Differential Expression of Inducible Caspase-9 (iC9) in Allogeneic T Cells Allows Selective Depletion of Activated T Cells Following Exposure to Rimiducid and Permits *in vivo* Allodepletion

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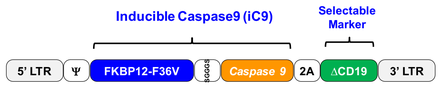
Abstract #3496

Background

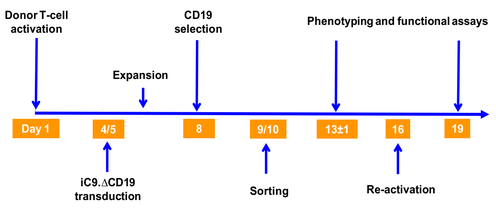
The inducible caspase-9 (iC9) suicide gene is a promising safety switch for cell therapies. The safety switch system consists of a bicistronic vector encoding a mutated FKBP12 binding protein linked to caspase-9 and truncated CD19 (ΔCD19) to allow selection of gene-modified T cells (SFG-iC9-ΔCD19). Exposure to rimiducid (AP1903, Rim) dimerizes iC9 resulting in apoptosis of gene-modified T cells.



Rivogenlecleucel or rivo-cel (formerly BPX-501) is a donor-derived allogeneic cell product consisting of T cells modified to express the inducible caspase-9 (iC9) safety switch, which can improve immune reconstitution and anti-viral, anti-tumor immunity following stem cell transplant. In instances of graft-versus-host disease (GvHD), activation of iC9 with rimiducid leads to rapid killing of alloreactive T cells and resolution of GvHD. However, gene-modified T cells re-expand in the host. Here we evaluate the relationship between transgene expression and sensitivity to rimiducid to understand differential apoptosis in patients treated with rivo-cel.

Methods

To evaluate the effect of transgene expression levels to the sensitivity of rimiducid-induced apoptosis, rivo-cel was sorted into 3 equal populations based on the intensity of CD19 staining (iC9-ΔCD19^{high}, iC9-ΔCD19^{med} and iC9-ΔCD19^{low}). Phenotyping and functional assays (i.e., apoptosis) were performed by flow cytometry, qPCR and Western blot before and after rivo-cel reactivation using anti-CD3/anti-CD28 antibodies. *In vivo* studies were performed by i.v. injection of control or rivo-cel co-expressing luciferase into NSG mice, followed by i.p. injection of a titrated dose of rimiducid (0.001 to 1 mg/kg), control drug (temsirolimus, Tem; 1 mg/kg) or vehicle. Bioluminescent imaging and flow cytometry were subsequently performed to assess *in vivo* depletion following iC9 activation.



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Results

Characteristics of rivo-cel

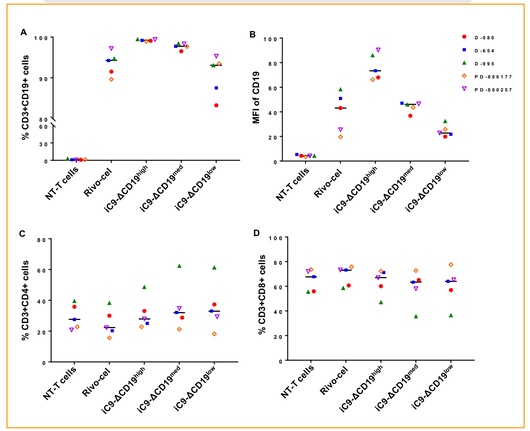

Figure 1. Detection of transgene expression by using CD19 surface marker in rivo-cel and sorted populations. Human T lymphocytes were transduced with iC9-ΔCD19 retrovirus, and positively selected for CD19 and analyzed for CD3$^+$CD19$^+$ expression on day 14 after activation. (A) Percentage of CD19$^+$ cells in CD3$^+$ cells in BPX-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-cel and sorted populations.

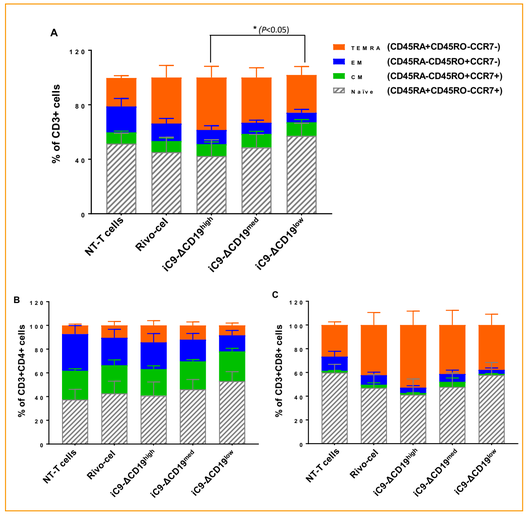

Figure 2. Composition of T cell subsets. Phenotype of T cell subsets according to memory markers in non-transduced cells, rivo-cel and sorted high, medium and low populations in: CD3$^+$ (A), CD4$^+$ (B) and CD8$^+$ (C) T cells.

Killing is correlated with the intensity of the iC9-ΔCD19 transgene expression

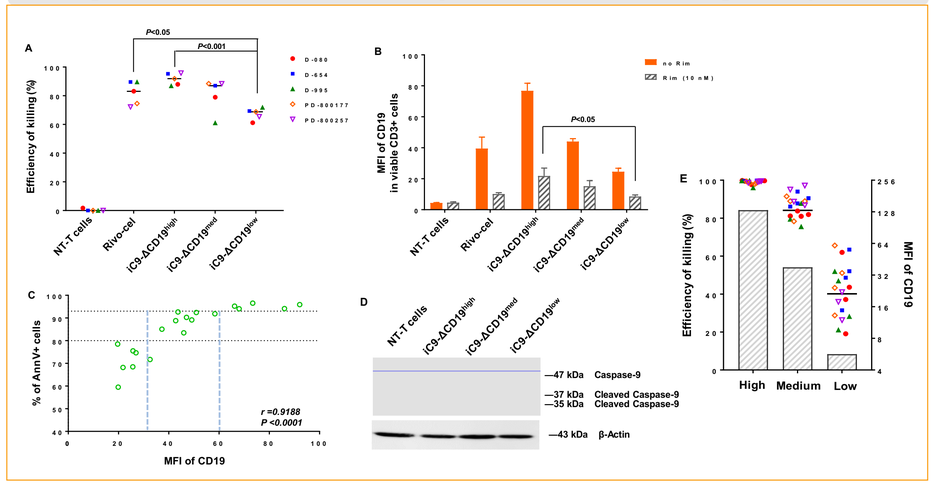

Figure 3. Apoptosis is correlated with iC9-ΔCD19 transgene expression. Cells were incubated for 4 hours in the presence of Rim and apoptosis was determined as the fraction of cells that were positive for Annexin V and/or 7-AAD. Killing efficiency in different populations (A), MFI of CD19 in cells with 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).

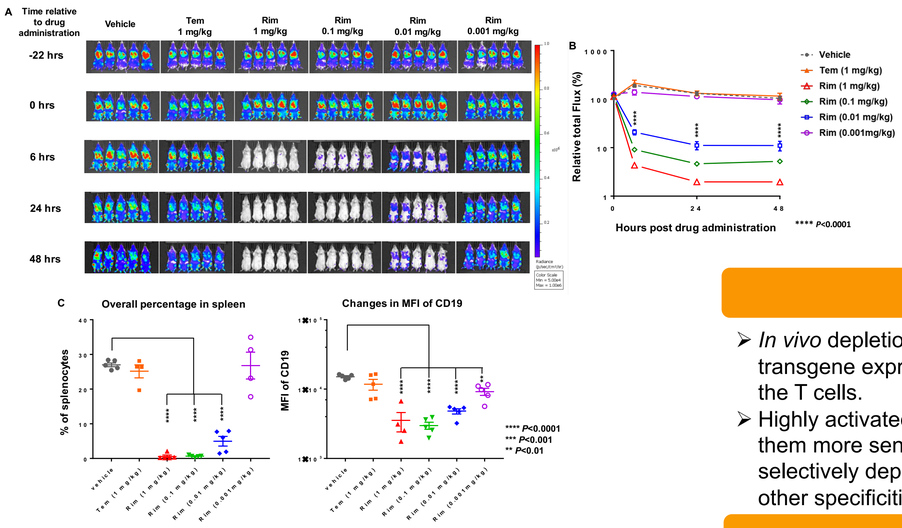

Figure 4. Rim-mediated elimination of rivo-cel *in vivo*. 1x10^7 EGFPluc$^+$-expressing rivo-cels were infused into NSG mice. Bioluminescence imaging using IVIS was done at different time points (A), and change in average radiance of each animal was performed using Living Image software. Values for average radiance were normalized to the initial radiance in each mouse (B). Residual rivo-cels from splenocytes were measured at 48 hours after drug administration by flow cytometry (C). Data represents mean ± SEM (n=5).

Killing can be enhanced when cells are activated

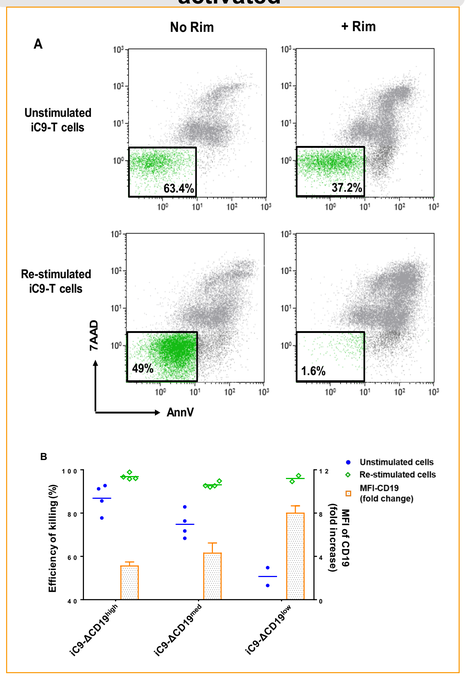

Figure 5. *In vitro* drug sensitivity is significantly increased after *ex vivo* activation. Representative example of dot plot of low iC9-ΔCD19 expressing cells. The percentage indicates the live cells in the population evaluated by 7AAD$^-$/AnnV$^-$ with or without anti-CD3/anti-CD28 reactivation (A). Fold change of MFI-CD19 is significantly increased after *ex vivo* activation, particularly in low iC9-ΔCD19 expressing cells (B).

Conclusions

- *In vivo* depletion of rivo-cel is dependent on the level of iC9-ΔCD19 transgene expression, which is regulated by the activation state of the T cells.
- Highly activated rivo-cel 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 specificities.

References

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