Background

Graft-Versus-Host-Disease (GVHD) is a life-threatening complication of allogeneic hematopoietic stem cell transplantation (HSCT). Animal models have demonstrated that Treg infusion could prevent otherwise lethal GVHD in mice given grafts from MHC-disparate donors. Here, we assessed the ability of clinical-grade isolated human Treg to attenuate experimental xenogeneic GVHD.

Material and methods

Human Treg were isolated from cytapheresis products with the Miltenyi CliniMacs system using a two-step procedure (CD8 and CD19 depletion followed by CD25 positive selection) in six independent experiments with six different healthy volunteer donors. Sub-lethally (2.5 Gy) irradiated NSG mice were given 2x10^6 cytapheresis product cells i.v. without (PBMC group) or with 1x10^6 Tregs (PBMC+Treg group), while other NSG mice received only 2x10^6 Treg (also in i.v.; Treg group). Mice in terminal stage GVHD were euthanised.

Results

After the selection, we obtained a CD25 enriched fraction including a median of 1.81 x 10^8 cells and containing 59 +/- 6% or 66 +/- 6% Treg defined as either CD45^-CD4^+CD25^highFoxP3^+ cells or CD45^-CD4^-CD25^-^CD127^- cells. In all experiments but the last (a technical problem dramatically impacts the efficiency of this selection), Treg co-transfusion significantly delayed death from xenogeneic GVHD. Specifically, median survivals in PBMC versus PBMC+Treg mice were 30 vs 56 days (p=0.015), 123.5 vs >162 days (p=0.23), 25.5 vs 70 days (p=0.012), 13 vs 16 days (p=0.038), 27 vs 49 days (p=0.061), and 46 vs 47 days (p=0.338) respectively. Further, none of the mice given only Treg experienced signs of GVHD, while, interestingly, the CD4^- cells found in these mice 27 days after transplantation were mainly conventional T cells (CD25^-FoxP3^- cells in human CD4^- total cells were only 2.1%, 3.1% and 17.7% in spleen, bone marrow and blood, respectively while 80.2% were grafted).

Conclusion

Treg infusion delayed the occurrence of xenogeneic GVHD without showing any toxicity in this murine model.

O.7 Erythropoietin therapy after allogeneic hematopoietic cell transplantation : a prospective randomised trial

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Based on the impairment of erythropoietin production after allogeneic hematopoietic cell transplantation (HCT), we previously reported in a phase-2 trial that recombinant human erythropoietin (rhEPO) therapy was very efficient when started one month after transplantation. We also demonstrated that anemia after non-myeloablative (NMHCT) HCT was less sensitive to rhEPO therapy than after conventional allogeneic HCT. This prompted us to confirm these findings in a prospective randomised trial.

One hundred and thirty-one patients were randomised (1:1) between no treatment (arm 1) or erythropoietin (Neorecormon) at the dose of 500 U/kg/week (arm 2). On the target Hb (13g/dL) has been attained, the dose of rhEPO was reduced by half, while it was withdrawn when Hb was = 14g/dL. Cohort A included 42 patients on day 28 after myeloablative HCT, cohort B 39 patients on day 28 after NMHCT, and cohort C 50 patients on day 0 of NMHCT. Primary endpoints included proportion of complete correctors (i.e. patients reaching Hb = 13g/dL) and median time to achieve Hb correction in each arm.

The proportion of complete correctors before day 126 post-transplant was 0% in group 1A vs 52.4% in group 2A, 0% in group 1B vs 69.5% in group 2B and 19.1% in group 1C vs 70.2% in group 2C. Median time to achieve Hb = 13g/dL was not reached in group 1B vs 49 days in group 2B; 363 and 59 days in groups 1A and 1B respectively and 363 and 87 days in groups 3A and 3B respectively (figure 1).

Conclusion

At long follow-up prolonged E.coli asparaginase therapy in conso-