CLINICAL TRANSLATION OF A NOVEL, POTENT AND WELL-CHARACTERIZED INDUCED REGULATORY T-CELL PRODUCT AGAINST GRAFT VERSUS HOST DISEASE

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Background

Immunotherapy with regulatory T cells (Tregs) stands as an alternative treatment for Graft-versus-Host Disease (GvHD), a major complication of allogeneic hematopoietic cell transplantation (allo-HCT). We have previously reported that HLA-G expression, known to promote feto-maternal tolerance, is induced by pharmacological hypomethylation of T cells, generating induced HLA-G$^+$ Tregs with suppressive properties.

Aim

To generate a fully characterized, safe and efficacious clinical-scale and GMP-grade product enriched in HLAG$^+$ T cells (iG-Tregs), and ultimately evaluate it in a phase I/II trial in patients undergoing HLA-matched sibling allo-HCT against GvHD.

Methods

Peripheral blood mononuclear cells from healthy donors were enriched for T-cells by adherence-based monocyte depletion, activated with anti-CD3/CD28 antibodies and treated for 3 days with decitabine. The safety and efficacy profile of the iG-Tregs product was assessed *in vitro* and *in vivo*. Polyclonal expansion of iG-Tregs was assessed by Immune-seq (Illumina). Sorted HLAG$^+$ cells were analysed by bulk (Illumina) and single-cell RNAseq (10X Genomics).

Results

iG-Tregs (n=22) contained 91.3% CD3$^+$ cells (median-Md) with 16.6% CD3$^+$HLAG$^+$ cells (2.4-28%) which was higher than PBS-Treated cells (controls) (1.4%, $p<0.0001$). A predominant CD4$^+$ fraction was observed versus controls (67.7% vs 57%).

Immunophenotyping of iG-Tregs revealed the expression of TIM3 and LAG3 inhibitory receptors stemming mostly from $HLAG^+$ cells. Except for IL13 (p=0.05), no other Th1/2/17 cytokines were increased in iG-Tregs supernatants, a finding which was validated by FACS.
Immunoprofiling of TRBV-TRBD-TRBJ gene rearrangements revealed that the extremely diverse and polyclonal TR gene repertoire of controls was retained in iG-Tregs, while treatment drove to repertoire renewal as only 4% (Md, 2%-4.7%) of the total identified T cell clones were shared amongst controls and $HLAG^+$ population's repertoire.
RNAseq revealed $HLAG^+$ cells to posses a distinct and homogenous transcriptional profile and upregulate the myeloid suppressive genes IDO-1, CCL17 and CCL22 compared to their $HLAG^-$ counterparts, while single-cell RNAseq uncovered underlying regulatory signatures.
Moreover, iG-Tregs demonstrated a favourable safety profile over controls *in vitro*, showing diminished alloreactivity against allogeneic targets (1.8%vs49% lysis) and their hyporesponsiveness after restimulation via reduction of intracellular pro-inflammatory cytokines (IL2/IFNγ/IL17a/TNFα). The diminished alloreactivity of iG-Tregs over controls was confirmed *in vivo* after infusion in NSG mice; 6/9(67%) iG-Tregs-treated mice survived until sacrifice whereas all 7 mice infused with controls developed GvHD from day 21 and succumbed by day 35 (Md). iG-Tregs' ability in preventing GvHD was tested by their co-infusion with T-cells; while T-cell infusion resulted in lethal GvHD by day 36 in all 3 control mice, the co-administration of iG-Tregs delayed (6/8 mice died by day 66) or even prevented (2/8) GvHD onset with a second infusion increasing survival to 40%.
Respectively, GMP-iG-Tregs were comparable with preclinical products regarding immunophenotype, immunoprofile, and absence of alloreactivity.

Summary/conclusion

iG-Tregs are enriched in regulatory $HLAG^+$ cells and can be robustly and reproducibly generated through a short and GMP-compatible protocol. iG-Tregs constitute a well-characterized product with the safety profile required for clinical translation, leading to the approval and initiation of a phase I-II clinical trial for GvHD (EUDRACT 2021-006367-26).