Adoptive cell therapy with Cytokine-Induced Killer cells retargeted with immunotoxins against HER-2 expressing breast cancer

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**BACKGROUND**

Cytokine-Induced Killer (CIK) cells are GDF-15+CD56- effector cells, easy to expand in clinically relevant numbers and endowed with T and NK cell phenotypes and functional properties. They express the FynSHI (CD56a) and are able to exert an Antibody-Dependent Cell-mediated Cytotoxicity (ADCC) if combined with monoclonal antibodies (mAbs).

**METHODS**

- **HER2xCD3 bsAb binds effectively on CIK and breast cancer cell lines**
  - HER2xCD3 bsAb binds to CIK cells with the 50% of CIK (a) and to cancer cells with the 50% of HER-2 in a dose-dependent manner (b). The bsAb binding is demonstrated also after 30 minutes of co-culture of CT26-labelled CIK cells with the bsAb HER2xCD3 together with Calcein Blue-labelled HCC1419 or SKBR3 cancer cells (c). The double positive population determined by flow cytometry analysis shows the percentage of this binding property.

- **CIK cells combined with HER2xCD3 bsAb rapidly kill target cells**
  - The real time cytotoxicity assay highlights that CIK cells combined with HER2xCD3 showed a rapidly remarkable antitumor activity compared to CIK cells with TRS (a) and even at a very low effector/target (E/T) ratio (b).

- **Retargeted CIK cells show a safe cytokines profile**
  - The cytokines released by HER2xCD3-retargeted CIK cells upon 20 hours co-culture with MCF-7 HER-2 positive cell line at a ratio of 10:1 is bsAb dose dependent and matched with a mononuclear profile. The profile seems not to be correlated with the Cytokines Release Syndrome (CRS), due to a low concentration of 4-6 and 8-5 released, supposing a safe profile of the treatment. The graphs indicated the mean±SD of the cytokines concentration with the comparison with the cytokines released by CIK+TRS (n=3), **P ≤ 0.001**, \*P ≤ 0.01, **P ≤ 0.05**, \*P ≤ 0.05.

- **HER2xCD3 bsAb reaches the tumor site**
  - The bsAb HER2xCD3 injected i.v. in a MCF-7 tumor bearing mice arrive efficiently at the tumor site, where reaches the maximum concentration after 8 hours post injection.

**CONCLUSIONS**

These results highlight the potentiality of using clinical grade bsAbs or recombinant immunotoxins to improve the cytotoxic activity of CIK cells against HER-2 positive tumor cells. This leads us to envisage new perspectives for adoptive immunotherapy to treat solid tumors where antigen-specific retargeting of immune cells can be achieved by the combination of non-antigen-specific effector cells with tumor-specific monoclonal antibodies and recombinant molecules.

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Authors declare conflicts of interest to declare.