A TOLL-LIKE RECEPTOR AGONIST REPRESENTS A POTENTIAL ADJUVANT IN FOURTH GENERATION CAR-T CELL-BASED IMMUNOTHERAPY

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 new approach to CAR-T cells to enhance their activity.

Methods
MC38 murine cancer cells were engineered to express GFP upon CD3 activation, indicating inducible protein production. The ligand was produced and released in the supernatant in its Golgi transport or a repetition of eight arginines, and killing and cytokines secretion assessed by luminescence assay.

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 high production and secretion, respectively. A repetition of the Nuclear Factor of Activated T cells (NFAT) with a synthetic TATA box was tested for the inducible production of the ligand only upon CAR-T cells activation.

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

Disclosure Information
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DEPIRING 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 appears 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.

Disclosure Information

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