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BACKGROUND: Precision medicine trials targeting the epidermal growth factor receptor (EGFR) in glioblastoma patients require selection for *EGFR*-amplified tumors. However, there is currently no golden standard in determining the amplification status of *EGFR* or *EGFRvIII* expression. Here, we aimed to determine which technique and which cut-offs are suitable to determine EGFR amplification status. **MATERIAL AND METHODS:** We compared fluorescent in-situ hybridization (FISH) and RT-qPCR data from patients screened for trial inclusion into the Intellance 2 clinical trial, with data from a panel-based next generation sequencing (NGS) platform (both DNA and RNA). **RESULTS:** By using data from >1000 samples, we show which cut-offs are optimal to determine *EGFR* gene amplification by FISH. Our data also show that gene amplification (as determined by FISH) correlates with *EGFR* expression levels (as determined by RT-qPCR) with ROC analysis showing an under the curve area of up to 0.902. *EGFR* expression as assessed by RT-qPCR therefore may function as a surrogate marker for *EGFR* amplification. Our NGS data shows that EGFR copy numbers can strongly vary between tumors with levels ranging from 2 to more than 100 copies per cell. Levels exceeding 5 gene copies can be used to define EGFR-amplification by NGS; below this level FISH detects very few (if any) *EGFR* amplified nuclei and none of the samples express *EGFRvIII*. **CONCLUSION:** Our data from central laboratories and diagnostic sequencing facilities, using material from patients eligible for clinical trial inclusion, help defining the optimal cut-off for various techniques to determine *EGFR* amplification for diagnostic purposes.

P11.09 *PAN*-RTK INHIBITION OF SLRIG1 MEDIATES AXL DOWNREGULATION IN GLIOBLASTOMA

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INTRODUCTION: Aberrant regulation of receptor tyrosine kinase (RTK) activity is characteristic of Glioblastoma (GBM). However, RTK-based targeted therapies have been largely unsuccessful in GBM patients, partially due to the complexity and redundance of RTK signaling. LRIG1 (Leucine-rich Repeats and ImmunoGlobulindomains protein 1) is known as an endogenous inhibitor of epidermal growth factor receptor (EGFR) during health and disease, however its mechanism of action is poorly understood. We previously showed that the soluble form of LRIG1 potently inhibits of GBM growth *in vivo*, irrespective of EGFR expression level and status, suggesting the involvement of other RTKs. Here, we aimed to shed light on the molecular mechanisms underlying its anti-cancer activity. **MATERIAL AND METHODS:** We generated a recombinant human soluble LRIG1 protein by expressing LRIG1 ectodomain in insect cells via baculovirus infection and subsequent His-tag purification. rh-sLRIG1 was applied in the medium of classical GBM cell lines, patient-derived GBM stem-like cells and patient-derived 3D tumor organoids. Using different cell-based assays, cell proliferation, invasion, cell morphology, as well as protein expression and protein-protein interactions were investigated. **RESULTS:** We find that recombinant sLRIG1 efficiently reduces proliferation, invasion and viability of GBM cells and patient-derived organoids, and modulates cytoskeleton proteins and cell shape. In line with previous data, the effect of recombinant sLRIG1 is independent of EGFR expression. Interestingly sLRIG1 regulates several RTKs by direct protein downregulation, including AXL, while EGFR expression is not affected. At the molecular level, we find that sLRIG1 interferes with AXL dimerization, while no protein interaction with EGFR is detected. **CONCLUSION:** We identify AXL as a novel LRIG1 target and provide evidence that sLRIG1-mediated RTK downregulation requires direct protein-protein interaction. These data pave the way for a potential therapeutic application of recombinant sLRIG1 in the inhibition of growth factor signaling in GBM.

P11.10 THE IFNΓ PATHWAY MEDIATES BRAIN METASTASIS FORMATION OF BREAST CANCER

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BACKGROUND: In previous work we showed the prominence of the T-cell response in the formation of brain metastases of primary ER negative breast cancers (Mustafa et al, Acta Neuropathol 2018). We also showed that breast cancer cells co-cultured with stimulated T lymphocytes overexpress Guanylate-binding protein 1 (GBP1) accompanying increased trespassing ability through an in vitro blood-brain barrier (BBB) model. In addition, we demonstrated a predilection for metastasizing to brain of breast cancer cells that were co-cultured with activated T cells in a mouse model. We now scrutinize the importance of the IFNγ pathway for tresspassing of the tumor cells through the BBB following T cell contact. **MATERIAL AND METHODS:** Anti-hIFN-γ-IgA antibodies were used to neutralize the IFNγ effects on the tumor cells. The effects on the tumor cells is only due to native IFNγ produced by activated T cells, not by recombinant IFNγ. Since the IFNγ expression itself enhances its expression by the T cells, we blocked IFNγ receptors prior to adding CD3+ T cell conditioned media to the breast cancer cells. The receptor blocking was achieved by antibodies to the IFNγα and IFNγβ subunits. Activation of the STAT1 pathway was monitored by GBP1 expression. For functional read-out the in vitro BBB model was used. **RESULTS:** The presence of T-lymphocyte-secreted IFNγ in the primary breast cancer microenvironment activates the STAT1-dependent IFNγ pathway in breast cancer cells, endowing them with an increased ability to trespass the in vitro BBB. Moreover, direct inhibition of soluble IFNγ, or blocking of the IFNγ-specific receptor in breast cancer cells significantly decreases their ability to cross the BBB. **CONCLUSION:** The results illustrate the specific action of T lymphocytes in the formation of cerebral metastasis involves the IFNγ signaling pathway as one of the crucial entangled pathways Subsequent studies should aim at the interference with the IFNγ pathway to develop preventive strategies against the formation of cerebral metastases of breast cancer.

P11.11 CIRCULATING PRO-ANGIOGENIC CELLS AND PROTEINS IN PATIENTS WITH GLIOMA AND ACUTE MYOCARDIAL INFARCTION: DIFFERENCES IN NEOVASCULARIZATION BETWEEN NEOPLASIA AND TISSUE REGENERATION

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BACKGROUND: Although extensive angiogenesis takes place in glial tumors, anti-angiogenic therapies have remained without the expected success. In the peripheral circulation of glioma patients increased numbers of endothelial precursor cells (EPCs) are present, potentially offering targets for anti-angiogenic therapy (Zheng et al., Ann Neurol, 2007). However, for an anti-angiogenic therapy to be successful, the therapy should specifically target glioma-related EPC subsets and secreted factors. Here we compared the EPC subsets and plasma factors in the peripheral circulation of patients with gliomas to acute myocardial infarctions (representing fysiologic regeneration). **MATERIAL AND METHODS:** We investigated the five most important EPC subsets and 21 angiogenesis-related plasma factors in peripheral blood samples of 29 patients with glioma, 14 patients with myocardial infarction and 20 healthy people as controls, by an advanced FACS protocol (Huizer et al., PlosOne 2018) and Luminex assay. **RESULTS:** In GBM patients all EPC subsets were elevated as compared to healthy subjects. In addition, HPC and KDR^+ cell fractions were higher than in MI, while $CD133^+$ and KDR^+CD133^+ cell fractions were lower. There were differences in relative EPC fractions between the groups: KDR^+ cells were the largest fraction in GBM while $CD133^+$ cells were the largest fraction in MI. An increase in glioma malignancy grade coincided with an increase in the KDR^+ fraction while the $CD133^+$ cell fraction decreased relatively. Most plasma angiogenic factors were higher in GBM than MI patients. In both MI and GBM, the ratio of $CD133^+$ HPCs correlated significantly with elevated levels of MMP9. In the GBM patients MMP9 correlated strongly with levels of all HPCs. **CONCLUSION:** In conclusion, the data demonstrate that EPC traffic in patients with glioma is different from that in normal tissue regeneration. Therefore, the effects of glioma extent beyond the brain, and future therapies aimed at lowering KDR^+ cells and HPCs may add to effective treatment.

P11.12 PERIOSTIN IS EXPRESSED BY PERICYTES AND IS CRUCIAL FOR ANGIOGENESIS IN GLIOMA

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BACKGROUND: The expression of the matricellular protein periostin has recently been associated with glioma progression and angiogenesis. The aim of the present study was to identify the cellular source of periostin ex-