sample size is small, this is the first report of the successful *in-vitro* & *in-vivo* culture of CSF-CTCs from pts with LMDz. Single cell analysis to determine how representative these models are and further *in-vivo* testing are in progress.

LPTO-04. GENERATION AND CHARACTERIZATION OF PATIENT-DERIVED PRECLINICAL MODELS FROM TUMOR CELLS ISOLATED FROM CEREBROSPINAL FLUID

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BACKGROUND: CNS metastases occur in 20–50% of lung cancer patients during their disease; leptomeningeal disease (LMD) representing 5–8%, classically carries a poor prognosis with a median overall survival ranging from 1–11 months. There is a paucity of patient-derived preclinical disease models using tumor cells isolated from the CSF. Models that faithfully recapitulate the biology of CNS tumors would offer new insights into the biology of the disease as well as provide the basis for developing more effective therapy. METHODS: To create more representative preclinical models to study LMD we isolated tumor cells from CSF of 5 patients with cytologically proven LMD and implanted the cells into the subcutaneous flank of immune-compromised mice. Where possible, cell lines were also generated from PDX tissues. Models were characterized by next generation sequencing (NGS), growth rates, expression of driver oncogenes and sensitivity to small molecule inhibitors. RESULTS: To date, one PDX (LUAD-0048A) and cell line model were successfully derived from CSF samples (NSCLC patient with *MET* amplification) and 4 are pending. *MET* amplification and mRNA overexpression were confirmed by quantitative PCR in the PDX tissue and the cell line. Western blot analysis indicated that over-expressed MET was phosphorylated in both PDX tissue and cell line. These results were confirmed by immunohistochemistry. Growth of LUAD-0048A cells were unaffected by 3 MET inhibitors (crizotinib, cabozantinib, glesatinib). Similarly, MET inhibitors did not induce apoptosis in the cells. CONCLUSION: LMD represents an aggressive metastatic event in lung cancer patients. Here we were able to successfully establish a PDX from the CSF of a patient with LMD and trial targeted therapies *in vivo*. Translational collaborations where patients with clinical suspicion of LMD undergo CSF sampling, NGS/ctDNA analysis, and PDX modeling are crucial in improving our understanding of this metastatic compartment and investigating novel treatment paradigms.

LPTO-05. FACTORS INFLUENCING RISK OF LEPTOMENINGEAL METASTASIS IN BREAST CANCER PATIENTS RECEIVING STEREOTACTIC RADIOSURGERY FOR LIMITED BRAIN METASTASES

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Leptomeningeal metastasis (LM) is a late stage manifestation of advanced breast cancer frequently managed with whole brain radiotherapy (WBRT) and/or intrathecal chemotherapy. A subset of breast cancer patients who undergo stereotactic radiosurgery (SRS) for limited brain metastases (BM) ultimately develop LM. We hypothesized that this subset of high-risk patients may be identified by patient, disease, and/or treatment parameters. Clinical records from 135 consecutive breast cancer patients from a single institution who underwent SRS for BM between February 2010 and March 2018 were retrospectively analyzed. Diagnosis of LM was determined radiographically and/or by cerebrospinal fluid analysis. Demographic data, clinical history, histopathology, BM features, systemic disease burden, and prior treatments were analyzed with Cox proportional hazards regression. In our cohort, 22 (16.3%) patients ultimately developed LM. With a median follow up of 18.9 (IQR 8.6–38.7) months after diagnosis of BM, the actuarial rate of LM at 18 months was 14.5% (95% CI, 7.0–21.4%). Median OS after diagnosis of LM was 7.3 (95% CI, 3.1–15.4) months. There was significantly increased risk of LM with ≥5 vs < 5 BM at BM diagnosis (33.0% vs 7.5% [18-month actuarial risk], HR 3.5, p=0.0045), and ≥7 vs < 7 cumulative number of BM treated (21.9% vs 11.1% [18-month actuarial risk], HR 2.7, p=0.023). Variables not significantly associated with the risk of LM included tumor receptor status (ER, PR, HER2, triple negative), graded prognostic assessment, KPS, extracranial metastases, total BM volume, prior WBRT, or prior surgical resection. In conclusion, patients with a larger number of brain metastases at BM diagnosis or ≥7 cumulative number of brain metastases treated appear to be at higher risk of developing LM and may benefit from stronger consideration of WBRT, intrathecal chemotherapy, and/or brain-penetrating systemic therapy.

LPTO-06. A NOVEL BRAIN-PERMEANT CHEMOTHERAPEUTIC AGENT FOR THE TREATMENT OF BREAST CANCER LEPTOMENINGEAL METASTASIS

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Leptomeningeal metastasis (LM), a spread of cancer to the cerebrospinal fluid and meninges, is universally and rapidly fatal due to poor detection and no effective treatment. Breast cancers account for a majority of LMs from solid tumors, with triple-negative breast cancers (TNBCs) having the highest propensity to metastasize to LM. The treatment of LM is challenged by poor drug penetration into CNS and high neurotoxicity. Therefore, there is an urgent need for new modalities and targeted therapies able to overcome the limitations of current treatment options. Quadriga has discovered a novel, brain-permeant chemotherapeutic agent that is currently in development as a potential treatment for glioblastoma (GBM). The compound is active in suppressing the growth of GBM tumor cell lines implanted into the brain. Radiolabel distribution studies have shown significant tumor accumulation in intracranial brain tumors while sparing the adjacent normal brain tissue. Recently, we have demonstrated dose-dependent *in vitro* and *in vivo* anti-tumor activity with various breast cancer cell lines including the human TNBC cell line MDA-MB-231. To evaluate the *in vivo* antitumor activity of the compound on LM, we used the mouse model of LM based on the internal carotid injection of luciferase-expressing MDA-MB-231-BR3 cells. Once the bioluminescence signal intensity from the metastatic spread reached (0.2 - 0.5) x 10^6 photons/sec, mice were dosed i.p. twice a week with either 4 or 8 mg/kg for nine weeks. Tumor growth was monitored by bioluminescence. The compound was well tolerated and caused a significant delay in metastatic growth resulting in significant extension of survival. Tumors regressed completely in ~ 28 % of treated animals. Given that current treatments for LM are palliative with only few studies reporting a survival benefit, Quadriga's new agent could be effective as a therapeutic for both primary and metastatic brain tumors such as LM. REF: https://onlinelibrary.wiley.com/doi/full/10.1002/pro6.43

LPTO-07. CARCINOEMBRYONIC ANTIGEN OF CEREBROSPINAL FLUID PREDICT PROGNOSIS OF LEPTOMENINGEAL METASTASIS FROM NON-SMALL CELL LUNG CANCER

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BACKGROUND: Leptomeningeal metastasis (LM) is a detrimental complication of patients with non-small cell lung cancer (NSCLC) and its incidence has increased due to recent improvements in survival. The aim of this study was to identify the clinicolpathological features and prognostic factors related to overall survival in NSCLC patients with LM. METHODS: Seventy-four consecutive patients diagnosed with LM from NSCLC between 2009 and 2018 in Guangdong Sanjiu Brain Hospital were retrospectively reviewed. RESULTS: Median KPS at diagnosis of LM were 60 (range, 20–90). Forty-seven (63.5%) patients harboring epidermal growth factor receptor (EGFR) or anaplasticlymphoma kinase (ALK) mutation while other twenty-seven patients (36.5%) were wild type or unknown status. Local treatment for LM consisted of whole-brain radiotherapy (WBRT) (52.7%), ventriculoperitoneal (VP) shunt (6.8%) and external drainage (6.8%). Systematic therapy for LM included EGFR or ALK tyrosine kinase inhibitors (TKI) (59.5%), chemotherapy (40.5%) and bevacizumab (8.1%). The median overall survival from diagnosis of LM to death was 8.1 months (95% confidence interval: 5.2 to 11.0). Patients with high Cerebrospinal Fluid (CSF) carcinoembryonic antigen (CEA) level (>50ng/ml) had worse prognosis compared with those low CSF CEA level (≤50ng/ml) ones (p=0.02). However, there was no significant difference in survival between patients with high serum CEA and those with low serum CEA (p=0.645). EGFR/ALK mutation and EGFR/ALK TKI after LM were also identified as variables that had prognostic influence on survival, while KPS, concurrent brain metastasis, WBRT and chemotherapy had no prognostic value for survival.