Abstracts

(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 Leuko- cytosis for purity (LUMP). Supervised 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. Ex-vivo 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.

BGSC.27. MELATONIN REDUCES MALIGNANCY OF BREAST CANCER BRAIN METASTATIC CELLS
Beatriz Fernandez G4, Katherine Rodriguez, Paula Schiaparelli, Carla Vazquez Ramos, Germaine Escames, Alfredo Quinones-Hinojosa, and Rachel Sarabia-Estrada

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 eventual 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 marker were 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-02. INTRATHECAL (IT) TRASTUZUMAB (T) FOR THE TREATMENT OF LEPTOMENINGEAL DISEASE (LMD) IN PATIENTS (PTS) WITH HUMAN EPIDERMAL RECEPTOR-2 POSITIVE (HER2+) CANCER: A MULTICENTER PHASE 1/2 STUDY
Priya Kumthekar, Andrew B Lassman, Nancy Lin, Sean Grimm, William Gradishar, Elena Pentsova, Surya Jeyapalan, Morris Groves, Melissa Melisko and Jeffrey Raizer

Patients with HER2+ breast cancer have frequent LMD. 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/improved clinical symptoms. PR received IT T via intraventricular Omumaya 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 pts in the phase 2. The median age was 51 (25-69), IT T was well tolerated with no treatment related deaths. 12 pts had received prior systemic/HER2+ LM in phase 1, 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 1.0-5.6) 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 (LMD) FROM MELANOMA TO IDENTIFY NOVEL TREATMENT STRATEGIES
Vincent Law, Brittany Evernden, Rajappa Kenchappa, John Puskas, Gisela Caceres, Elena Rychova, Inna Smalley, Arnold Etame, Solmaz Sahebjam, Anthony Magliocco, Keiran Smalley and Peter Forsyth

BACKGROUND: Approximately 5% of melanoma pts develop LMDs. There are essentially no models of LMDs available for therapeutic development. Here we report, the in-vitro & in-vivo cultivation 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-vitro from 1 pt; labeled human Braf V600E WM164 cells were injected IT in as a control. RESULTS: CSF-CTCs: 12 LMD pts and 8 melanoma pts without LMD were studied. All but 1 LMD pts (92%) had CSF-CTCs (avg: 2148±60; range 23 – 3105 CTCs/ml). In contrast, 3/8 (37%) melanoma Brain Mets pts without LMD had CSF-CTCs but fewer of them (avg: 0.31; range 0.13 - 0.6 CTCs/ml CSF). CSF-CTCs Profiles: 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 (~10% of samples). Ceritinib, used as a FAK inhibitor, with MEKi was effective in-vitro (~10% of samples) and prolonged survival in-vivo in LMDs (mouse survival: >32 days vs control: 18 days; p=7.81e-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 LMDs. Single cell analysis to determine how representative these models are and further in-vitro testing are in progress.