TREG AVATARS: ADOPTIVE CELL THERAPY TO PREVENT AND CURE TYPE 1 DIABETES, GEORGIA FOUSTERI

State-of-art and future development/perspectives of the research area at international level (max 2000 characters):

Adoptive cellular therapies (ACT) with FOXP3-expressing T regulatory cells (Tregs) to prevent and cure autoimmune disease and graft rejection are the next frontier in translational medicine. We aim to leverage the regulatory properties of Tregs to restore immune tolerance in high-risk individuals and those undergoing beta cell replacement. ACT with antigen-specific Tregs was shown to be a more promising approach because fewer cells are needed to suppress targeted cells. There are several strategies to generate antigen-specific Tregs. Among those, overexpression of a transgenic T cell receptor (TCR) or a chimeric antigen receptor (CAR) by means of genetic engineering stands out as the most promising approaches. Most T cell engineering approaches rely on viral vectors, such as retroviral and lentiviral (LV) vectors, while non-viral vector-based transposon genetic engineering is taking flight due to its reduced costs and higher safety profile.

Actual lines of research (as is) of the Diabetes Research Institute (max 2000 characters):

i) We aim to generate CAR (islet-specific, B-cell-specific, HLA-specific) Tregs leveraging either the bidirectional LV system or the Sleeping Beauty (SB) transposon system. In collaboration with Prof. Bonini, Magnani, Gaipa, Brusko we aim to develop the following Treg avatars:
-Allogeneic islet transplantation
• HLA-A2-specific CAR Tregs
• CXCR5-expressing HLA-A2-specific CAR Tregs
-T1D prevention
• Islet-specific TCR Tregs
• CXCR5-expressing Islet-specific TCR Tregs
• CD19 and CD22 CAR Tregs
ii) We aim to identify culturing conditions for optimizing Treg expansion and in vivo survival properties and function (in collaboration with Dr. P. Monti).
iii) We aim to test the production of iPSC-derived Tregs in collaboration with Dr. Themeli.
iv) We aim to test the production of xeno (swine)-Tregs in collaboration with Prof. Piemonti and Dr. Citro.

Strengths of the research area (as is) of the Diabetes Research Institute (max 2000 characters):

Our therapeutic approaches leveraging the tolerogenic properties of “Treg avatars” aim at preventing T1D in susceptible individuals and at promoting pancreatic islet engraftment in those with established disease. It is generally accepted that antigen-specific Tregs, including TCR- and CAR-Tregs, are superior to polyclonal Tregs in their suppression. Tregs need to migrate to disease-related target organs to exert maximal effects of suppression. Thus, antigen-specific-Tregs will be more potent in suppression than polyclonal Tregs since antigen-specific Tregs tend to migrate to a target organ harboring a specific antigen. Compared with non-specific immunosuppression mediated by polyclonal Tregs, inhibition mediated by antigen-specific likely generates fewer side effects as they promote disease-specific immunosuppression.

Weaknesses of the research area (as is) of the Diabetes Research Institute (max 2000 characters):

There are some disadvantages or limitations in genetically engineered Tregs. First and foremost there are no clinically-available data on the safety of this approach. It remains to be determined whether CAR-Tregs could induce adverse reactions. Second, we have limited data on the survival and functionality of these cells in vivo. Reduced survival and functional exhaustion likely will limit their efficacy in suppression. Additionally, it is still unclear what co-stimulatory molecule (e.g., CD28 vs. 3-1BB vs. OX40) will improve CAR Treg persistence and performance in the context of T1D. Another important aspect of these approaches is the issue of generating easy-to-produce, off-the-shelf Treg products instead of donor-derived cells. Mitigation of this issue may derive from using pandonors (HLA-selected donors), iPSC-derived Tregs (in development), or xeno Tregs (e.g., from genetically-modified pigs) as we are planning to develop. An alternative will be the in vivo engineering of CAR Tregs, a new field that will open up in the next 5-10 years.

Short-medium term OSR/UniSR goals (0-18 months): milestones and deliverables (max 1000 characters):

i) Production of human and murine Treg avatars (with Magnani, Bonini, Gaipa, Brusko)
• HLA-A2-specific +/- CXCR5 CAR Tregs with the bidirectional LV system.
• Islet-specific TCR +/- CXCR5 Tregs with the bidirectional LV or RV system.
• CD19 and CD22 CAR Tregs using the SB transposon system.
ii) In vitro phenotypic and functional analysis of Tregs, optimization of transduction, and culture conditions.
iii) Testing of culture protocols (with Dr. P. Monti)
iii) Development of protocols for iPSC-derived Tregs (with Dr. Themeli)
iv) Testing of xeno Treg isolation, transduction and culture protocols (with Piemonti, Citro).

Medium term OSR/UniSR goals (18-36 months): milestones and deliverables (max 1000 characters):

i) In vivo testing of mouse Tregs efficacy in murine models of T1D and islet transplantation
ii) In vitro and un vivo testing of human Treg function and survival such as in humanized models of graft-vs-host disease (GvHD).
iii) Phenotypic and functional in vitro testing of iPSC-derived Treg. Inclusion of CAR/TCR transduction protocols.
iv) Xeno Treg in vivo and ex vivo testing (mouse models of T1D, humanized GvHD, VIO, or similar).

Long term OSR/UniSR goals (36-60 months): milestones and deliverables (max 1000 characters):

i) Decipher in vivo mechanisms of action mouse Tregs efficacy in murine models of T1D and islet transplantation
ii) Upscaling and GMP production of human Treg avatars. Identification of possible responder groups (h1)

iii) iPSC-derived Treg in vivo experimentation –GMP?
i) Xeno Treg GMP product development.

Investments of the Diabetes Research Institute (e.g. personnel, space, technology) to achieve the short-medium-long term goals (max 2000 characters):

. Successful implementation of aim 2 will require:
1. Launch of a special institutional program on Treg cell therapy
2. Crowdfunding initiative for sustained funding, access to funding opportunities by non-profit VC
3. Financial advisors, perhaps hiring project managers
4. Foster training and career development for young investigators
5. Hiring of molecular biology scientists, technicians, statisticians
6. Hiring of supportive/administrative personnel (secretary, reporting, IACUC, IRB protocol support, public engagement and communication, web page and social media curators, fundraising)
7. Space for lab and offices for scientists, bioinformaticians, and administrative personnel
8. Core islet transplantation facility
9. Collaboration with industry and experts for generation of off-the-shelf" Treg avatars
10. GMP facility for Treg development and up-scale manufacturing
11. Flow cytometer dedicated to DRI
12. Launch clinical trial: better, more transparent, and effective engagement and collaboration with clinicians
13. Funds to support clinical personnel, for example, nurses.