jected in orthotopic GBM bearing mice. Tumor size was measured once a week through in vivo bioimaging system. RESULTS: 0.022 MOI, the IC50 of T601, showed high cytotoxicity of T601. Moreover, the significantly decreased cell viability under the combined treatment of 5-FC and 0.22MOI T601 showed intact anti-tumoral function. In MTD assay, except for 107 group, no significant weight loss was found. However, in 107 pfu group, mean body weight decreased around 10% and animal fatality happened on day 9. According to the MTD result, certain amount of virus was intratumorally injected. In all treatment group, the tumor size was significantly shrined. At the same time, the survival rate of mice under viral treatment was significantly extended. CONCLUSION: In summary, T601 exhibited efficient anti-tumoral function and acceptable side effect. T601 treatment prolonged the survival period of GBM mice with acceptable neurotoxicity, demonstrating that T601 contains necessary criterial for intra-tumoral injection. Ultimately, this project provided basic reference information of dose for future clinical trial.

P13.16 METASTATIC POTENTIAL OF SYSTEMIC GLIOBLASTOMA STEM CELL LINES *IN VIVO*

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BACKGROUND: Despite aggressive tumor behavior, extracranial metastases rarely develop in glioblastoma (GBM) patients. Two potential explanations have been suggested: 1) The blood-brain-barrier functions as a physical barrier that prevents the dissemination of GBM cells out of the central nervous system (CNS) or 2) that extracranial metastasis do occur, but the patients die before extracranial metastases manifest themselves. The first theory has been questioned based on the fact that circulating tumor cells (CTC) were found in blood samples of GBM patients without systemic metastases. To date it has not been proven if CTCs are able to reenter the brain and to what extent they are able to form systemic extracranial metastatic lesions. Therefore, the current study aimed at analyzing the dissemination patterns and the underlying mechanisms associated with the ability of GBM CTCs to form extracranial metastases. MATERIAL AND METHODS: Five highly characterized human GBM stem cell (GSC) lines (P3, BG5, BG7, GG6, GG16), displaying GBM CNV patterns, were intracranially implanted in a first cohort, then transduced with a lentiviral Firefly Luciferase-eGFP vector and injected into the left cardiac ventricle of NOD/SCID mice in a second cohort. Mice were observed closely and tumor burden was assessed using *in vivo* as well as *ex vivo* bioluminescence imaging, MRI and PET. Mice were euthanized when the objective endpoint criteria (tumor burden) was met, then organs were harvested and fixed for further analysis. RESULTS: First, a detailed characterization of the GSC line invasion patterns were assessed when grown as orthotopic xenografts *in vivo* dividing them into three categories: 1) Highly invasive without apparent angiogenesis (BG5) 2) Invasive with perivascular infiltration and angiogenesis (P3, BG7 and GG16) and 3) Angiogenic and highly circumscribed (GG6). Following intracardial injection, (7 out of 8) P3 animals developed extracranial and intracranial tumors with a distinctive pattern. Brain, adrenal gland, ovary and liver were amongst the organs most susceptible for tumor growth in the P3 group. For the BG5 and BG7 cell lines, no metastases were observed whereas only 1 animal out of 10 developed metastases in both groups GG16 and GG6. CONCLUSION: Only one out of 5 GSC lines exhibited a strong metastatic potential when injected into the left cardiac ventricle. Compared to other tumors which exhibit a strong metastatic potential from the circulation, GSC lines do only to a very limited extent show this potential reflecting observations made in the clinic.

P13.17 *CD95* GENE SILENCING AFFECTS GROWTH AND INVASIVENESS OF GLIOMA-INITIATING CELLS IN A CD95L-INDEPENDENT MANNER

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BACKGROUND: CD95 (Fas/APO-1) holds a dual role of potential relevance in tumor development. CD95-CD95 ligand (CD95L) signaling regulates apoptotic cell death in CD95-expressing cells, but non-apoptotic, tumor-promoting CD95-CD95L signaling has been likewise described. Therapeutic stimulation of apoptotic CD95 signaling is associated with major clinical side effects. However, inhibition of tumor-promoting CD95 signaling may represent a promising treatment strategy for human cancers where potential tumor-promoting CD95 functions include invasiveness and cancer cell stemness, including glioblastoma. MATERIAL AND METHODS: In this study, CD95 and CD95L expression was characterized in human glioma-initiating cells (GIC) *in vitro* and *in vivo*. *CD95* and *CD95L* gene knockout (KO) GIC were generated by means of CRISPR-Cas9 and the effects of gene silencing were evaluated by assessing growth, clonogenicity, invasiveness and tumorigenicity in nude mice. RESULTS: CD95 expression and sensitivity to exogenous CD95L-induced apoptosis were confirmed in selected GIC *in vitro*. CD95L expression was not detected. Upon *CD95* KO, all GIC acquired resistance to CD95L-induced apoptosis. Furthermore, despite the confirmed absence of CD95L expression *in vitro*, *CD95* KO S-24 GIC revealed decreased cell growth, inferior sphere forming capacity and decreased invasiveness. These data suggested a CD95L-independent tumor-promoting role of CD95 in S-24 GIC. *In vivo*, however, *CD95* KO did not prolong the survival of glioma-bearing mice. Analyses of further GIC models are ongoing. CONCLUSION: These data demonstrate that, unlike CD95, CD95L is not expressed in cultured human GIC and that CD95-CD95L interactions are not required for tumor-promoting CD95 signaling. Although *CD95* KO is detrimental for S-24 GIC *in vitro*, *CD95* KO alone does not affect survival in S-24 human GIC xenograft-bearing mice.

P13.18 INHIBITION OF EPIDERMAL GROWTH FACTOR RECEPTOR AND PLATELET-DERIVED GROWTH FACTOR RECEPTOR-ALPHA EXERTS SYNERGISTIC EFFICACY IN GLIOBLASTOMA

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BACKGROUND: Despite our understanding of the genetic changes that precipitate gliomagenesis, targeted therapy has failed in glioblastoma (GBM) with median survival not significantly improved over the past two decades. Epidermal growth factor receptor (*EGFR*) alterations, including amplification and activating mutations, are among the most common genetic changes in GBM, occurring in more than half of cases. EGFR is located on Chr. 7, and Chr. 7 gain is one of the earliest events precipitating gliomagenesis. Various EGFR inhibitors, including tyrosine kinase inhibitors, monoclonal antibodies, vaccines, and CAR-T cells have failed in GBM due to intrinsic heterogeneity and receptor tyrosine kinase bypass pathways that mediate therapeutic resistance. New targeted therapeutic approaches to leverage synergistic combinations are desperately needed to improve GBM prognosis. Using the TCGA and other GBM databases, we have previously demonstrated that the presence of *PDGFRA*amplification in patients with *EGFR*-amplified GBM carries significantly worse survival. *EGFR* and *PDGFRA* co-expression occur in more than one-third of GBM patients. The PDGFRA ligand PDGFA is also located on Chr. 7, and its expression is significantly increased with Chr. 7 gain and *EGFR* copy number increase. Therefore, Chr. 7 gain inherently leads to co-activation of both EGFR and PDGFRA signaling pathways. MATERIALS AND METHODS: We used models of patient-derived glioblastoma cells to test combined inhibition of epidermal growth factor receptor and platelet-derived growth factor receptor-alpha *in vitro*. RESULTS: Using patient-derived GBM models with Chr. 7 gain, we found that combined inhibition of both EGFR and PDGFRA using a variety of FDA-approved EGFR-targeted agents (Erlotinib, Gefitinib, Dacomitinib, Neratinib, and Osimertinib) and Crenolanib, respectively, leads to synergistic cytotoxicity *in vitro*. We found that inhibition of either EGFR or PDGFRA alone led to receptor cross-activation, and EGF and PDGF-AA-induced receptor tyrosine kinase activation was blocked by Neratinib and Crenolanib. Immunoprecipitation experiments and proximity ligation assays demonstrated that combined inhibition prevents EGFR and PDGFRA heterodimerization and pathways of therapeutic resistance. This combined inhibition led to decreased activation of downstream signaling pathways, including phosphatidylinositol 3-kinase and mitogen-activated protein kinase. CONCLUSIONS: We show that combined inhibition of EGFR and PDGFRA exerts synergistic cytotoxicity in GBM and prevents resistance pathways that emerge during single-agent targeted therapy against these receptor tyrosine kinases. These pathways are targetable with FDA-approved agents that could be used in patients with GBM with Chr. 7 gain.

P13.19 BI-MODULAR G-QUADRUPLEX DNA-CRYPTO-APTAMERS DIMINISH VIABILITY OF GLIOMA PRIMARY CELL CULTURES OF PATIENTS

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BACKGROUND: G-quadruplex oligonucleotides (GQs) exhibit specific anti-survival activity in human cancer cell lines; they can selectively inhibit the viability/proliferation. The most studied, AS1411, had been in clinical trials. This anti-proliferative ability of GQs could be translated into glioma, which currently has poor prognosis and low-efficiency therapeutic treatments for