A Novel IL-2 Mutein Induces Regulatory T Cell-Rich Microenvironment and Leads to Alloantigen-Specific Graft Acceptance

Yoshikazu Ganchiku[1] , Thiago de Jesus Borges[1], Rodrigo Benedetti Gassen[1], Orhan Efe[1], Zach Shriver[2], Greg J. Babcock[2], Leonardo V. Riella[1]

[1]Center for Transplantation Sciences, Massachusetts General Hospital, Boston, MA, [2]Visterra, Inc., Waltham, MA

MASSACHUSETTS GENERAL HOSPITAL
CENTER FOR TRANSPLANTATION SCIENCES

HARVARD MEDICAL SCHOOL TEACHING HOSPITAL

Background

Low-dose IL-2 therapy is recognized as an effective approach for *in vivo* expansion of Tregs.

Drawbacks:
- Short half-life[1]
- Off-target effects[2]

More stable and selective IL-2 analogs are needed[2-5]

Novel IL-2 mutein (mIL-2): Engineered fusion protein:
- Selectively binds to high-affinity IL-2 receptors
- Conjunction with the human IgG1 Fc domain

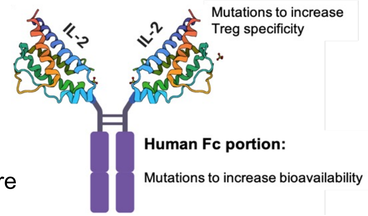


Fig 1. Illustration of our novel modified IL-2 mutein molecule
The regions in which the mutations were performed to increase Treg specificity and half-life.

Experiment summary

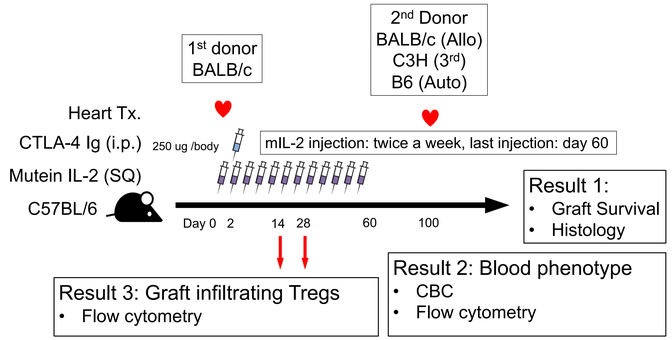
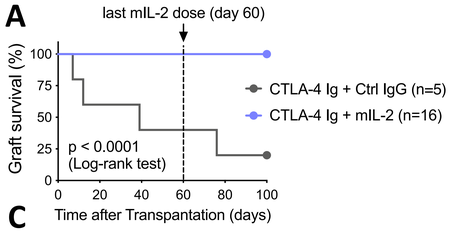
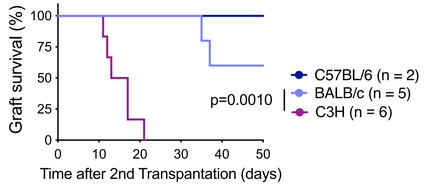


Fig 2. Experimental summary. BALB/c heart grafts were transplanted into the abdomen of C57BL/6 recipients. mIL-2 were administered subcutaneously on day 0, 2 and twice a week thereafter until day 60. 250 ug of CTLA-4 Ig was intraperitoneally injected on day 2. In mIL-2 group with accepted 1st grafts, 2nd heart grafts were transplanted into the right cervical area on day 100. Graft survival was evaluated by palpation.

Result 1: mIL-2 treatment induced graft acceptance





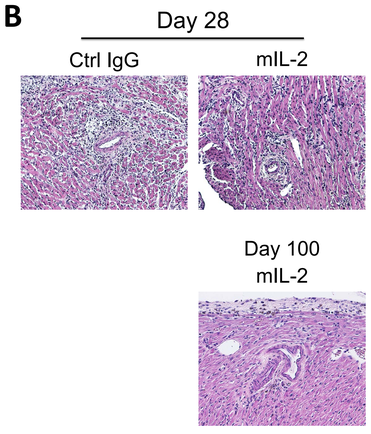
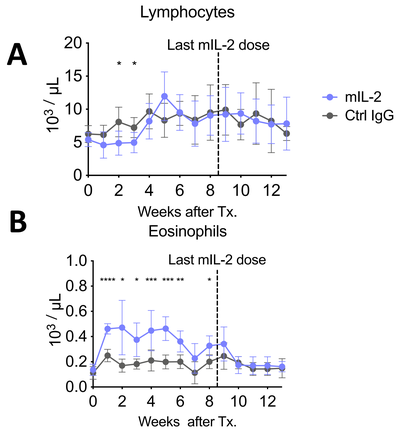


Fig 3. mIL-2 treatment with CTLA-4 Ig induced alloantigen-specific graft acceptance. (A) Graft survival of the first BALB/c graft. By day 60, mIL-2 or control IgG was injected subcutaneously twice a week. After day 60, any intervention was done. (B) Graft histology at day 28 (control group and mIL-2 group) and day 100 (mIL-2 group). (C) Graft survival of the second graft without any immunosuppression. The survival of both 1st and 2nd allograft was observed by palpation.

Result 2: mIL-2 expanded peripheral blood Tregs



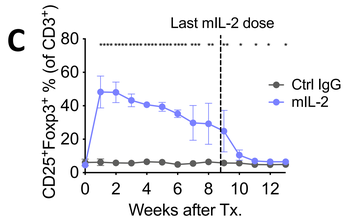


Fig 4. mIL-2 treatment changed the phenotype of circulating immune cells
(A) Blood count of lymphocytes.(B) eosinophils.
(C) Proportion of CD4 Tregs (Foxp3⁺).

Result 3: mIL-2 expanded graft-infiltrating Tregs

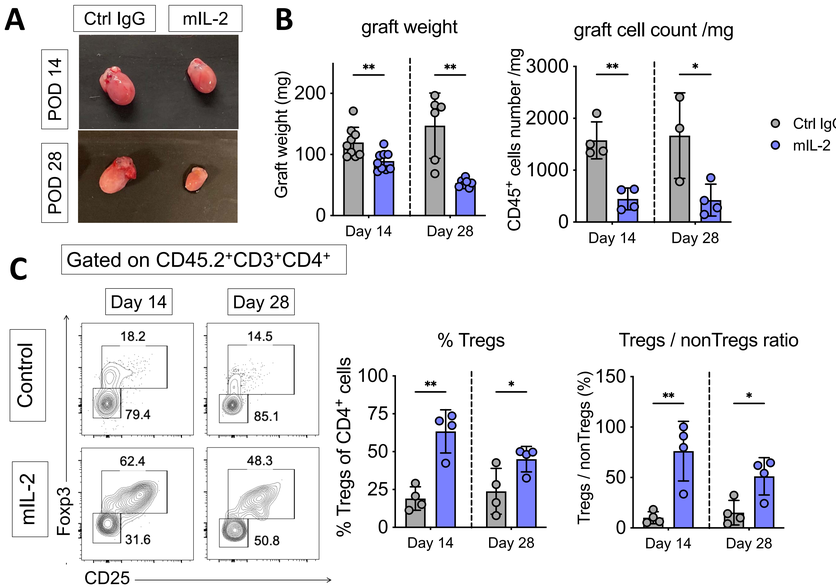


Fig 5. mIL-2 treatment attenuated graft infiltration and expanded graft-infiltrating Tregs. (A) Graft photos at the time of procurement. (B) Graft weight (left) and CD45⁺ cell count (right). (C) The proportion of graft infiltrating Tregs. Left: representative images, center: the proportion of Tregs, right: Tregs/nonTregs ratio.

Conclusions

- mIL-2 can be used without serious adverse events.
- mIL-2 induced antigen-specific allograft acceptance.
- mIL-2 expanded CD4⁺ Tregs in both peripheral blood and the graft.

References

1. M. T. Lotze et al. In vivo administration of purified human interleukin 2. I. Half-life and immunologic effects of the Jurkat cell line-derived interleukin 2. *J. Immunol.* **134**, 157–166 (1985).
2. R Hernandez et al. Engineering IL-2 for immunotherapy of autoimmunity and cancer . *Nat Rev Immunol* **22**, 614-628 (2022).
3. Y Yamada et al. Biased IL-2 signals induce Foxp3-rich pulmonary lymphoid structures and facilitate long-term lung allograft acceptance in mice. *Nat Commun.* **14**, 1383 (2023).
4. J. H. Xie et al. Mouse IL-2/CD25 fusion protein induces regulatory T cell expansion and immune suppression in preclinical models of systemic lupus erythematosus. *J Immunol.* **207**, 34-43 (2021).
5. L Khoryati et al. An IL-2 mutein engineered to promote expansion of regulatory T cells arrests ongoing autoimmunity in mice. *Sci Immunol.* **5**, eaba5264 (2020).

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