domain and 4-1BB costimulatory domain in the advanced CAR gene to exhibit excellent in vivo persistence and anti-tumor effect of advanced CAR-T cells. In addition to the advanced CAR gene, TGF-β-converter is introduced, which converts the inhibitory proline-rich globular domain of TGF-β into an autoactivating cytokine overexpressed in the hostile tumor microenvironment, into the activation signal of the cytokine IL-18 in advanced CAR-T cells. DAY9 NSG mice bearing orthotopically xenografted GBM cell lines (1 x 10⁴ U251MG expressing an ex vivo target antigen, whereas recombined with exogenous cytokine release, T cell proliferation and target cell apoptosis. We evaluated the performance of PDMP-CAR-T cells in GBM patient derived organoids (PDO). MATERIAL AND METHODS: PDOs were generated from freshly resected GBM tissue and could be cultured successfully up to several months. We assessed PDMP expression of PDOs via immunohistochemistry (IHC) prior to treatment. PDMP-CAR T cells were generated from peripheral blood mononuclear cells of healthy donors via lentiviral transduction and expansion. The transduction rate was assessed by flow cytometry prior to in vivo injection. PDOs doped with transduced T cells served as controls. PDOs were incubated with a preset number of PDMP-CAR T cells at a CD4⁺/CD8⁺ ratio of 1:4 and were examined microscopically to register morphological disintegration after 48h. Immunofluorescence staining was conducted to detect proliferating CD4⁺/CD8⁺ T cells (CD4⁺/Ki67⁺) after 72h and cytokine release of IFN-γ was determined via ELISA after 20h. RESULTS: In total, PDOs from three patients were treated with PDMP-CART cells. All of them expressed the antigen according to IHC staining. All incubated PDOs showed clearly diminished proliferation to total dissolving, whereas control PDOs stayed intact. CD4⁺ CART cells showed extensive proliferation at a mean rate of 79.8% ± 6.59%, which was significantly for two PDOs in comparison to control PDOs (p = 0.48, p = 0.01). All PDOs exhibited cytokine release in all PDOs (p < 0.01). CONCLUSION: We here describe PDMP as a promising target and proved effectiveness of PDMP-CAR-T cells in an ex vivo 3D model. Additional ex vivo models like tumor slice cultures might be crucial to establish this cell therapy approach and its effect in vivo. In future, PDOs might be tested in these ex vivo as well as in vivo GBM models alone and in a combined approach with CARs targeting additional antigens in order to overcome tumor heterogeneity.

P06.03.A. COMBINATION OF EGFRVIII CAR T CELL THERAPY AND PARACRINE GAM MODULATION FOR THE TREATMENT OF GBM T. A. Martus¹, N. Tatari¹, M. Ritz², T. Shekaran³, P. Schnassmann⁴, E. Kaymak⁵, G. Hagemann², M. Nerreter³, N. Hagemann², M. N предоставлено для определения влияния эффектов обеих 6 в vitro и в предклинических моделях. Однако, туморная гетерогенность, антигенные уклонения и сложные взаимодействия с окружающей микроокружением (TME) тормозят их клиническую реализацию. Таким образом, разработаны ex vivo моделей, моделирующих межклеточные интеракции и иммунообильные трансплантации, которые могут помочь в исследовании новых терапевтических подходов. В этом исследовании было проведено сравнение эффективности тетрафункционального CAR-T поколения (Advanced CAR-T) с ex vivo CAR-T поколения (Ex vivo CAR-T) в модели DAY9 ортотопического GBM модели.

P06.05.A. REPEATED INTRACRANIAL ADMINISTRATION OF PILIMUMAB AND NIVOLUMAB IN PATIENTS WITH RECURREN T Glioblastoma (RGB): A MULTI-COHORT ADAPTIVE PHASE II CLINICAL TRIAL


BACKGROUND: Perioperative intracranial (iC) administration of pilumunab (iP) and nivolumab (iNO) in combination with IV NIVO was deemed feasible and safe without dose limiting AEs. A potential survival benefit (Duerinck et al. JITC 2021). In subsequent cohorts, combination of iC administration with biweekly intracavitary (iC) via an Ommaya reservoir administration of increasing doses of iN and iP was safe and feasible. Ex vivo analysis of CAR T cell receptor repertoire confirmed the necessity of a suitable target antigen. When activated, CAR-T cells initiate the triad of antigen-specific cytokine overexpression in the hostile tumor microenvironment, into the action signal of the cytokine IL-18 in advanced CAR-T cells. DAY9 NSG mice bearing orthotopically xenografted GBM cell lines (1 x 10⁴ U251MG expressing an ex vivo target antigen, whereas recombined with exogenous cytokine release, T cell proliferation and target cell apoptosis. We evaluated the performance of PDMP-CAR-T cells in GBM patient derived organoids (PDO). MATERIAL AND METHODS: PDOs were generated from freshly resected GBM tissue and could be cultured successfully up to several months. We assessed PDMP expression of PDOs via immunohistochemistry (IHC) prior to treatment. PDMP-CAR T cells were generated from peripheral blood mononuclear cells of healthy donors via lentiviral transduction and expansion. The transduction rate was assessed by flow cytometry prior to in vivo injection. PDOs doped with transduced T cells served as controls. PDOs were incubated with a preset number of PDMP-CAR T cells at a CD4⁺/CD8⁺ ratio of 1:4 and were examined microscopically to register morphological disintegration after 48h. Immunofluorescence staining was conducted to detect proliferating CD4⁺/CD8⁺ T cells (CD4⁺/Ki67⁺) after 72h and cytokine release of IFN-γ was determined via ELISA after 20h. RESULTS: In total, PDOs from three patients were treated with PDMP-CART cells. All of them expressed the antigen according to IHC staining. All incubated PDOs showed clearly diminished proliferation to total dissolving, whereas control PDOs stayed intact. CD4⁺ CART cells showed extensive proliferation at a mean rate of 79.8% ± 6.59%, which was significant for two PDOs in comparison to control PDOs (p = 0.48, p = 0.01). All PDOs exhibited cytokine release in all PDOs (p < 0.01). CONCLUSION: We here describe PDMP as a promising target and proved effectiveness of PDMP-CAR-T cells in an ex vivo 3D model. Additional ex vivo models like tumor slice cultures might be crucial to establish this cell therapy approach and its effect in vivo. In future, PDOs might be tested in these ex vivo as well as in vivo GBM models alone and in a combined approach with CARs targeting additional antigens in order to overcome tumor heterogeneity.

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