The inducible caspase-9 (iC9) suicide gene is a promising safety switch for cell therapies. The safety switch system consists of a bicistronic vector encoding a mutated FKBPL2 binding protein linked to caspase-9 and truncated CD19 (ΔCD19) to allow selection of gene-modified T cells (SFG iC9-ΔCD19). Exposure to rimiducid (AP19903, Rim) dimerizes iC9 resulting in apoptosis of gene-modified T cells.

**Background**

The ability to selectively eliminate activated cells on an as-needed basis is an important tool in adoptive cell therapy. Inducible caspase-9 (iC9) suicide gene is an attractive safety switch candidate to trigger apoptosis in T cells ex vivo while sparing unstimulated T cells in vivo. This strategy might be applied to both autologous and allogeneic cell therapies, although the latter is more challenging due to the risk of graft-vs-host disease (GvHD). We have previously established an inducible suicide system in which the expression of iC9 is induced by a small molecule, rimiducid (Rim), which rapidly activates the caspase pathway.

**Methods**

To evaluate the effect of iC9 on transgene expression levels, we established a colony consisting of 3 equal populations based on the intensity of CD19 staining (SFG iC9-ΔCD19, iC9-ΔCD19* and iC9-ΔCD19Δ). Phenotypic and functional assays (i.e., apoptosis) were performed by flow cytometry, qPCR and Western blot before and after rimiducid reactivation using FKBPL2 and C28 antibodies. In vivo studies were performed by i.v. injection of control or rimiducid co-expressing luciferase into NGS mice, followed by i.p. injection of a titrated dose of rimiducid (0.001 to 1 mg/kg), control drug (luminal), or vehicle. Bioluminescent imaging and flow cytometry were subsequently performed to assess in vivo depletion following IC9 activation.

**Results**

Figure 1. Detection of transgene expression by using CD19 surface marker in rivo-cell and sorted populations. Human T lymphocytes were transduced with iC9-ΔCD19 retrovirus, and positively selected for CD19 and analyzed for CD3/CD19 expression on day 14 after activation. (A) Percentage of CD19+ cells in CD3+ cells in BXP-501 and sorted populations. The mean fluorescence intensity (MFI) of CD19 in different populations (B). Percentage of CD4+ (C) and CD8+ (D) T cells in CD3+ population in non-transduced cells, rivo-cell and sorted populations.

Figure 2. Composition of T cell subsets. Phenotype of T cell subsets according to memory markers in non-transduced cells, rivo-cell and sorted high, medium and low populations in: CD3+ (A), CD4+ (B) and CD8+ (C) T cells.

Figure 3. Apoptosis is correlated with the intensity of the iC9-ΔCD19 transgene expression. Cells were incubated for 4 hours in the presence of Rim and apoptosis was determined as the fraction of cells that were positive for Annexin V and/or 7-AAD. (A) Percentage of apoptotic cells with the MFI of CD19 (C). Caspase-mediated apoptosis, which is regulated by the activation state of CD28 reactivation (A). Fold change of MFI of CD19 in cells with or without Rim treatment (B), correlation of percentage of apoptotic cells with the MFI of CD19 (C). Caspase-9 protein level in non-transduced T cells, and sorted high, medium and low CD19-expressing populations was measured by western blot (D), and computation of killing efficiency according to the MFI of CD19 (E).

Figure 4. Rim-mediated elimination of rivo-cell in vivo. 1x10^6 EGPFR+ rivo-cell expressing rivo-cells were infused into NGS mice. Bioluminescent imaging using iC9 was done at different time points (A), and change in average radiance of each animal was performed using Living Image software. Values for average radiance were normalized to the initial radiance in each mouse (B). Residual rivo-cells from splenocytes were measured at 48 hours after drug administration by flow cytometry (C). Data represents mean ± SEM (n=5).

**Conclusions**

- In vivo depletion of rivo-cell is dependent on the level of iC9-ΔCD19 transgene expression, which is regulated by the activation state of the T cells.
- Highly activated rivo-cell express higher levels of iC9, which makes them more sensitive to rimiducid-induced apoptosis, and serves to selectively deplete GvHD-causing T cells while sparing T cells with other phenotypes.

**References**