37. *IN VIVO* FUNCTIONAL GENOMIC SCREEN TO IDENTIFY NOVEL DRIVERS OF LUNG-TO-BRAIN METASTASIS

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Brain metastasis, the most common tumour of the central nervous system, occurs in 20–36% of primary cancers. In particular, 40% of patients with non-small cell lung cancer (NSCLC) develop brain metastases, with a dismal survival of approximately 4–11 weeks without treatment, and 16 months with treatment. This highlights a large unmet need to develop novel targeted therapies for the treatment of lung-to-brain metastases (LBM). Genomic interrogation of LBM using CRISPR technology can inform preventative therapies targeting genetic vulnerabilities in both primary and metastatic tumours. Loss-of-function studies present limitations in metastasis research, as knocking out genes essential for survival in the primary tumour cells can thwart the metastatic cascade prematurely. However, gene overexpression using CRISPR activation (CRISPRa) has the potential for overcoming dependencies of gene essentiality. We theorize that an *in vivo* genome-wide CRISPRa screen will identify novel genes that, when overexpressed, drive LBM. We have developed a patient-derived orthotopic murine xenograft model of LBM using primary patient-derived NSCLC cell lines (termed LTX cells) from the Swanton Lab TRACERx study. We are now poised to transduce LTX cells with a human genome-wide CRISPRa single guide RNA (sgRNA) library, and to subsequently inject the cells into the lungs of immunocompromised mice. We will then track the process of LBM using bioluminescent and MRI imaging until mice reach endpoint. Sequencing of primary lung tumours and subsequent brain metastases promises to uncover enriched sgRNAs, which may represent novel drivers of primary lung tumour formation and LBM. To the best of our knowledge, this study is the first *in vivo* genome-wide CRISPRa screen focused on identifying novel drivers of LBM, and can inform future preventative therapies to improve survival outcomes for NSCLC patients.

38. INNOVATIVE USE OF A CUSTOM MOBILE APPLICATION (APP) BY A BRAIN METASTASES PROGRAM FACILITATES MULTIDISCIPLINARY MANAGEMENT OF PATIENTS AND DECREASED LENGTH OF HOSPITAL STAY (LOS)

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INTRODUCTION: Patients with Brain Metastases (BM) are complex, mandating multidisciplinary care. Our BM patients are discussed at in-person, weekly Brain Tumor Boards (BTB). However, BM patients diagnosed outside weekly BTBs wait several days for the next BTB, causing delays in generating multidisciplinary plans-of-care, prolonging LOS. We created a custom mobile app for our Brain Metastases Program to have a 'Brain Metastases Virtual Tumor Board' (BMVTB) discussion, in real-time, resulting in faster plans-of-care, decreasing LOS. METHODS: The current pathway for navigating multidisciplinary discussions for patients with BM was examined by members of our Brain Metastases Program. We identified the need for all disciplines to participate in a BMVTB, outside of our in-person, weekly BTB. We developed a secure app that can be downloaded on any provider's mobile device. The app includes a digital BM treatment algorithm for providers to understand comprehensive, data-driven, BM management. The app also gives our multidisciplinary Brain Metastases Program access to a BMVTB messenging tool to securely communicate and generate real-time consensus plans-of-care. Using a Vizient Clinical Database, we retrospectively calculated LOS index (observed LOS/expected LOS) for 184 BM patients over 21 months, creating a baseline. After launching our app and BMVTB workflow we prospectively evaluated LOS index in 45 BM patients over 6 months. RESULTS: Over 21-months, 184 patients demonstrated baseline LOS index of 1.073. After launching our mobile app and BMVTB workflow, 45 patient admissions over 6-months demonstrated LOS index of 0.850. Using Levene's test for equal variances, LOS variance with the app and BMVTB was lower than LOS variance at baseline ($p = 0.049$). This demonstrates a 38% reduction in LOS when the app and BMVTB generated real-time plans-of-care. CONCLUSION: We demonstrated utility of a custom BM app coupled with a BMVTB to generate real-time plans-of-care for BM patients, reducing LOS.

39. CHARACTERIZING NOVEL INHIBITORS OF BRAIN METASTASIS-INITIATING CELLS

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BACKGROUND: The incidence of brain metastases (BM) is tenfold higher than primary brain tumors. BM commonly originate from primary lung, breast, and melanoma tumors with a 90% mortality rate within one year of diagnosis. Current standard of care for BM includes surgical resection with concurrent chemoradiation, but does not extend median survival past 16 months, posing a large unmet need to identify novel therapies against BM. METHODS: From a large in-house biobank of patient-derived BM cell lines, the Singh Lab has generated murine orthotopic patient-derived xenograft (PDX) models of lung, breast, and melanoma BM that recapitulate the stages of BM progression as seen in human patients. Using these three PDX models, we identified a population of "pre-metastatic" brain metastasis-initiating cells (BMICs) that are newly arrived in the brain but have yet to form detectable tumors. Pre-metastatic BMICs are not detectable in human patients but are important therapeutic targets with the potential to prevent BM in at-risk patients. RESULTS: RNA sequencing of pre-metastatic BMICs from all three PDX primary tumor models with subsequent Connectivity Map analysis identified novel compounds that have the potential of killing all three types of BMICs. In particular, we identified two compounds that have selective killing of BMICs *in vitro* from all three primary tumor cohorts while sparing non-cancerous cells. We further characterized their ability to inhibit the self-renewal and proliferative properties of BMICs. Ongoing *in vivo* work will investigate the compounds' preclinical utilities in preventing BM. CONCLUSION: Identification of novel small molecules that target BMICs could prevent the formation of BM completely and dramatically improve the prognosis of at-risk cancer patients.

40. AN UPDATE ON THE DEVELOPMENT OF A NEW TOOL TO ASSESS RESPONSE IN LEPTOMENINGEAL METASTASIS

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In 2016, a standardized scorecard to aid in the evaluation of MRI findings during the course of disease was proposed by the RANO leptomeningeal metastasis (LM) subcommittee. In this scorecard, LM main features and the different CNS metastases types were to be reported as present or absent, dimensions of measurable nodules were to be noted, and changes from the previous MRI were to be scored from -3 to +3. A feasibility and validation study of this scorecard was performed by asking 19 experts to evaluate response in 22 patients diagnosed with and treated for LM. The outcome was disappointing in that the scorecard appeared to be too complicated (Le Rhun et al. Neuro-Oncology 2019;21:648–658). Specific challenges were (i) to understand that the form should be used to rate the current MRI and to compare it with the previous one, (ii) to use the proposed rating with "minus" or "plus" options to assess the change and (iii) to derive a sum score that does not take into consideration (per instruction) changes for the items "hydrocephalus", "brain metastases" and "parenchymal medullary metastases". In addition to the apparent challenges for experienced raters to use the LANO scorecard instructions without further instructions, we identified additional weaknesses. These include to eliminate epidural metastases from response assessment, to include the definition of a nodule, the distinction of leptomeningeal versus parenchymal brain disease, and to include parallel, but clearly separate criteria to document brain parenchymal disease. The revised, published proposal for a new scorecard has been used to rate another series of LM cases by experienced neuro-oncologists from March to May 2020. First analyses of this novel feasibility and validation study will be available in August.

41. PROGNOSTIC VALIDATION OF THE EANO ESMO CLASSIFICATION OF LEPTOMENINGEAL METASTASIS

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BACKGROUND: The EANO ESMO guidelines have proposed a classification of leptomeningeal metastases (LM) based on clinical (typical/atypical), cytological (positive/negative/equivocal) and MRI (A linear, B nodular,

C linear and nodular, D normal or hydrocephalus only) presentation. Type I LM is defined by the presence of tumor cells in the cerebrospinal fluid (CSF) (confirmed LM) whereas type II LM is defined by typical clinical and MRI signs (probable or possible LM). Here we explored the clinical utility of these EANO ESMO LM subtypes. PATIENTS AND METHODS: We retrospectively assembled data from 254 patients with newly diagnosed LM from different solid tumors, including as main primary tumors breast cancer (n=98, 45%), lung cancer (n=65, 25.5%) and melanoma (n=51, 13.5%). Survival curves were estimated using the Kaplan-Meier method and compared by Log-rank test. RESULTS: Median age at LM diagnosis was 56.5 years (range 20–82 years). Typical clinical LM symptoms or signs were noted in 225 patients (88.5%); only 13 patients (5%) were clinically asymptomatic. The most common MRI subtype was A seen in 117 patients (46%). Types B (n=33, 13%), C (n=54, 21%) and D (n=50, 19.5%) were less common. Tumor cells were observed in the CSF in 186 patients (73%) whereas the CSF was equivocal in 24 (9.5%) and negative in 44 (17.5%) patients. Patients with confirmed LM had inferior outcome than patients with probable or possible LM (p=0.0063). Type I patients had inferior outcome than type II patients (p=0.0019). Nodular disease was a negative prognostic factor in type II LM, but not in type I LM (p=0.0138). CONCLUSION: The presence of tumor cells in the CSF appears to have a greater prognostic role than the neuroimaging presentation. EANO ESMO LM subtypes are highly prognostic and should be considered in the design of clinical trials.

42. IDENTIFICATION OF BRAIN METASTASIS VULNERABILITIES USING METPLATFORM

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The diagnosis of brain metastasis involves high morbidity and mortality and remains an unmet clinical need in spite of being the most common tumor in the brain. Exclusion of these cancer patients from clinical trials is a major cause of their limited therapeutic options. In this study, we report a novel drug-screening platform (METPlatform) based on organotypic cultures which allows identifying effective anti-metastasis agents in the presence of the organ microenvironment. We have applied this approach to clinically relevant stages of brain metastasis using both experimental models and human tumor tissue (by performing patient-derived organotypic cultures). We identified heat shock protein 90 (HSP90) as a promising therapeutic target for brain metastasis. Debio-0932, a blood-brain barrier permeable HSP90 inhibitor, shows high potency against mouse and human brain metastases from melanoma, lung and breast adenocarcinoma with distinct oncogenomic profiles at clinically relevant stages of the disease, including a novel model of local relapse after neurosurgery. Furthermore, we have also used METPlatform to perform unbiased proteomics of brain metastases in situ. By applying this analysis to brain metastases treated with the chaperone inhibitor, we uncovered non-canonical clients of HSP90 as potential novel mediators of brain metastasis and actionable mechanisms of resistance driven by autophagy. Combined therapy using HSP90 and autophagy inhibitors showed synergistic effects compared to sublethal concentrations of each monotherapy, demonstrating the potential of METPlatform to design and test rationale combination therapies to target metastasis more effectively. In conclusion, our work validates METPlatform as a potent resource for metastasis research integrating drug-screening and unbiased omic approaches that is fully compatible with human samples and questions the rationale of excluding patients with brain metastasis from clinical trials. We envision that METPlatform will be established as a clinically relevant strategy to personalize the management of metastatic disease in the brain and elsewhere.

43. DELAYS IN ADJUVANT STEREOTACTIC RADIOSURGERY REDUCE LOCAL CONTROL FOR RESECTED BRAIN METASTASES

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OBJECTIVE: For resected brain metastases (BM), stereotactic radiosurgery (SRS) is often offered to minimize local recurrence (LR). Although the aim is to deliver SRS within a few weeks of surgery, a variety of socioeconomic, medical, and procedural issues can cause delays. We evaluated the relationship between timing of postoperative SRS and LR. METHODS: We retrospectively identified a consecutive series of BM patients managed with resection and adjuvant SRS, recommended within two weeks of surgery, at our institution from 2012–2018. We assessed the correlation between time to SRS, as well as other demographic, disease, and treatment variables, and LR, distant recurrence (DR), and overall survival (OS). RESULTS: 133 patients met inclusion criteria. Median age was 64.5 years. Approximately half of patients had a single BM, and median BM size was 2.9 cm. Gross total resection was achieved in 111 (83.6%) patients, and >90% received fractionated SRS. Median time to adjuvant SRS was 37.0 days and LR rate was 16.4%. The factor most predictive of LR was time from surgery to SRS. Median time from surgery to SRS was 34.0 days for patients without LR, versus 61.0 days for those with LR (p<0.01). LR was 2.3% with SRS administered ≤4 weeks postoperatively, compared to 23.6% if delayed >4 weeks (p<0.01). Local recurrence-free survival (LRFS) was also improved for patients who had SRS at ≤4 weeks (p=0.02). Delayed SRS was also predictive of DR (p=0.02), but not OS. CONCLUSIONS: We demonstrate that the strongest predictor of failure of postoperative SRS for BM is the delay to SRS. A cut-off of 4 weeks is a reliable predictor of increased LR. Every effort should be made to perform SRS within 4 weeks of surgery, and if this cannot be achieved, other RT modalities, such as brachytherapy or preoperative SRS, should be strongly considered.

44. EFFECT OF STEREOTACTIC RADIOSURGERY ON NON-SMALL CELL LUNG CANCER BRAIN METASTASIS: CONTINUED CORRELATIVE RADIOBIOLOGIC ANALYSIS OF DNA AND RNA GENOMIC PROFILES FROM PHASE-II CLINICAL TRIAL NCT03398694

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BACKGROUND: Stereotactic radiosurgery (SRS) is an increasingly common modality used with or without surgery for the treatment of brain metastases (BM). However, the effects of SRS on tumors *in vivo* is unknown. METHODS: Patients were treated with SRS prior to surgery as per clinical trial NCT03398694. Resected tumor was divided into two groups: 'center' and 'periphery' with respect to SRS treatment. Tissue were analyzed by DNA and RNA sequencing and compared between the two and to non-radiated tumor. RESULTS: DNA analysis showed at the individual level, matched comparison of SRS samples from the center or periphery of the same tumor had mutational burden differences. RNA analysis revealed no differentially expressed genes between center and periphery, but there were 62 and 192 differentially expressed genes between the center or periphery and non-radiated control, respectively. At an individual level, matched center and periphery tumor had an average of 16641 differentially expressed genes. Comparing total number of up- and downregulated genes with SNP and Indel mutations of matched patient samples, in patients with higher mutational burdens in peripheral tumors as compared to center there was a higher number of upregulated genes. Reciprocally, when mutation burden was higher in center tumor, total number of genes that were either up- or downregulated were about the same. Pooled analysis revealed significant downregulation of oncogenes, such as TP63 and RECQL4, in the SRS group. DO enrichment analysis also revealed pathways related to NSCLC and lung carcinoma significantly altered in radiation cohort. CONCLUSION: In summary, this study demonstrates that SRS alters the molecular and genomic profile of NSCLC BM. It results in downregulation of oncogenes and pathways related to lung cancer. Additionally, by sampling the tumor at the center and periphery, there are differential effects of the dose gradient on the cellular and molecular response to ionizing radiation.

45. DELAY OR FAILURE TO ADMINISTER STEREOTACTIC RADIOSURGERY TO THE CAVITY AFTER SURGERY FOR BRAIN METASTASES. AN INTENTION-TO-TREAT ANALYSIS

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BACKGROUND: Data regarding the efficacy of adjuvant stereotactic radiosurgery (SRS) for resected brain metastases (BM) is often limited to patients completing SRS within a specified timeframe. We performed an intention-to-treat analysis to determine local recurrence (LR) for all BM patients referred for SRS. METHODS: We retrospectively identified resected BM patients referred for SRS between 2012 and 2018. Patients were divided based on delay to SRS into four categories: 1) ≤4 weeks, 2) >4–8 weeks, 3) >8 weeks, and 4) never received. We investigated the relationship between