52P - The inducible Medigene T cell receptor (IM-TCR) controls cytotoxicity of tumor-specific TCR-modified T cells with improved avidity through control of TCR surface expression

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Abstract

Background: Adoptive cell therapy (ACT) is used successfully as a highly personalized cancer treatment. It encompasses the ex vivo expansion of patient tumor infiltrating lymphocytes (TIL therapy), as well as the genetic modification of patient lymphocytes with chimeric antigen receptors (CAR-T therapy) or tumor antigen-specific T cell receptors (TCR-T therapy). The high efficacy of TCR-T cells comes with the risk of rare severe side effects due to off-target recognition.

Methods: Inducible Medigene TCRs (IM-TCRs) allow full control of TCR surface expression without impairing cytotoxicity against tumor cells. Mutations introduced into the C-terminus of the TCR β chain prevent receptor pairing by the TCR α chain and thereby restraining the interactions with CD3 subunits. The mutated α and β chains are modified to carry a truncated estrogen receptor domain at the C terminus allowing homodimerization.

Results: The addition of the dimerization agents 4OH-Tamoxifen or Endoxifen forces fast pairing of the modified α and β chains followed by rapid expression of the TCR on the cell surface. Clinical range doses of Endoxifen sufficiently induce enough TCR membrane expression to drive specific recognition of pMHC ligands and T cell responses comparable to the non-modified wild type TCR. Removal of dimerizer results in rapid downregulation of TCR surface expression and prompt incapacity of the T cell to recognize antigen and exert responses.

Conclusion: Engineering tumor antigen-specific TCRs as IM-TCRs enables tight control of TCR surface expression that provide a safety net to deviate off-target activity of the TCR away from the tumor microenvironment and opens a new avenue for early safety assessment of TCRs or application in challenging clinical settings where overexpansion of T cells could be detrimental.

Figure 1. Surface expression of IM-TCR is only detected after treatment with dimerizer. Without dimerizer, the expression of the IM-TCR on the cell surface is absent (left panel). Only after adding the dimerizing agent, the IM-TCR is expressed on the cell surface and can be detected (right panel).

Figure 2. IM-TCR expression is already induced at low concentrations of dimerizer and stable for at least 48h in Jurkat-76 cells. A single dose of 0.5µM dimerizer is already sufficient to reach a half maximal IM-TCR expression within less than 4h. Furthermore, maximal expression is detected at least 24h after treatment. The expression is stable for at least 48h and is comparable to the expression level of the wt TCR.

Figure 3. IM-TCR is rapidly down-regulated and functionally silenced after removal of dimerizer using a Jurkat-76 NFAT-GFP reporter cell line. Functionality of the IM-TCR expressed in a Jurkat-76 NFAT-GFP reporter cell line can be demonstrated in a co-culture containing LCL targets presenting relevant peptide. Hence, TCR activity is indicated by NFAT-GFP expression in presence of the dimerizer. Already one hour after removing the dimerizer, IM-TCR functionality is no detectable anymore, when pre-treated with 0.5µM dimerizer. Treatment with 1µM dimerizer results in loss of functionality within less than 4h.

Figure 4. Only IM-TCR-transduced CD8+ T cells treated with dimerizer can control growth of A375 melanoma cells in a co-culture. In absence of dimerizer, IM-TCR-transduced T cells are not able to control the growth of NFAT-GFP expressing A375 melanoma cells in a co-culture experiment (light red line). The growth curve of these A375 targets is comparable to those co-cultured with mock-transduced T cells (grey and black lines). Upon treatment with dimerizer, IM-TCR-transduced T cells show highly functional and capable to control the growth of A375 target cells within a few hours of co-culture (dark red line), comparable to the control-transduced T cells (purple line).

Summary

- The IM-TCR technology makes use of specific mutations within the constant regions of the TCR to prevent natural pairing of α and β chains
- A dimerizing domain added to the C-terminus of the TCR α and β chain enables controlled expression of the TCR upon treatment with dimerizer
- Surface expression of IM-TCR takes place shortly after induction and is rapidly down regulated after removal of the dimerizer
- IM-TCR functionality of CD8+ T cells is preserved as shown by elimination of A375 melanoma target cells
- These results show that the IM-TCR technology is a useful tool to improve the safety at TCR-T cell immunotherapies