Design, characterization and preclinical validation of a combinatorial CAR-based immunotherapy against colorectal cancer with HER2 amplification

Marco CORTESE - University of Turin

Adoptive Cell Therapy (ACT) based on CAR-T cells has led to successful treatment of some hematological malignancies, but it remains extremely challenging for solid tumors, mostly because of “on-target off-tumor” toxicity, as observed in the case of anti-HER2 CAR-T cells treatment of CRC with HER2 amplification. To enable ACT against HER2amp CRC, was therefore considered a combinatorial strategy based on the synNotch-based artificial regulatory network. A synthetic Notch receptor was employed in which the extracellular domain is an anti-HER2 scFv and the intracellular domain contains the GAL4VP64 artificial transcription factor. Engagement of the anti-HER2 domain by target cells drives GAL4VP64 cleavage and translocation to the nucleus, where it drives expression of a CAR under a GAL4UAS. In this way, only cells co-expressing both HER2 and the CAR target are killed. As a CRC-specific CAR target CEA was selected. CEA expression is restricted to the digestive tract and is increased in cancer. As effector cells for the system, was selected the natural killer cell line NK-92. NK-92 cells transduced with the two lentiviral vectors encoding HER2-synNotch and inducible CEA-CAR were repeatedly sorted in the OFF and ON state to select those with the best CAR induction after synNotch engagement. Subsequently, cloning of sorted cells led to identification of an optimally responsive clone (5F). *In vitro*, the 5F clone displayed selective cytotoxicity against HER2amp/CEA+ CRC cells, with minimal killing activity against HER2amp/CEA- cells, or against HER2-/CEA+ cells. Additional assays on 3D organoids highlighted better recruitment and infiltration by clone 5F respect to NK-92 WT cells, only in HER2amp models. *In vivo*, the clone 5F significantly impaired tumor growth in two different HER2amp CRC models. To further improve survival, tumor penetration and *in vivo* efficacy of the NK-92-5F clone, a more complex system was built in which HER2-synNotch engagement drives not only expression of the CEA-CAR but also of IL-2. 5F-IL-2 cells displayed a further increase of cytotoxicity *in vitro*, also at a particularly low effector: target ratio (1:50). *In vivo*, 5F-IL-2 cells drastically increased survival of mice carrying HER2 amp CRC xenografts with respect to the parental 5F clone. The observed selective efficacy both *in vitro* and *in vivo* of the HER2-synNotch/CEA-CAR system, and its future evolutions, opens a perspective for possible clinical applications in cases of HER2amp CRC displaying primary or secondary resistance to HER2/EGFR blockade.