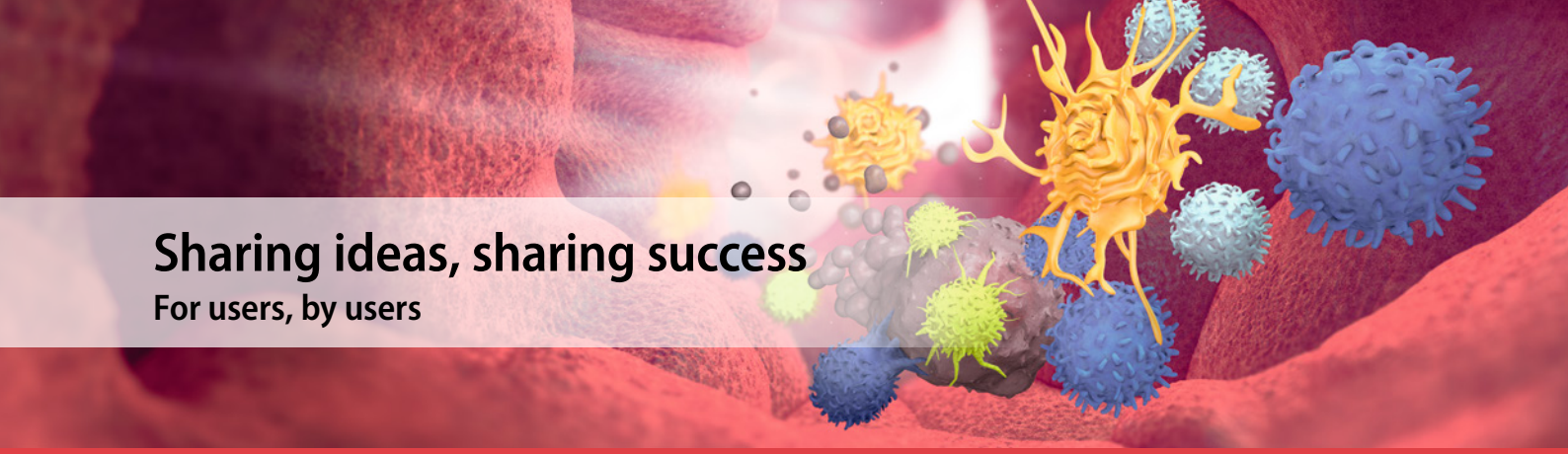

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Adoptive transfer of pan T cells separated with MACS® Technology

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Background

The chemotherapeutic agent Azacitidine (AzaC) alleviates graft versus host disease (GVHD) in both mouse models and human clinical trials, whilst the graft versus leukemia effect is preserved. Cooper and colleagues were able to show in their experimental studies that regulatory T (Treg) cells are required in the donor graft for AzaC to optimally protect against GVHD[1]. Opposed to the T effector (Teff) cells, Treg cells are resistant to the antiproliferative effects of AzaC. Accordingly, it was shown that lethally irradiated mice survived significantly longer after bone marrow (BM) transplantation and the induction of GVHD, via the transfer of donor pan T cells when functional natural regulatory T (nTreg) cells were present.
The results demonstrate that Treg cells are essential for AzaC to fully protect against GVHD and have important clinical implications for future clinical trials testing AzaC as a novel method of GVHD prophylaxis in humans.
This specific T cell transfer experiment shows by using B6.Foxp3$^{DTR/GFP}$ mice, in which Treg cells can be specifically ablated through administration of Diphtheria toxin (DT), Treg cells are required in the donor graft for AzaC to optimally protect against GVHD.
T cells used for the transfer were isolated on the autoMACS® Pro Separator using the Pan T cell Isolation Kit II, mouse.

Materials and methods

Materials

- Pan T cell Isolation Kit II, mouse
- autoMACS Pro Separator
- Balb/c 925cG lethally irradiated recipient mice
- TCD BM donor mice: C57BL/6
- Pan T cell donor mice: B6.Foxp3$^{DTR/GFP}$

Method

1. Balb/c mice were lethally irradiated and left either untreated or infused with T cell depleted bone marrow (TCD BM) from C57BL/6 donor mice.
2. On day 11 post TCD BM infusion, GVHD was induced by transfer of 1×10^7 pan T cells isolated using MACS® technology (Pan T cell Isolation Kit II, mouse).
3. Pan T cells were transferred from B6.Foxp3$^{DTR/GFP}$ donor mice. Both Teff cells and Treg cells were included.
4. Mice were treated with DT, AzaC, a combination of both or left untreated (phosphate buffered saline (PBS) as a control).

Results

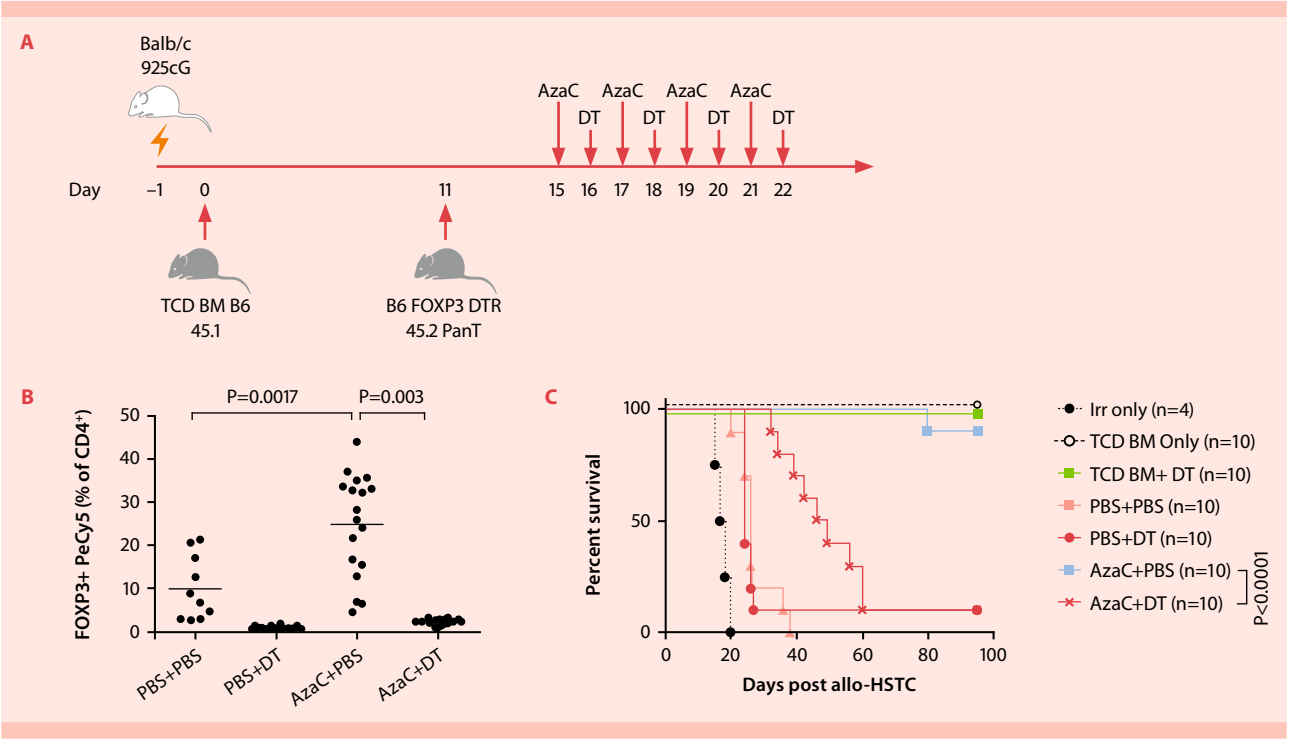

Figure 1: Depletion of Foxp3$^{DTR/GFP}$ Treg cells by DT reduces the protective effect of AzaC in an allotransplant model.

To assess the role of Treg cells in the alleviation of GVHD by AzaC *in vivo*, B6.Foxp3$^{DTR/GFP}$ pan T cells (both Teff cells and Treg cells) were infused into lethally irradiated Balb/c recipients to induce GVHD. The recipient mice were then treated with PBS, DT, AzaC or a combination of both AzaC and DT (fig. 1A).

Mice treated with DT resulted in the depletion of donor FOXP3+ Treg cells. Two treatments with AzaC resulted in a 2.5 fold increase in Treg cells. Mice treated with both AzaC and DT again resulted in the depletion of both nTreg cells and AzaC generated Treg cells (fig. 1B).

Mice treated with either PBS or DT alone developed severe GVHD and survived an average of 26 and 24 days respectively. In contrast, 90% of the AzaC treated mice survived the full length of the study (100 days) and displayed no visible GVHD related symptoms. The treatment of mice with AzaC+DT showed prolonged survival compared to PBS treated mice ($p<0.001$), but in comparison to the AzaC treated mice, had significantly greater mortality (median survival = 47.5 days, $p<0.0001$) (fig. 1C).

Conclusions

Pan T cells isolated with the Pan T cell isolation Kit, II from donor mice can be transferred into recipient mice to study the effects of GVHD and potential treatments.

References

1. Cooper, M, L. *et al.* (2017) Azacitidine mitigates GvHD via differential effects on the proliferation of T effector and nTregs *in vivo*. J Immunol. 198(9): 3746–3754.