"Tr1-dendritic cells and uses thereof"

Background and Description of Invention. A large panel of immunosuppressive drugs is now available to prevent acute GvHD and allograft rejection. These agents have been associated with numerous and rather significant toxicities. Moreover, continuous drug administration leads to a sustained general depression of immune responses. All these effects are due to the non-selective mode of action of the immunosuppressive drugs. A valid alternative to immunosuppressive regimens for prevention of GvHD and allograft rejection is the induction of tolerance to the alloantigens expressed by the recipient or by the graft. This tolerance-induction strategy should selectively target only a small fraction of potentially alloreactive T cells and leave the remaining T cells of the immune system functionally intact.

Peripheral T-cell tolerance can be induced and maintained by a variety of mechanisms, including deletion, induction of T-cell hypo-responsiveness, and differentiation of T regulatory (Tr) cells. Tr cells include a wide variety of cells which all have a unique capacity to inhibit effector T-cell responses. Addition of IL-10 during dendritic cells (DC) differentiation induces a new subset of tolerogenic Tr1-DC which can be used to generate anergic Tr1. Tr1-DC are CD14+CD11c+CD11b+, express CD83, CD80, and CD86, and secrete high levels of IL-10 but low amounts of IL-12. Importantly, IL-10/IL-12 ratio is maintained upon activation with LPS and IFN-g. Tr1-DC are refractory to activation and are potent Tr1 cells inducers *in vitro*.

- Gene signature of human Tr1 cells are object of a new patent application (WO2013192215). The patented technology is available for licensing worldwide. Patent applications pending in US, Europe and Canada.
- CD4+ T cell that produces high levels of IL-10 (CD4IL-10 T cells), by lentiviral vector-mediated gene transfer, for use in the treatment and/or prevention of a tumor that expresses CD13, HLA-class I and CD54 and/or for use in inducing Graft versus tumour (GvT) (Locafaro et al., Molecular Therapy 2017) are object of a new patent application (WO2016146542). CD4IL-10 T cells (i) mediate anti-tumor effects against myeloid cells; (ii) control GvHD by inhibiting the proliferation of human T cells in the periphery; (iii) do not compromise the GvL effect mediated by allogeneic T cells against leukemia blasts.

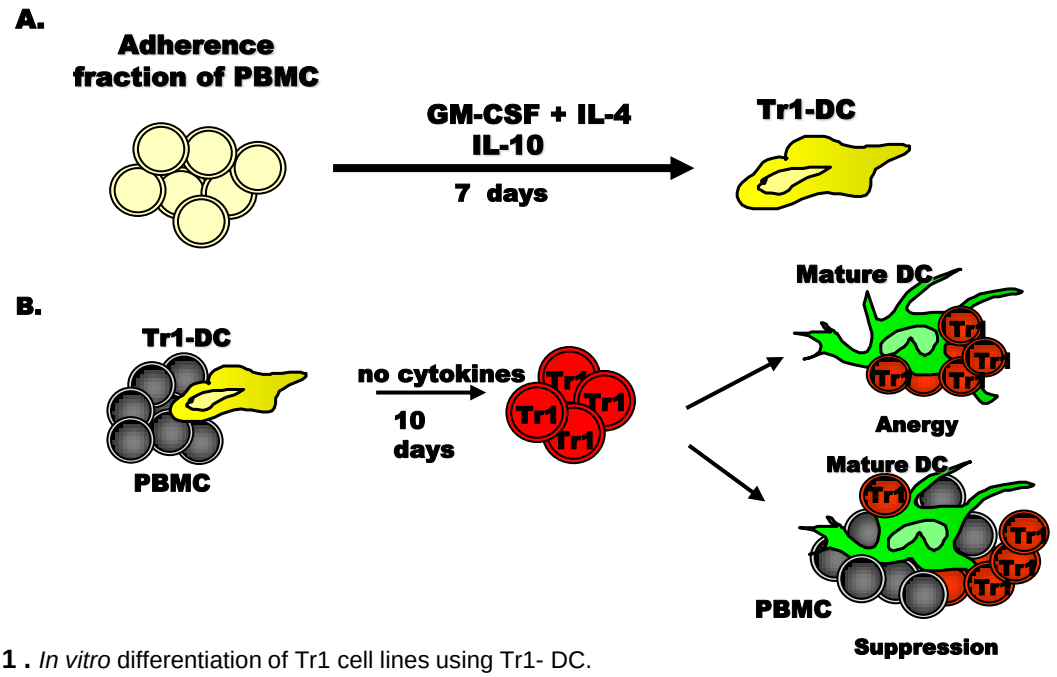

Figure 1 . *In vitro* differentiation of Tr1 cell lines using Tr1- DC.
A. Tr1-DC are differentiated from CD14+ monocytes by culturing with IL-4 and GM-CSF for 7 days in the presence of exogenous IL-10. B. Tr1 cell differentiation using Tr1-DC. Total PBMC are stimulated with allogeneic Tr1-DC at 10:1 ratio for 10 days. The resulting Tr1 cell lines are anergic in response to mature allogeneic DC, and suppress responses of autologous PBMC activated with mDC.

Patent information. An international patent application was published as WO2007131575. Patent granted in US and Canada; and US divisional pending. The second patent (WO2016146542) is pending in US, Europe and Canada. Both patents are available for licensing worldwide.

Stage of Development. IL-10 promotes the differentiation of a new subset of tolerogenic dendritic cells (Tr1-DC) which can be used to generate anergic Tr1 cells with limited *in vitro* manipulation and suitable for potential clinical use to restore peripheral tolerance. The ability of Tr1-DC obtained by the present method to induce anergic allo-antigen specific Tr1 cells was evaluated. In addition, the potential of Tr1-DC to induce T-cell anergy with limited in vitro manipulation in haplo-identical and HLA-matched un-related donors was investigated.

Potential Applications and Competitive Advantages. Peripheral blood naive $CD4^+$ T cells stimulated with allogeneic Tr1-DC are profoundly anergic and acquire regulatory function. These T cells are phenotypically and functionally similar to Tr1 cells, since they secrete high levels of IL-10 and TGF-b and suppress T-cell responses. Collectively these data indicate that Tr1-DC are a novel subset of tolerogenic DC that secrete high levels of IL-10 and low levels of IL-12, and are refractory to activation and maturation *in vitro*.

- Tr1-DC induce anergic T cells in short term cultures; anergic T cells induced by Tr1-DC are regulatory T cells phenotypically and functionally similar to Tr1 cells.
- Tr1-DC display low stimulatory capacity, and, importantly, a single round of stimulation with Tr1-DC is sufficient to induce Tr1 cells.
- Tr1-DC induce anergic T cells in pairs with different HLA disparities which can be used as cellular therapy to prevent GvHD and organ allograft rejection.

Relevant Publications.

- Roncarolo MG, Gregori S, Bacchetta R, Battaglia M. Tr1 cells and the counter-regulation of immunity: natural mechanisms and therapeutic applications. *Curr Top Microbiol Immunol. 2014*
- Bacchetta R, Lucarelli B, Sartirana C, Gregori S, Lupo Stanghellini MT, Miqueu P, Tomiuk S, Hernandez-Fuentes M, Gianolini ME, Greco R, Bernardi M, Zappone E, Rossini S, Janssen U, Ambrosi A, Salomoni M, Peccatori J, Ciceri F, Roncarolo MG. Immunological Outcome in Haploidentical-HSC Transplanted Patients Treated with IL-10-Anergized Donor T Cells. *Front Immunol. 2014*
- Gagliani N, Magnani CF, Huber S, Gianolini ME, Pala M, Licona-Limon P, Guo B, Herbert DR, Bulfone A, Trentini F, Di Serio C, Bacchetta R, Andreani M, Brockmann L, Gregori S, Flavell RA, Roncarolo MG. *Nat Med.* 2013
- Gregori S, Tomasoni D, Pacciani V, Scirpoli M, Battaglia M, Magnani CF, Hauben E, Roncarolo MG. Differentiation of type 1 T regulatory cells (Tr1) by tolerogenic DC-10 requires the IL-10-dependent ILT4/HLA-G pathway. *Blood.* 2010.

We seek a potential commercial partner with a strong pipeline in cellular immunotherapy protocols to further explore Tr1-dendritic cells and uses thereof for the generation of anergic Tr1 cells.

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