P06.06.B. STANDARDIZATION OF THERAPY AND MANUFACTURING USING TUMOR-ASSOCIATED ANTIGEN-STIMULATED AUTOLOGOUS DENDRITIC CELLS CO-CULTURED WITH CYTOKINE-INDUCED KILLER CELLS IN CANCER IMMUNOTHERAPY

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BACKGROUND: The application of DC-CIK in the field of cancer immunotherapy has been shown to be an effective treatment. However, the cost of DC-CIK treatment is prohibitive for many patients, and the lack of standard manufacturing processes and treatment strategies are the main limitations. MATERIAL AND METHODS: Our experiments used tumor lysate instead of tumor cell line as tumor-associated antigen source with DCs co-culture. We provide the most efficient method for obtaining autologous DC-CIK cells from peripheral blood. Flow cytometry was used to evaluate DCs activation, CBA assay was used to quantify cytokines secreted by CIK cells, and the antitumor activity of DC-CIK was evaluated in vitro by K562 cell line. RESULTS: We demonstrate that the manufacturing process of employing frozen Peripheral Blood Mononuclear Cells (PBMCs) can balance patient's comfort and economic benefits. DC-CIK can effectively upgrade the immunological specificity of CIK cells to tumors in the presence of tumor-associated antigen. In vitro experiments showed that when the number of DC: CIK cells was co-cultured in 1:20 ratio on the 14th day, the amount of cytokine secreted by CIK cells was the largest, and the antitumor immune effect was the most potent. When the number of CIK: K562 cells was in 25:1 ratio, the cytotoxic activity of CIK on K562 cells was the highest. CONCLUSION: We developed an efficient activated fashion of DC:CIK, established the optimal ratio of DC-CIK immunologic activity and the best cytotoxic model of CIK to K562 cells.

P06.07.A. NATURAL KILLER CELLS LYSE GLIOBLASTOMA STEM CELLS AND INCREASE THEIR SENSITIVITY TO CHEMOTHERAPY

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BACKGROUND: Glioblastoma is the most common and lethal brain tumor in the adult population and immunotherapy is playing an increasingly central role in the treatment of many cancers. Nevertheless, the search for effective immunotherapeutic approaches for glioblastoma patients continues. In this study, we aimed to explore the therapeutic potential of allogeneic highly activated super-charged natural killer (NK) cells in glioblastoma. MATERIAL AND METHODS: Chromium release- and calcein release-based cytotoxicity assays, ELISA, ELISPOT, and multiplex cytokine assays were used to determine NK cell cytotoxicity against glioblastoma stem cells (GSCs) and secretion of cytokines. Cell surface marker expression using flow cytometry and cell growth *in vitro* and *in vivo* were measured to determine GSC phenotype. NK cell killing and penetration in 3D were measured using confocal microscopy of GSC tumorospheres. RESULTS: Super-charged NK cells efficiently lysed patient-derived GSCs in 2D and 3D models potentially reversing the immunosuppression observed in patients. NK-cells secreted IFN-γ, upregulated GSC surface expression of CD54 and MHC class I and increased sensitivity of GSCs to chemotherapeutic drugs. Co-localization of NK cells with GBM cells in perivascular niches in glioblastoma tissues and their direct contact with GSCs in tumorospheres suggests their ability to infiltrate glioblastoma tumors and target GSCs. CONCLUSION: Allogeneic super-charged NK cells appear to be a potential therapeutic approach for glioblastoma by selectively killing therapy-resistant cancer stem cell population, increasing their immune-related surface markers and enhancing their sensitivity to chemotherapy. Due to GSC heterogeneity and plasticity personalized immunotherapeutic strategies should be developed to effectively target glioblastomas.

P06.08.B. RADIATION THERAPY ENHANCES ANTI-TUMOR ACTIVITY OF A MET CAR T-BASED IMMUNOTHERAPY FOR GLIOBLASTOMA

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BACKGROUND: Glioblastoma is the most frequent primary brain tumor with dismal prognosis after standard treatment with surgery, and chemoradiation (the Stupp protocol). After a decade of failed clinical trials, tumor-treating fields have been first to show the added benefit of improved overall survival compared to the Stupp protocol (20.9 months vs 16.0 months). However, GBM remains a devastating disease, with almost inevitable recurrence, and limited options for second-line therapy. Radiation therapy (RT), is a standard therapy option for GBM, and it is used in most GBM cases affecting tumor through induction of DNA-damage. Recently, RT has been investigated as a mediator of T cell-based therapies in the context of immunosuppressive GBM microenvironment. The findings have shown promise in combination of T cell-based therapies, such as chimeric antigen receptor (CAR) T cell therapies, in improving the tumor infiltration, and penetration with immune cells. MET is a relevant oncogene in the context of GBM, being involved in stem-like properties, radiation response and resistance. Hence, MET appeared to be a plausible target for combination with RT. In our research, we use MET-targeting CAR T cells (MET-CAR T cells) combined with radiation, and hypothesize synergistic interaction for GBM treatment. MATERIAL AND METHODS: We used adherent (2D) and stem-like (3D) human GBM cell lines with different levels of MET expression. For MET-CAR T cell generation we did retrovirus-mediated transduction of activated human T cells and sorted the CAR-positive cells. We co-cultured MET-CAR T cells with GBM cells with or without RT, and assessed the killing and cytokine production in CAR T cells. RESULTS: Our results indicated that 5Gy radiation combined with MET-CAR T cells increases their potential in tumor cell killing. We observed increased CAR T cells effect at lower CAR T to target cells ratios when combined with radiation, even when radiation treatment did not lead to a significant decrease in viability. This phenomenon was similar across different types of cell lines (adherent, stem-like), different levels of MET expression, and different sensitivity to CAR T cells. We investigated the underlying mechanisms via intracellular cytokine measurement. We observed the most prominent response in TNF-α-expression. We also observed an increase in Granzyme B expression in co-culture with some of the GBM cell lines, especially in CD8+ subpopulation of CAR T cells. IFN-gamma expression increased in some adherent glioma cell lines but not in stem-like cell lines. CONCLUSION: In conclusion, our data demonstrates the potency of MET-CAR T cells against GBM, and increased efficiency when combined with radiation. The suggested mechanism is the increased activation of T cells in TNF-α-dependent-manner. To validate these results we are testing our setup in an orthotopic mouse GBM model.

P07 ADVANCED NEUROSURGICAL TECHNIQUES

P07.01.A. TOPICAL FLUORESCENT PROBE FOR VISUALIZATION OF GLIOBLASTOMA

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BACKGROUND: Glioblastoma (GBM) is the most aggressive form of cancer that originates within the central nervous system (CNS). GBM represents 15% of all primary brain tumors, but the cause is unclear, and there is no clear way to prevent it. At the moment, various sophisticated and specific surgical procedures are being used, but there are relatively few simple methods. MATERIAL AND METHODS: In this work, we used a turn-on type fluorescent probe that can sense cysteine (Cys) amino acid in the GBM site, and developed topical treatment methods for image-guided surgery (IGS). RESULTS: The probe can distinguish the GBM cells and disease sites in clinical samples from individual patients. Besides, the probe has no short or long-term toxicity and immune response. CONCLUSION: The present findings hold promise for the application of the probe to a relatively simple and straightforward following of GBM at clinical sites.

P07.02.B. NEURO-ONCOLOGICAL AUGMENTED REALITY PLANNING (NOA-P)

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BACKGROUND: When preparing for the resection of an intracranial lesion, neuronavigation with a tracked pointer is most often used to determine lesion borders and the optimal approach. This can sometimes prove challenging, especially for deep-seated lesions. Augmented Reality (AR), directly displaying the lesion on the patient's skin, can simplify and improve this step. MATERIAL AND METHODS: We developed a system for inside-out infrared tracking that does not require an external tracking camera or external computer and allows for heads-up displaying an AR scene on the Microsoft HoloLens II. Twenty patients planned for the resection of an

intracerebral lesion were included in our study. After patient registration, the lesion outlines were marked on the patient's skin by different participants, consecutively using the Brainlab neuronavigation system and the HoloLens. Each registration on both systems provided a registration transform that was compared for accuracy and consistency. The performance of the participants was measured in terms of duration and accuracy and compared to expert registration and delineation. RESULTS: Both registration and delineation were significantly faster with AR (p=0.02 and p<0.001, respectively, and p<0.001 for the total duration), taking 79.23±17.48 and 39.58±39.10 seconds while neuronavigation required 96.61±24.54 and 90.80±44.09 seconds. AR had a registration offset of 3.3mm and 3.4°, and was more consistent compared to neuronavigation. AR facilitated more accurate and detailed lesion delineation, while neuronavigation often overestimated lesion size. CONCLUSION: Augmented reality provides a faster and more accurate alternative for resection planning. Lesion delineation is more intuitive while remaining high in accuracy. Future research should focus on further intraoperative implementations.

P07.03.A. ADVANCES IN ROBOTIC NAVIGATED LASER CRANIOTOMY. AN IN-VIVO NON-RECOVERY ANIMAL STUDY

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BACKGROUND: We previously described a new frameless stereotactic intervention using robotic guided laser beam for depth electrode placement. This study tested the feasibility of a new cutting strategy for angulated precision bone channels as well as improved cut-through detection using optical coherence tomography (OCT) and a new generation of co-axial live video feed. MATERIAL AND METHODS: Preoperative CT scans were performed to plan trajectories for bone channels angulated 45, 60, and 90° relative to the surface. The animals were prepared under general anesthesia by a trained veterinarian conforming European requirements and Good Laboratory Practice regulations. A new cutting strategy was implemented consisting of two circular paths and three different ablation phases. After cut-through detection bolts and depth electrodes were inserted. Before termination ad-hoc planned laser craniotomies were performed to evaluate possible cortex damage. RESULTS: 70 robotic guided laser beam precision bone channels were cut in four pig specimens. Bolts and depth electrodes were implanted solely guided by the trajectory given by the laser precision channels. The new cutting strategy showed no irregularities for either cylindrical (n=38, 45°=10, 60°14, 90°=14) or anti-conical (n=33, 45°=11, 60°=13, 90°=9) bone channels. Angulation and hole diameter showed no significant difference between cylindrical and anti-conical cutting strategies. The updated co-axial camera live video feed in addition to OCT reliably detected cut-through in 80% of cases. Insertion of bolts with firm fit was achieved in 94% of bone channels. All four anesthesia protocols showed no irregularities. No unintended damage to the cortex was detected after laser guided craniotomy. CONCLUSION: The new cutting strategy showed promising results in 70 precision bone channels for angulated cylindrical and anti-conical channels in a large in-vivo non-recovery animal study. OCT signal and a new co-axial camera proved its feasibility for cut-through detection. Robotic guided laser beam techniques proved its feasibility for the placement of depth electrodes and might be a suitable option to optimize the burr hole for biopsies.

P07.04.B. HEME BIOSYNTHESIS FACTORS AND 5-ALA INDUCED FLUORESCENCE: ANALYSIS OF MRNA AND PROTEIN EXPRESSION IN FLUORESCING AND NON-FLUORESCING GLIOMAS

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BACKGROUND: The intraoperative visualization of adult-type diffuse gliomas with 5-aminolevulinic acid (5-ALA) induced fluorescence is widely used in the neurosurgical field. While visible 5-ALA induced fluorescence is found in the majority of high-grade gliomas, most low-grade gliomas lack visible fluorescence during surgery. Recently, the heme biosynthesis pathway was identified as crucial influencing factor for presence of visible fluorescence since it metabolizes 5-ALA to fluorescing Protoporphyrin IX (PpIX). However, the exact alterations within the heme biosynthesis pathway resulting in visible 5-ALA induced fluorescence in gliomas are still unclear. The aim of the present study was thus to compare the mRNA and protein expression of promising intramitochondrial heme biosynthesis enzymes/transporters in glioma tissue samples of different fluorescence behavior. MATERIAL AND METHODS: A total of 19 strongly fluorescing and 21 non-fluorescing tissue samples from neurosurgical adult-type diffuse gliomas (WHO grades II-IV) were included in the current analysis. In these samples, we investigated the mRNA expression by quantitative real time PCR and protein expression using immunohistochemistry of the intramitochondrial heme biosynthesis enzymes Coproporphyrinogen Oxidase (CPOX), Protoporphyrinogen Oxidase (PPOX), Ferrochelatase (FECH) and the transporter ATP-binding Cassette Subfamily B Member 2 (ABCG2). RESULTS: Regarding mRNA expression analysis, we found a significantly decreased ABCG2 expression in fluorescing specimens compared to non-fluorescing samples ($p=0.001$), whereas no difference in CPOX, PPOX and FECH was present. With respect to protein expression, significantly higher levels of CPOX ($p=0.005$), PPOX ($p<0.01$) and FECH ($p=0.003$) were detected in fluorescing samples. Similar to mRNA expression analysis, the protein expression of ABCG2 ($p=0.001$) was significantly lower in fluorescing samples. CONCLUSION: Distinct alterations of the analyzed heme biosynthesis factors were found primarily on protein level. Our data indicate that heme biosynthesis pathway activity in general is enhanced in fluorescing gliomas with upregulation of PpIX generating enzymes and decreased ABCG2 mediated PpIX efflux outweighing the also increased further metabolization of PpIX to heme. Intramitochondrial heme biosynthesis factors thus constitute promising pharmacological targets to optimize intraoperative 5-ALA fluorescence visualization of usually non-fluorescing tumors such as low-grade gliomas.

P07.05.A. IMPLANTABLE ELECTROPHORETIC DEVICES FOR LOCAL TREATMENT OF INOPERABLE BRAIN TUMOURS

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Glioblastoma (GBM) is the most malignant primary brain tumour in adults, with a median overall survival of fewer than 18 months after initial diagnosis. For over five decades, research has been focused on developing new anticancer therapies for GBM, including anti-neoplastic agents, molecular targeted drugs, immunotherapeutic approaches, and angiogenesis inhibiting compounds; however, the prognosis of patients has hardly improved and temozolomide remains the only chemotherapy shown to improve patient survival in randomized clinical trials. A fundamental limitation of the success of chemotherapy in brain cancer therapies is the blood-brain barrier which significantly reduces the concentration of chemotherapeutic agents delivered into a tumour.

MATERIAL AND METHODS: Therapeutic strategies that control drug release spatially and temporally represent a significant step forward in terms of reducing side effects and improving treatment efficacy and will thus have a significant clinical impact. Electrophoretic drug delivery devices, which use electric fields to enhance drug transport, represent one such strategy.

Results

Here, we present an implantable device that enables highly spatially selective delivery of charged drug molecules directly into brain tumours. Our device combines a microfluidic system for drug transport with embedded electrodes which enable electrophoretic transport of drug molecules into the target tissue. This allows delivery of chemotherapeutic agents without transport of bulk solvent preventing issues arising from intracranial pressure gradients. We have shown that the device can be implanted safely without any limitation. We have tested the device's capabilities to deliver a wide range of small, medium, and large chemotherapeutic agents without limitations. Currently, we are investigating the delivery of cisplatin in GBM-bearing mice.

Conclusion While electrophoretic drug delivery was first described in the early 20th century and has been used since primarily for transdermal drug delivery, we believe that our approach is one of the first times this has been demonstrated for brain cancer therapy.

P07.06.B. THE IMPACT OF USING INTRAOPERATIVE ULTRASOUND ON SURGICAL RESECTION OF HIGH-GRADE GLIOMA: A SYSTEMATIC REVIEW AND META-ANALYSIS.

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BACKGROUND: Despite operative and adjuvant therapies, high-grade glioma (HGG) remains incurable, with the extent of surgical resection being one of the modalities that can improve patient survival. Enabling maximal safe and minimising post-operative neurological morbidity is a key aim of surgical resection. Numerous intraoperative surgical adjuncts are used at surgery and intraoperative ultrasound (IoUS), is one such adjunct. IoUS is a cost-effective, easy to use, repeatable surgical adjunct, safe for the patient and potentially available in all centres. Although it's commonly used, no up to date systematic review exists collating and quantifying the level of evidence, delineating its impact on the extent of surgical resection. MATERIAL AND METHODS: A systematic review was conducted according to the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines. The study was registered with the PROSPERO data-