Background and Description of Invention. In vivo induction of antigen-specific Treg has been a long-sought goal of experimental medicine. Here we report in vivo induction of antigen-specific Treg and active immune tolerance against foreign antigens by hepatocyte-targeted Integrate-Deficient Lentiviral Vectors (IDLV). In particular, the present invention describes a new strategy exploiting endogenous miR-142 regulation in combination with IDLV.

Figure 1. GFP-expressing IDLV.ET.GFP.142T (grey bars) vs. ICLV.ET.GFP.142T (black bars) injected intravenously to adult mice (n=20 for IDLV mice and n=4 for ICLV mice in three independent experiments) and measured GFP expression and vector DNA contents in the liver at different times post-injection.

Figure 2. Transgene-specific tolerance after IDLV liver gene transfer. Quantification of the CD8+ GFP200-208-pentamer-positive T cells infiltrating the liver of mice treated with the indicated IDLV (IDLV.PGK n=9; IDLV.PGK.142T n=6; IDLV.ET.142T n=3) after antigen re-challenge (by intramuscular vaccination with GFP-encoding plasmids) 6 weeks after IDLV treatment.

Figure 3. Induction of transgene-specific Tregs by IDLV treatment CD4+ cells isolated from OTII Ly5.2 Foxp3-GFP transgenic mice were FACS-sorted to remove GFP+ cells obtaining an homogeneous population of CD4+ non-regulatory T cells with a unique antigen specificity (OVA323-339 presented in IAp molecule). (A) Tregs-depleted OTII CD4+GFP- (2.5×times;106/mouse) were adoptively transfer intravenously into naïve C57BL/6 Ly5.1 recipient mice one day before the injection of IDLV.PGK (n=3) or IDLV.ET.142T (n=3) encoding for OVA. Three weeks after IDLV administration livers were harvested and infiltrating lymphocytes isolated. OVA-specific induced Tregs were measured as GFP+ cells gated on CD4+Ly5.2+. (B) A representative histogram and (C) mean % induced Tregs +/- SEM is reported.

These results demonstrate a new strategy of tolerance induction which exploits miR-142-regulation in combination with transient gene delivery. This approach renders treated mice unresponsive to vaccination against strong neoantigens including a therapeutic protein (factor IX in hemophilia B mice) at 6 weeks after IDLV delivery (Fig. 2) and induces the conversion of naïve into antigen-specific Tregs in the liver as assessed at three weeks after IDLV delivery (Fig. 3). This strategy allows induction of long-lasting tolerance to transgene-encoded antigens without the need for long-term transgene expression and while reducing risks associated with vector integration.


Stage of Development. In vivo proof of principle has been achieved with different model antigens (GFP, ovalbumin) and a therapeutic protein (coagulation factor IX in hemophilia B mice). Further validation for more specific therapeutic application of this technology are ongoing.
Potential Applications and Competitive Advantages. This platform enables efficient liver gene transfer for a window of time and induces immune tolerance to the encoded antigen in a “hit and run” approach, without the need for long-term integration, thus providing enhanced safety as compared to Integrase-Competent Lentiviral Vectors (ICLV). Hepatocyte-targeted IDLV are promising new vectors for a broad range of applications, and primarily for

- induction of antigen-specific Tregs in “inverse vaccination” strategies to
  - tolerate individuals to protein replacement therapies (such as in lysosomal storage disorders and hemophilias or other plasma protein deficiencies). One of the major complications of protein replacement therapies is the development of neutralizing antibodies against the therapeutic protein. Thus patients undergoing these therapies would strongly benefit from the induction of tolerance to the therapeutic protein.
  - prevent or revert the development of autoimmune diseases (such as multiple sclerosis, diabetes) and allergic diseases.
- reversible hepatic gene transfer of therapeutic proteins of therapeutic proteins, such as interferon (IFN) and other cytokines, in chronic viral hepatitis or hepatic tumors; gene-based delivery may provide therapeutic concentrations of the factor at the disease site with limited systemic exposure and only for a defined window of time.

Relevant Publications.


We seek an industrial partner focused on protein replacement therapies and/or treatment of autoimmune disorders to further develop this technology for clinical applications.

For further information on this project please contact:

**Business Contact**
Paola Vella
Head, Office of Biotechnology Transfer
San Raffaele Hospital and Scientific Institute
Tel: +39 02 2643 4281
Fax: +3 90 2643 8138
E-mail: vella.paola@hsr.it

**Scientific Contacts**
Prof. Luigi Naldini
Director San Raffaele Telethon Institute for Gene Therapy
San Raffaele Hospital and Scientific Institute
Tel: +39 02 2643 4681
Fax: +39 02 2643 4621
E-mail: naldini.luigi@hsr.it