enrichment of CD4+(including the regulatory subtype), CD8 and CD56-T and depletion of natural killer cells. Differences across serum- and tissue-derived clusters were present but less prominent than their plasma counterparts. No consistent increase in immune cell proportions or other clonotypic features within each tumor type (sex, age, histotypes, invasion) was ever observed. CONCLUSION: Our results suggest that PitNETs are characterized by differential TME and systemic immune subtypes which also distinguish these tumors. One mechanism by which PitNETs differ from their non-PitNET counterparts is the extent of their expression of immune checkpoints. No differences were observed in plasma, suggest that the systemic response to the presence of the tumor is distinct from the immune response noted in the TME. Tumor immune subtyping may allow the stratification of SIT according to immunotherapy response vulnerabilities.

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BACKGROUND: Novel immunotherapies based on targeting of specific immune checkpoints have proven to be effective treatments for a variety of immunogenic tumors, but melanoma brain metastases (BM) remain an unmet oncological need with an overall 2-year survival rate lower than 10%. Tumour immune microenvironment has been demonstrated to play a key role in BM establishment and development, but data regarding the specific milieu of melanoma BM is limited. MATERIAL AND METHODS: Gene expression profiles of 55 samples of primary melanoma and BM were evaluated using the nCounter PanCancer IO 360 Panel (NanoString Technologies) targeting 770 mRNA involved in tumor immune microenvironment modulation. TUMOR primary melanomas and their 10 matched BM, 25 unmatched BM, and 10 locally advanced control melanomas without evidence of BM after >5 year follow up. RESULTS: Among BM samples, most patients (25/45) were males and median age at BM diagnosis was 61.2 years with a median time to BM development of 2.1 years. Median OS from BM diagnosis was 1.3 years. Several genes resulted significantly downregulated in BM compared to primary melanomas, including SERPINB3 (p<0.001), ARGL (p<0.0007), S100A8 (p<0.001), S100A9 (p<0.001), S100A12 (p=0.0037), IL1RN (p=0.0012), CCL21 (p=0.0012), CCL2 (p=0.0012) and CCL13 (p=0.0017), respectively. Conversely, C7 was upregulated (p<0.001). Downregulated signatures in BM involved those associated with multiple immune cell populations, including neutrophils, dendritic cells, mast cells and Treg, as well as inflammatory chemokines, the CTLA4 associated with multiple immune cell populations, including neutrophils, dendritic cells, mast cells and Treg, as well as inflammatory chemokines, the CTLA4 and PD-1 pathways. Analysis according to tumour mutational burden (TMB) identified those BM with a significantly greater TMB. RESULTS: Among BM samples, most patients (25/45) were males and median age at BM diagnosis was 61.2 years with a median time to BM development of 2.1 years. Median OS from BM diagnosis was 1.3 years. Several genes resulted significantly downregulated in BM compared to primary melanomas, including SERPINB3 (p<0.001), ARGL (p<0.0007), S100A8 (p<0.001), S100A9 (p<0.001), S100A12 (p=0.0037), IL1RN (p=0.0012), CCL21 (p=0.0012), CCL2 (p=0.0012) and CCL13 (p=0.0017), respectively. Conversely, C7 was upregulated (p<0.001). Downregulated signatures in BM involved those associated with multiple immune cell populations, including neutrophils, dendritic cells, mast cells and Treg, as well as inflammatory chemokines, the CTLA4 associated with multiple immune cell populations, including neutrophils, dendritic cells, mast cells and Treg, as well as inflammatory chemokines, the CTLA4 and PD-1 pathways. Analysis according to tumour mutational burden (TMB) identified those BM with a significantly greater TMB.

OS08.4.A. ANALYSIS OF MELANOMA BRAIN METASTASIS IMMUNE MICROENVIRONMENT THROUGH MULTIPLEX GENETIC PROFILING

BACKGROUND: Novel immunotherapies based on targeting of specific immune checkpoints have proven to be effective treatments for a variety of immunogenic tumors, but melanoma brain metastases (BM) remain an unmet oncological need with an overall 2-year survival rate lower than 10%. Tumour immune microenvironment has been demonstrated to play a key role in BM establishment and development, but data regarding the specific milieu of melanoma BM is limited. MATERIAL AND METHODS: Gene expression profiles of 55 samples of primary melanoma and BM were evaluated using the nCounter PanCancer IO 360 Panel (NanoString Technologies) targeting 770 mRNA involved in tumor immune microenvironment modulation. TUMOR primary melanomas and their 10 matched BM, 25 unmatched BM, and 10 locally advanced control melanomas without evidence of BM after >5 year follow up. RESULTS: Among BM samples, most patients (25/45) were males and median age at BM diagnosis was 61.2 years with a median time to BM development of 2.1 years. Median OS from BM diagnosis was 1.3 years. Several genes resulted significantly downregulated in BM compared to primary melanomas, including SERPINB3 (p<0.001), ARGL (p<0.0007), S100A8 (p<0.001), S100A9 (p<0.001), S100A12 (p=0.0037), IL1RN (p=0.0012), CCL21 (p=0.0012), CCL2 (p=0.0012) and CCL13 (p=0.0017), respectively. Conversely, C7 was upregulated (p<0.001). Downregulated signatures in BM involved those associated with multiple immune cell populations, including neutrophils, dendritic cells, mast cells and Treg, as well as inflammatory chemokines, the CTLA4 and PD-1 pathways. Analysis according to tumour mutational burden (TMB) identified those BM with a significantly greater TMB.

OS08.4.A.1. ADENOVIRUS-MEDIATED DELIVERY OF THE MHC-II TRANSACTIVATOR CIITA MUTANT TO GROWTH IN IMMUNOCOMPETENT GIOBLASTOMA ORGANOIDS

BACKGROUND: Although immunotherapies represent an encouraging approach against cancer, to date none translated to the clinical benefit in glioblastoma. Our approach to overcome immune suppression is to increase anti-tumor immune responses via adenovirus (Ad)-mediated delivery of the MHC-II Transactivator (CIITA) gene. CIITA-induced MHC-II expression is anticipated to convert GBM cells into surrogate antigen presenting cells able to prime T helper cells, therefore promoting CD4+ and CD8+ mediated immunity. MATERIAL AND METHODS: We generated AdVs containing wild type CIITA (Ad-CIITA) using a replication-defective serotype5 adenoviral backbone. AdVs containing a mutated, non-functional version of CIITA (Ad-CIITA mutant) and an empty CMV promoter (Ad-null) were used as controls. AdV-mediated MHC-II expression was monitored at mRNA, protein and cell surface level. For the functional assessment of anti-tumor immune responses, we developed an advanced human GBM organoid model consisting of GBM organoids cultured in either human peripheral blood mononuclear cells (PBMCs) or isolated CD3+ T cells. T cell mediated tumor cell killing was monitored over time via live cell imaging and flow cytometry. RESULTS: We successfully constructed and produced a CIITA-armed AdV that induces MHC-II expression in infected GBM cells, indicating the efficient expression of transgenic active CIITA for at least 72 hours post infection. In immunocompetent human GBM organoids, Ad-CIITA infection of tumor cells led to prominent organoid disruption and tumor cell death, an effect that was not observed in the absence of PBMCs or CD3+ T cells. Tumor organoids infected with non-Ad-CIITA AdVs were unaffected.

BACKGROUND: Globlastoma (GBM) is the most common type of adult malignant brain tumor, with a median survival of only 21 months. This is linked to the highest rate of resistance to conventional therapy, including radiation (RT) and chemotherapy (CHT). DNA-PK, a DNA repair protein known to be transcriptionally activated by retinoblastoma protein (RB), was similarly found to be downregulated in ARF4-knockdown conditions. We then performed an unbiased proteomics screen to identify which genes were up-regulated and which were down-regulated in ARF4-knockdown conditions. We further investigated via live-cell imaging of transferrin receptors, a promoting factor in glioblastoma. The unknown genes driving the development of this resistance, we performed a genome-wide CRISPR knockdown screen comparing a DMSO-treated population over 14 days. Results showed an overall 200 novel genes, including a previously unstudied gene ARF4—inolved in retrograde trafficking to the nucleus. Here, we set out to characterize the mechanism by which ARF4 may be acting to promote chemoresistance.

BACKGROUND: von Willebrand factor (vWF) and platelet factor 4 (PF4) are involved in the process of tumor cell dissemination and metastasis formation. Syndecan-1 (SDC1) is a transmembrane glycoprotein that functions as a coreceptor for vWF and PF4. New therapeutic strategies targeting tumor cell dissemination and metastasis formation need to be developed. Here, we investigated the potential role of SDC1 in the process of tumor cell dissemination and metastasis formation in glioblastoma. METHODS: To evaluate the role of SDC1 in glioblastoma cell dissemination and metastasis formation, we used 2D and 3D cell culture systems, in vitro and in vivo xenograft models, and human tissue samples. SDC1 expression was downregulated in glioblastoma cells as compared to normal brain tissue. Furthermore, glioblastoma cells with higher SDC1 expression showed increased tumor cell dissemination and metastasis formation in vitro and in vivo. Additionally, glioblastoma cells with higher SDC1 expression showed reduced chemoresistance and improved survival in xenograft models. In human tissue samples, glioblastoma cells with higher SDC1 expression showed increased tumor cell dissemination and metastasis formation. Furthermore, patients with higher SDC1 expression had shorter overall survival time than those with lower SDC1 expression. CONCLUSION: These findings suggest that SDC1 may play a crucial role in the process of glioblastoma cell dissemination and metastasis formation. Further investigation is needed to determine the potential therapeutic target for glioblastoma.
OS098.A. INTEGRIN-SPECIFIC CAR T CELLS FOR THE TREATMENT OF GLIOBLASTOMA
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BACKGROUND: Current standard of care for glioblastoma patients has limited therapeutic efficacy and novel innovative treatment strategies are urgently needed. One such strategy is chimeric antigen receptor (CAR) T cell therapy, which has shown great success in hematological malignancies. In glioblastoma, integrins are overexpressed in several neoplasms and have already been used as therapeutic targets for small molecule inhibitors and antibodies, which did not cause major toxicities. Herein, we propose αvβ₅ integrin as an ideal target for CAR T cell therapy in glioblastoma. MATERIAL AND METHODS: CARs were generated by transducing primary human T cells from healthy donors with a lentiviral vector expressing a second-generation CAR. Activity and specificity of CAR T cells were determined by co-culture assays with different glioma cell lines. Efficacy of CAR T cells to control tumor growth in vivo was investigated in clinically relevant orthotopic xenograft glioma mouse models. Additionally, we generated CAR T cells from T cells from a glioblastoma patient and measured their activity against the patient's autologous tumor cells.

RESULTS: A newly generated integrin-targeting CAR T cells exerted strong anti-tumor activity in vitro. Long-term and repetitive killing assays as well as cytokine-release measurements demonstrated highest activity of αvβ₃ and αvβ₈ integrin-specific CAR T cells. Antigen specificity of these cells was confirmed, as glioma cells with a CRISPR/Cas9-mediated knockout of the target antigen were resistant to CAR T cell-mediated cytotoxicity. Intratumoral injection of αvβ₅ or αvβ₈ CAR T cells significantly prolonged the survival and cured a substantial fraction of glioma-bearing mice in two different xenograft models. When used in a patient-derived model, CAR T cells exerted strong anti-glioma activity. Intratumoral injection of CRISPR/Cas9-mediated knockout of the target antigen were resistant to CAR T cells.

CONCLUSION: The treatment effect was abrogated in different genetic immunodeficient mouse models. The treatment combination of L19TNF and lomustine was well tolerated in the first patients treated within a phase I/ II clinical trial and we observed partial tumor responses also in patients with an unmethylated MGMT promoter. CONCLUSION: The combination of L19TNF and lomustine demonstrated promising anti-glioma activity and patients in the second cluster also had deficits in information processing speed, and the last cluster showed patients with impairments across all domains.

OS099 SURVIVORSHIP AND LATE EFFECTS

OS099.A. COGNITIVE FUNCTIONING OF PATIENTS WITH DIFFUSE GLIOMA SURVIVING A STABLE CASE M. Gortert,1, J. G. Röthemüller,2, V. Belgers1,2, M. R. van Lingen1, P. C. De Witt Hamer2, L. Douw1,2, M. Klein1,3; Amsterdam UMC location Vrije Universiteit Amsterdam, Anatomy and Neurosciences, De Boelelaan 1117, Amsterdam, Netherlands, Amsterdam UMC location Vrije Universiteit Amsterdam, Medical Psychology, De Boelelaan 1117, Amsterdam, Netherlands, Amsterdam UMC location Vrije Universiteit Amsterdam, Neurology, De Boelelaan 1117, Amsterdam, Netherlands, 1Amsterdam UMC location Vrije Universiteit Amsterdam, Neurology, De Boelelaan 1117, Amsterdam, Netherlands.

BACKGROUND: Glioma patients often experience cognitive problems, which are associated with decreased functional independence and health-related quality of life. However, the prevalence and nature of cognitive impairment in these patients is relatively underreported, particularly during stable disease. In this study we determine the prevalence of cognitive deficits and explore the cognitive profiles of patients with diffuse glioma at least two months after tumor treatment. MATERIAL AND METHODS: A total of 112 patients (mean age: 43 years) without clinical or radiological progression with a grade II-IV glioma were included in this observational cohort study. Cognitive functioning was assessed using neuropsychological tests (attention, information processing speed, verbal memory, working memory, and executive functioning). Cognitive impairment was defined as a z-score of 1.5 SD below that of healthy controls. Hierarchical cluster analysis was used to examine cognitive profiles. RESULTS: In total, 46 patients (41%) had cognitive impairment. More than one domain was impaired in 25 of these patients (54%). The domains were affected in decreasing order of frequency: working memory in 31%, information processing speed in 22%, attention in 19%, verbal memory in 12%, and executive functioning in 11%. Osborne was found to be associated with cognitive clusters with an increasing number of cognitive domains impaired. The first cluster displayed patients with only working memory deficits, patients in the second cluster also had deficits in information processing speed, and the last cluster showed patients with impairments across all domains.

CONCLUSION: Cognitive impairment is highly prevalent in patients with diffuse glioma during stable disease. Working memory and information processing speed are most frequently affected. Importantly, our analyses show evidence for three subgroups in cognitively impaired glioma patients. Working memory seems an important driver in cognitive impairment given the involvement in all subgroups.


BACKGROUND: It is not clear whether Mood Disorders (MD) and poor Health Related Quality of Life (HRQoL) in glioma patients correlate with clinical and histomolecular features of the tumor or with secondary symptoms associated with treatment. This study assessed the prevalence of MD and decline in HRQoL (with a specific interest in sexuality) in glioma patients, identifying the clinical, imaging and treatment factors associated with these variables. MATERIAL AND METHODS: 260 glioma patients (190 lower grade) were evaluated. Patients were prospectively followed-up from admission to 12 months after