CHARACTERIZATION AND GMP MANUFACTURING OF EPIGENETICALLY REPROGRAMMED HLA-G EXPRESSING, T REGULATORY CELLS (iG-Tregs)

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Immunotherapy with T-regulatory cells (Tregs) stands as an alternative treatment for graft-versus-host disease (GvHD), a major complication of allogeneic hematopoietic cell transplantation. We have shown that HLA-G expression, known to allow the maternal-fetal tolerance, can be induced by pharmacological hypomethylation (Decitabine-Dec) of T-cells, generating T-cells with immunoregulatory properties (iG-Tregs). Herein, we elucidated the molecular characterization of iG-Tregs, assessed their safety and efficacy *in vitro* and *in vivo* and validated their GMP manufacturing. iG-Tregs were generated after a 3-day exposure of anti-CD3/CD28-activated T cells to Dec. RNA-seq revealed a distinct expression profile of FACS-sorted HLAG^{+}CD4+cells over their HLAG^{-}CD4+ counterpart, in terms of regulatory genes (CCL17/CCL22/CXCL9) and the myeloid suppressor gene IDO-1. iG-Tregs demonstrated a favorable safety profile over untreated-control cells in vitro, by producing decreased levels of GvHD-associated cytokines upon PMA/ionomycin stimulation (IL-2/IFNγ/IL-17a) and exerting diminished alloreactivity against allogeneic PHA-blasts ($p<0.0001$). Safety was also confirmed *in vivo*; all control mice succumbed by d35 from histologically confirmed GvHD whereas 67% of iG-Treg-treated mice survived until sacrifice (d84) ($p=0.0019$). As regards efficacy, co-administration of iG-Tregs with donor lymphocyte infusions (DLIs) delayed or prevented the onset of GvHD versus DLI-treated-mice. Two large-scale iG-Treg products were generated and validated under GMP conditions; an average of $8.6x10^7$ iG-Tregs were produced from 2 healthy volunteers, enriched for HLA-G^{+}cells (26±0.04%) and lacking alloreactivity. Overall, we demonstrate the feasibility of GMP generating clinically relevant doses of well-characterized iG-Tregs. A close to initiation, phase I/II clinical trial (EUDRACT:2021-006367-26) will evaluate iG-Tregs for the prevention and treatment of steroid-refractory GvHD.