ADOPTIVE CELL THERAPY WITH CYTOKINE-INDUCED KILLER CELLS RETARGETED WITH IMMUNOTOOLS AGAINST HER-2 POSITIVE BREAST CANCER

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Background Cytokine-Induced Killer (CIK) cells are a heterogeneous population of CD3+CD56+ effector cells easy to expand from PBMCs in clinically relevant numbers, which are endowed with T and NK cells phenotypic and functional properties. They show an MHC-unrestricted cytotoxicity and exert Antibody-Dependent Cell-mediated Cytotoxicity (ADCC) when combined with monoclonal antibodies (mAbs). In the present study, CIK cells were combined either with Trastuzumab (TRS) or with the engineered TRS V90leC13, which bears two amino acid substitutions (S239D/I332E) and lacks Fc fucosilation or with a bispecific antibody (bsAb) directed against HER-2 and CD3 (HER2xCD3).

Methods CIK cells were obtained from PBMCs by the addition of IFNγ, OKT3 and IL-2. The effector cell cytotoxicity and the dose-dependent activity of HER2xCD3 and TRS V90leC13 were evaluated with a 4-hours Calcein-AM assay and with a 72-hours real-time cell analysis against HER-2-expressing breast cancer cell lines. The concentration of cytokines produced upon the co-culture of CIK cells with target cells was assessed with a multiplex assay. The biodistribution of the bsAb was evaluated in NSG mice upon the chemical conjugation of HER2xCD3 with a fluorophore.

Results The combination of CIK cells with HER2xCD3 or TRS V90leC13 resulted in a significant improvement of the antigen-specific cytotoxic activity against breast cancer cell lines when compared to the combination of CIK cells with clinical mAb TRS. In particular, the real time analysis showed that even at a very low effector/target (E/T) ratio, such as 0.1:1 E/T ratio, CIK cells combined with HER2xCD3 showed a remarkable cytotoxicity, completely restraining target cells growth. Interestingly, TRS-resistant tumor cell lines showed to be sensitive to HER2xCD3-directed CIK cell lytic activity. Moreover, bsAb resulted to be effective also at very low concentrations, and the cytokines released from CIK cells matched with a proinflammatory profile, with no significant concentration of cytokines correlated with Cytokines Release Syndrome (CRS), such as IL-6 and IL-5. The analysis of the in vivo biodistribution showed that the bsAb arrives efficiently at the tumor site where accumulates and reaches the maximum concentration 8 hours after i.v. injection.

Conclusions Taken together, these results highlight the potentiality of using recombinant immunotools to improve the antigen-specific cytotoxic activity of CIK cells against HER-2 positive tumor cells.

REFERENCES

Ethics Approval Patient samples were obtained after written informed consent from Padua Hospital, Italy (Ethical Committee act n. 3529/AO/14). All the procedures involving animals and their care were in conformity with institutional guidelines that comply with national and international laws and policies (D.L. 26/2014 and subsequent implementing circulars), and the experimental protocol (Authorization n. 118/2019-PR) was approved by the Italian Ministry of Health.