Background Chimeric Antigen Receptor (CAR)-T cell therapy is very effective in the treatment of B cell leukemia but still inefficient in solid cancer treatment. Immunosuppression in tumor microenvironment (TME), tumor heterogeneity and immune escape dampen the efficacy of CAR-T cells in these tumor types. To overcome these issues, here we propose a Toll-like Receptor (TLR) ligand. We assume that this mediator functions as a ‘danger signal’ that can activate immune cells in the TME and promotes the generation of an inflammatory milieu. Thus, we aim to combine direct CAR-dependent antitumor activity and TLR-mediated immunostimulation in one tool.

Materials and Methods MC38 murine cancer cells were engineered to express a truncated form of the human Epidermal Growth Factor Receptor (trEGFR) and used as a target. A second-generation CAR was synthesized (cetuximab scFv - CD8 hinge - 4.1BB - CD3z) and cloned in the MSCV retroviral vector. Murine 2nd generation CAR-T cells were engineered and killing cytokines secretion assessed by luminescence-based assay and ELISA upon co-culture with target cells. Different constructs were tested combining the TLR ligand to different export signals. Colorimetric assays and western blot analyses were performed to evaluate its activity and its production and secretion, respectively. A repetition of the Nuclear Factor of Activated T cells (NFAT) with a synthetic TATA box (synTATA)(1) was tested for the inducible production of the TLR ligand only upon CAR-T cells activation.

Results EGFR-targeted murine CAR-T cells recognized and killed target cells after 48 hours of co-culture. Meanwhile, TLR ligand constructs were cloned and expressed in HEK293T cells. Analysis of supernatants and cell lysates revealed high production and secretion of the glycosylated ligand when coupled with the IgK leader sequence, indicating Golgi transport. Whereas, when coupled with the cell penetrating peptides transportan or a repetition of eight arginines, the ligand was produced and released in the supernatant in its non-glycosylated form, bypassing Golgi. All the secreted ligands stimulated TLR-sensor cells, with different intensities and kinetics. TLR activation appeared to be dependent on ligand production and its glycosylation status as well. Finally, murine T cells transfected with the NFAT synTATA promoter expressed GFP upon CD3 activation, indicating inducible protein production.

Conclusions EGFR-targeted CAR-T cells activity, ligand-dependent TLR stimulation and NFAT synTATA inducible protein production represent valuable building blocks for the production of 4th generation CAR-T cells. Next steps contemplate the construction of a vector encoding for both CAR and inducible TLR ligand, and to test its functionality in terms of improved CAR activity and reshaping of immune cell landscape in solid tumors both in vitro and in an immunocompetent mouse tumor model.

REFERENCES


P09.07 DECDIPHERING THE NATURE OF THE COOPERATION BETWEEN MONOCYTES/MACROPHAGES AND CD8+ T CELLS DURING IMMUNOTHERAPY-INDUCED TUMOR REGRESSION

Background The success of immunotherapy is associated with a remodeling of the entire tumor microenvironment. In this context, a positive cooperation between CD8+ T cells and the activated tumor-infiltrating macrophages and monocytes appear necessary for an optimal tumor regression. However, as the mechanisms of such interactions are still unknown, we aimed to uncover the precise identity of the cooperating monocytes/macrophages and CD8+ T cells as well as the nature of their interplay.

Model In the transplanted PyMT mammary tumor model, we sorted monocytes/macrophages and CD8+ T cells from tumors regressing after STING agonist therapy to perform scRNAseq on these two populations.

Results We discovered that the monocyte/macrophage compartment is divided in three main macrophages subsets and two monocyte-like subsets and that the CD8+ T cells comprise a large diversity of phenotypes (effector, naïve, memory-like, regulatory cells). We have followed the dynamic changes of these populations across the stages of tumor regression and noticed that monocyte-like subsets are preferentially enriched upon regression. In parallel, the CD8+ memory subsets were increased during the regression at the expense of regulatory-like populations.

Perspectives We are currently examining the predicted functions of the different subsets as well as their spatial distribution in the tumor microenvironment, to uncover the mechanisms by which these cells cooperate in tumors treated by immunotherapy.