

8 MULTIPARAMETER CHARACTERIZATION OF CAR T CELLS

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Background Adoptive cell transfer of chimeric antigen receptor (CAR) modified T cells has demonstrated great therapeutic success against certain hematological malignancies. However, a substantial number of patients experienced relapse at some point after treatment with the underlying mechanisms not fully understood. Emerging data suggest that the undesired clinical outcome is related to different aspects, which include: the tumor heterogeneity, the tumor microenvironment, as well as intrinsic characteristics of the CAR T cells. In this work, we aimed to understand the diversity of CAR T cells generated from different donors, using multiparameter in vitro characterization.

Methods Leukapheresis from healthy donors were collected to generate CAR T cells using the GMP-compliant CliniMACS Prodigy[®] platform, enabling an automated and closed engineering of CAR T cells in a highly reproducible manner. We performed an in-depth characterization of the resulting CAR T cells by exploring differences in the immunophenotype, cell fitness and effector function of the freshly prepared as compared to frozen CAR T cell samples. Specifically, we designed several flow cytometry panels for the extensive characterization of immunophenotypes of interest such as: proliferative capacity, differentiation, activation and exhaustion. Cell fitness status was determined by the rate at which cells undergo apoptosis following stress. Finally, effector function was determined by the ability of the activated CAR T cells to secrete proinflammatory cytokines including IFN- γ , TNF- α and IL-2. The associations between these different parameters were analyzed using comprehensive statistical approaches.

Results With our established workflow, over 20 healthy-donor derived CAR T cells were generated and characterized. We have observed donor-dependent variations and responses for most of the explored parameters. In general, the freezing and thawing process negatively affected cell fitness and effector function of the CAR T cells and resulted in altered immunophenotypes. Additionally, correlations between certain immunophenotypes and cell fitness/effector function were identified.

Conclusions Collectively, we established a workflow for multiparameter characterization of CAR T cells and assessed the intrinsic variability of CAR T cells for both research and clinical application.

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