17-19 NOVEMBRE 2021
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Congrès commun avec la SFBCT
Centre de Congrès Prouvé Nancy

Etude des mécanismes de prévention de la maladie du greffon contre l'hôte (GVHD) par le cyclophosphamide dans un modèle de souris humanisées

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☑ Je n'ai pas de lien d'intérêt potentiel à déclarer

Introduction

- Cyclophosphamide (PTCy) is increasingly used as GVHD prophylaxis
- It's mechanisms of action besides depletion of alloreactive cells have remained debated

Review > Front Immunol. 2020 Apr 9;11:636. doi: 10.3389/fimmu.2020.00636. eCollection 2020.

Post-transplantation Cyclophosphamide: From HLA-Haploidentical to Matched-Related and Matched-Unrelated Donor Blood and Marrow Transplantation

Louis Williams [1], Frank Cirrone [2], Kelli Cole [2], Maher Abdul-Hay [2], Leo Luznik [3], Ahmad Samer Al-Homsi [2]

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PMID: 32373119 PMCID: PMC7177157 DOI: 10.3389/fimmu.2020.00636

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Abstract

Following allogeneic blood and marrow transplantation (BMT), graft-versus-host disease (GvHD) continues to represent a significant cause of treatment failure, despite the routine use of conventional, mainly calcineurin inhibitor-based prophylaxis. Recently, post-transplant cyclophosphamide (PTCy) has emerged as a safe and efficacious alternative. First, omitting the need for ex-vivo T-cell depletion in the setting of haploidentical transplantation, growing evidence supports PTCy's role in GvHD prevention in matched-related and matched-unrelated transplants. Through improved understanding of GvHD pathophysiology and advancements in drug development, PTCy emerges as a unique opportunity to develop calcineurin inhibitor-free strategies by integrating agents that target different stages of GvHD development.

Sci Transl Med. 2013 Nov 13;5(211):211ra157. doi: 10.1126/scitranslmed.3006960.

Aldehyde dehydrogenase expression drives human regulatory T cell resistance to posttransplantation cyclophosphamide

Christopher G Kanakry [1], Sudipto Ganguly, Marianna Zahurak, Javier Bolaños-Meade, Christopher Thoburn, Brandy Perkins, Ephraim J Fuchs, Richard J Jones, Allan D Hess, Leo Luznik

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Free PMC article

Abstract

High-dose, posttransplantation cyclophosphamide (PTCy) is an effective strategy for preventing graft-versus-host disease (GVHD) after allogeneic blood or marrow transplantation (alloBMT). However, the mechanisms by which PTCy modulates alloimmune responses are not well understood. We studied early T cell reconstitution in patients undergoing alloBMT with PTCy and the effects of mafosfamide, a cyclophosphamide (Cy) analog, on CD4(+) T cells in allogeneic mixed lymphocyte reactions (MLRs) in vitro. Patients exhibited reductions in naïve, potentially alloreactive conventional CD4(+) T cells with relative preservation of memory CD4(+)Foxp3(+) T cells. In particular, CD4(+)CD45RA(-)Foxp3(+hi) effector regulatory T cells (Tregs) recovered rapidly after alloBMT and, unexpectedly, were present at higher levels in patients with GVHD. CD4(+)Foxp3(+) T cells from patients and from allogeneic MLRs expressed relatively high levels of aldehyde dehydrogenase (ALDH), the major in vivo mechanism of

1. PTCy mitigates xenoGVHD

NSG mice

D0: 20x10^6 human PBMCs i.v.

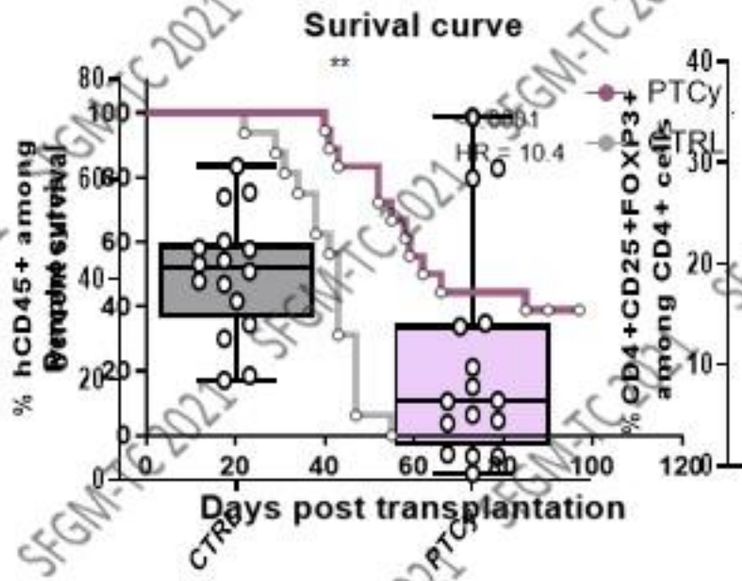
D3: cyclophosphamide 100 mg/kg i.p injection

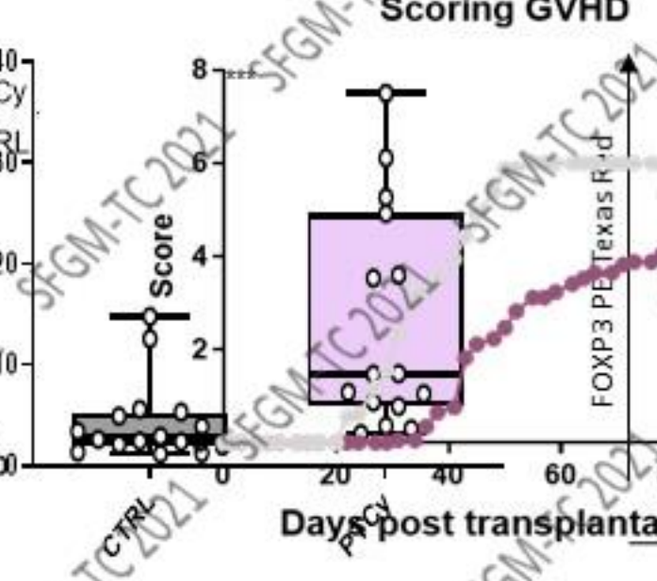
D21: Facs analysis for blood immune reconstitution

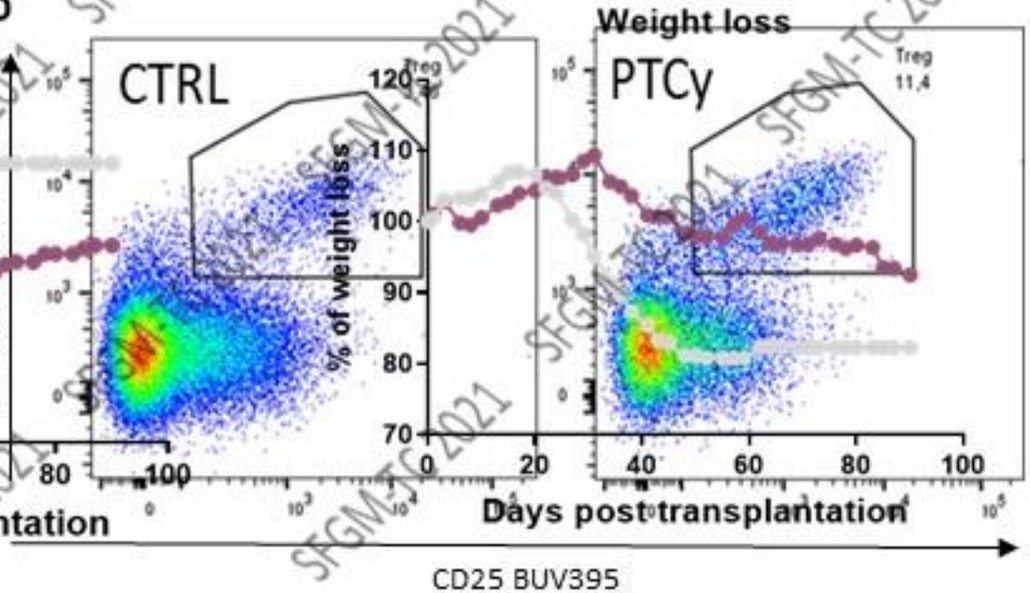
Survival and scoring

GVHD score:
- Hunching (2)
- Anemia (2)
- Mobility loss (2)
- Weight loss (2)

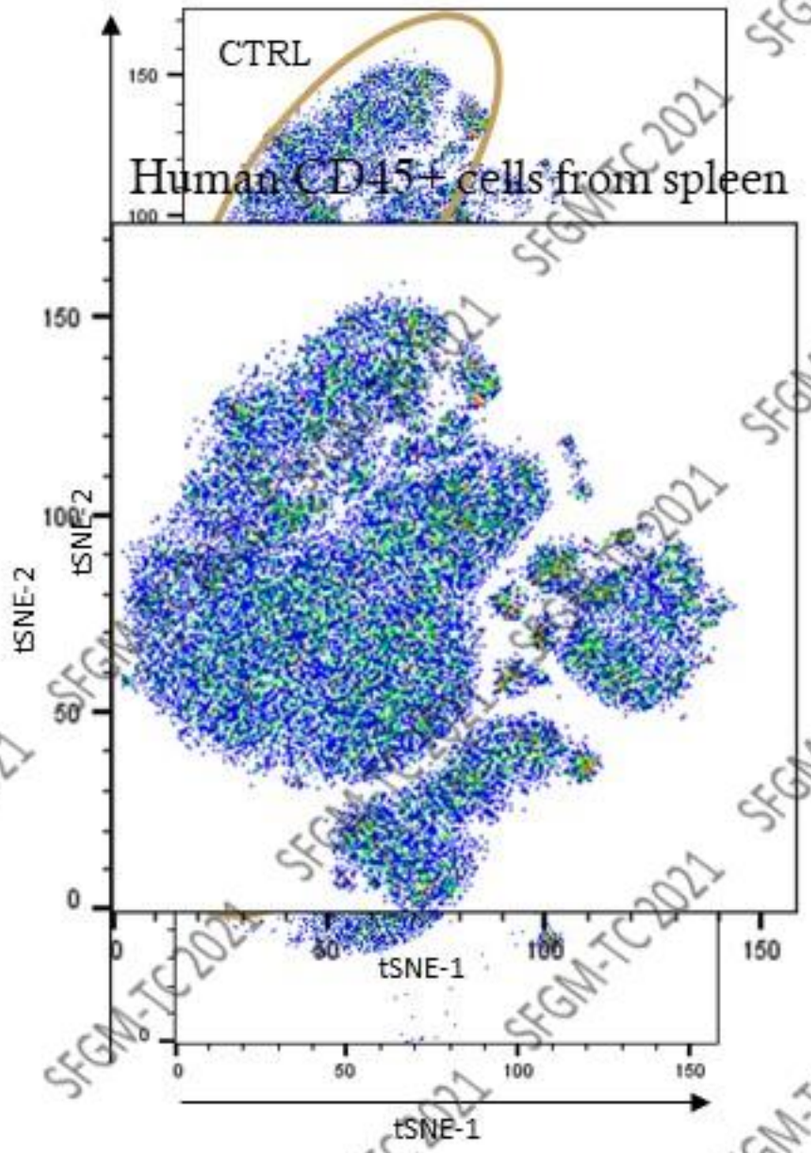
→ Sacrifice score 6/8



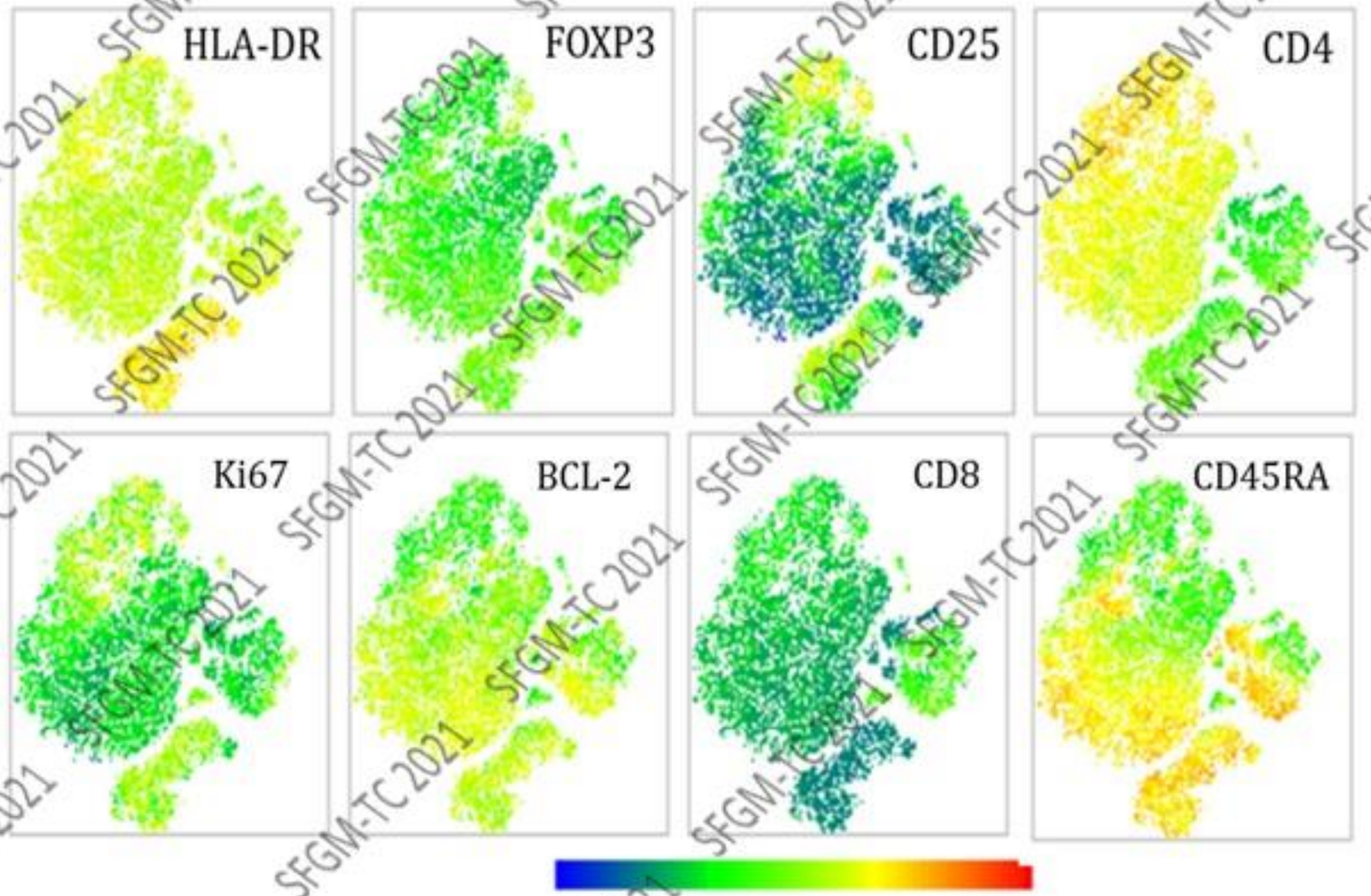




2. PTCy kills proliferative and activated T cells

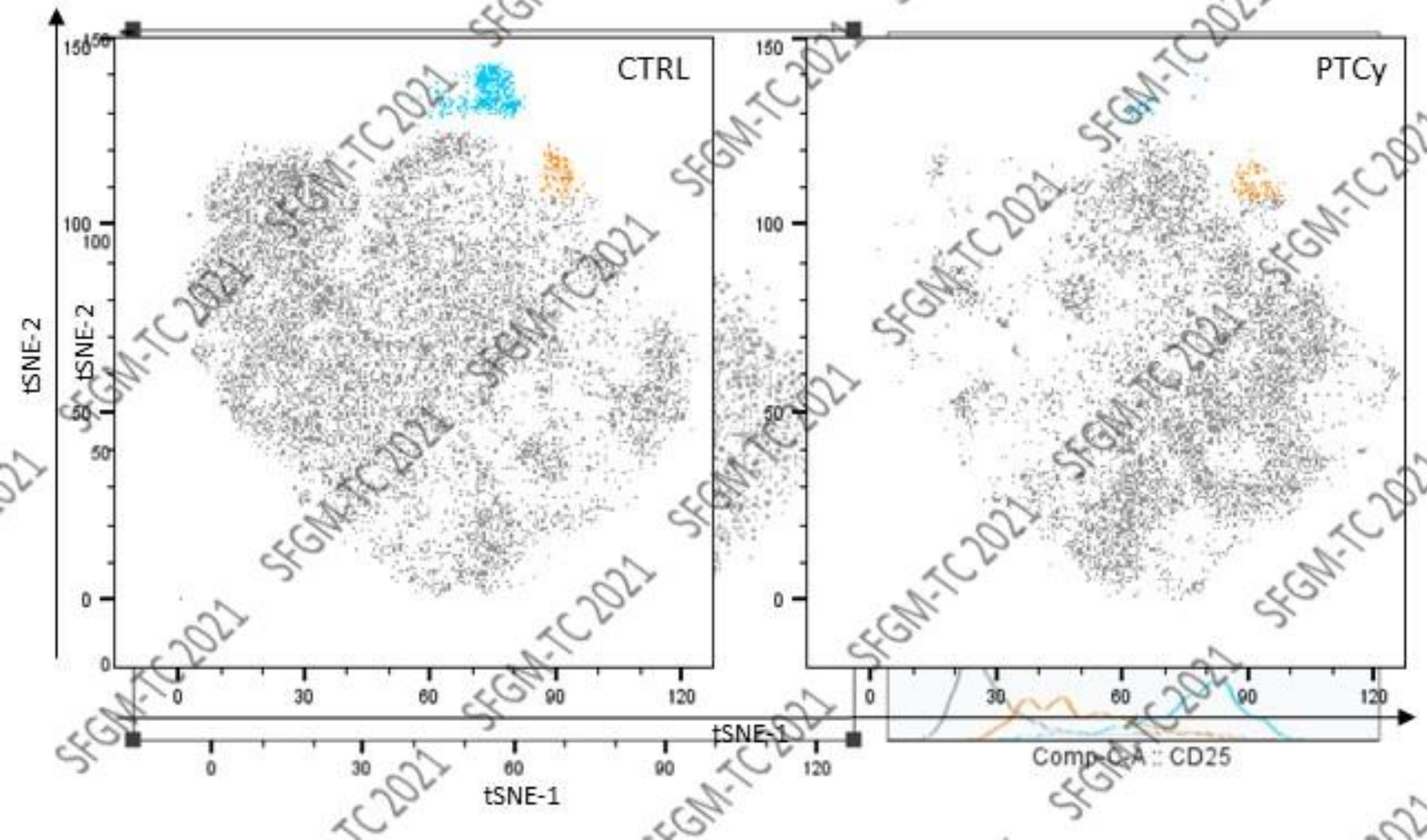


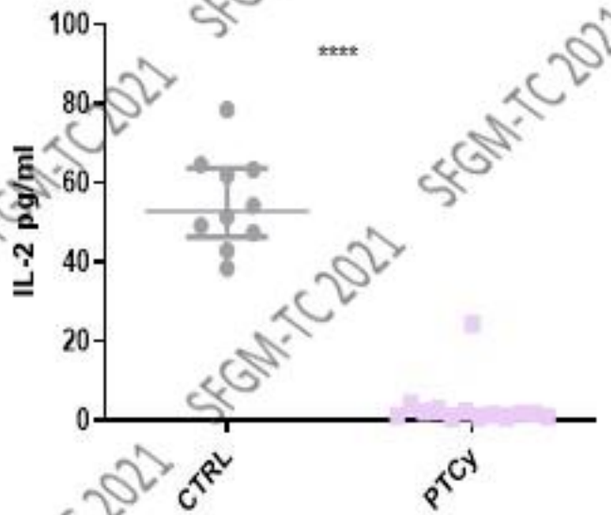
- PTCy kills proliferative and activated T cells and increases the frequencies of naive and BCL-2^{+} T cells



3. PTCy affects activated Treg cells *in vivo*

- Activated anf proliferative Treg with a higher expression of FOXP3 , CD25 and KI67 are killed by PTCy
- Naive and resting Treg with a higher BCL-2 expression are resistant to PTCy





4. Il-2 does not protect Treg from PTCy

NSG mice

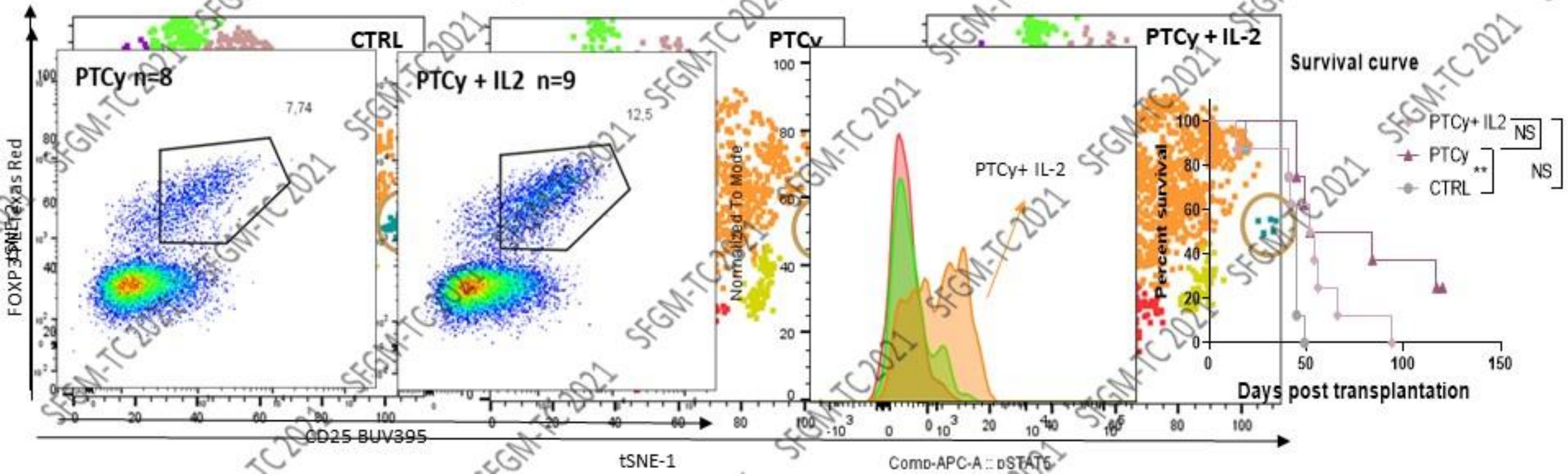
D0: 20x10^6 human PBMCs i.v.

D2- D15 ➔ low dose IL-2 25 000 IU ip injection/mice

D3: cyclophosphamide 100 mg/kg i.p injection

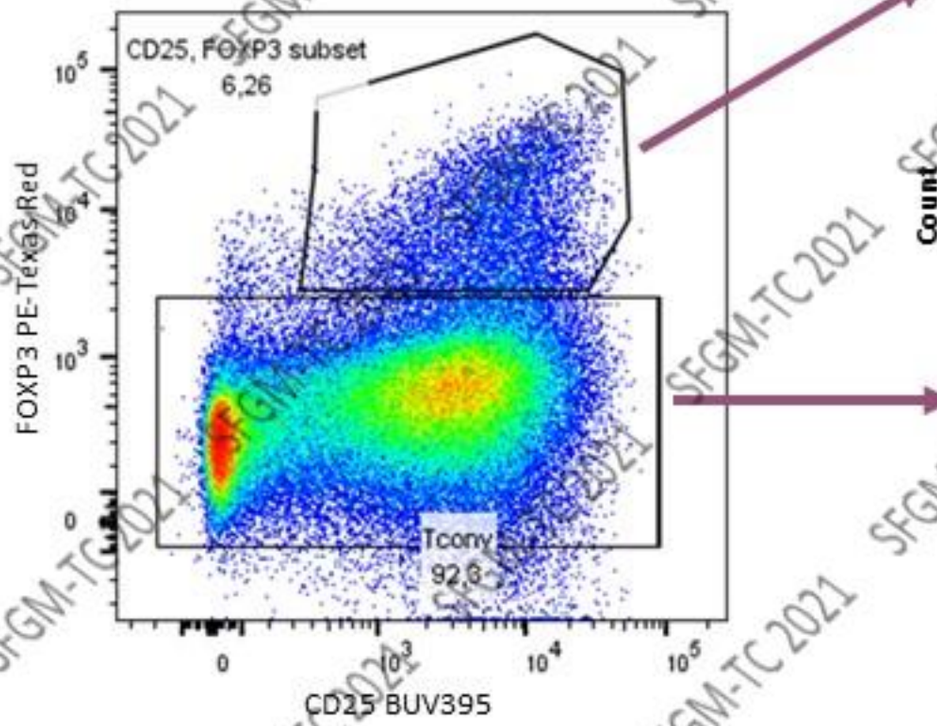
D7-14-21-28: Facs analysis for blood immune reconstitution

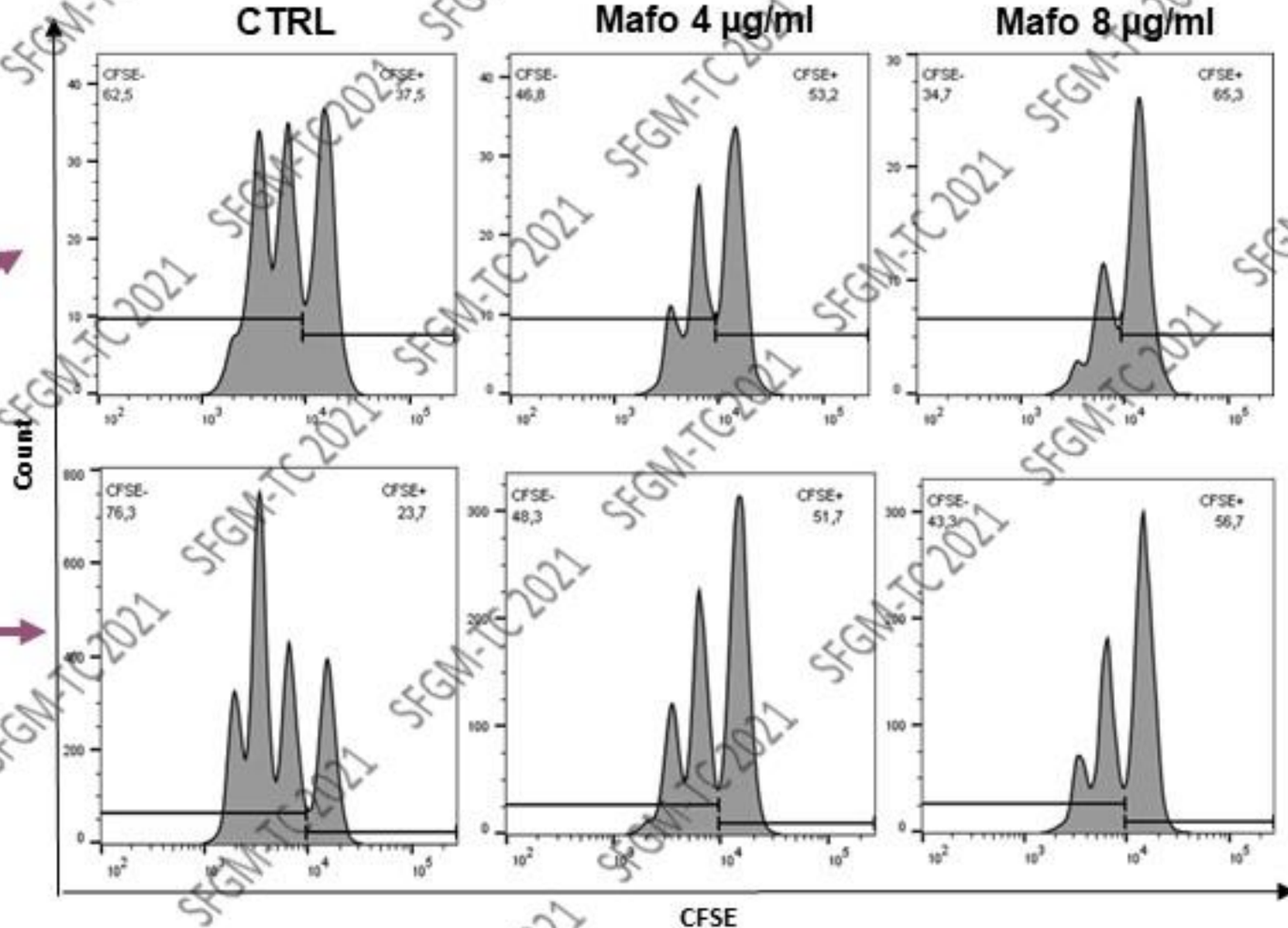
Survival and scoring



5. Proliferative Treg are impacted by PTCy *in vitro*

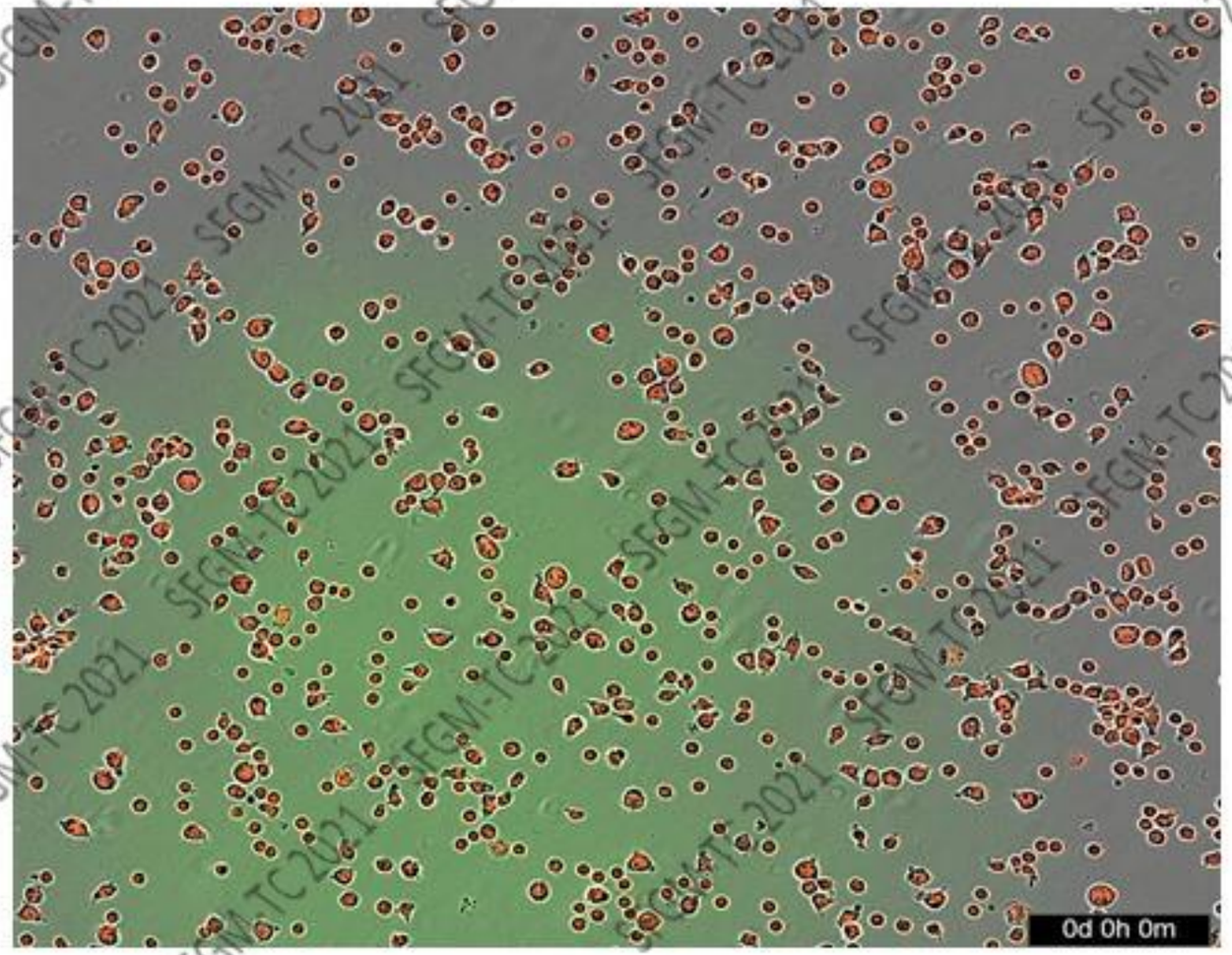
- T cells were stained with CFSE and stimulated with CD3/CD28 beads + IL-2 to induce activation and proliferation. After 48h of culture, they were treated or not with Mafosfamide (4-8 µg/ml)
- CFSE intensity were assessed in Treg and Tconv





5. Proliferative Treg are impacted by PTCy *in vitro (2)*

- Tconv and Treg were activated with anti CD3/CD28 beads + IL-2 and incubated with Mafosfamide 4 or 8 µg/ml for 48h:
  - Draq5 (red) = living cells
  - AnnV- FITC (green) = apoptotic cells



6. PTCy did not abrogate GVL effect

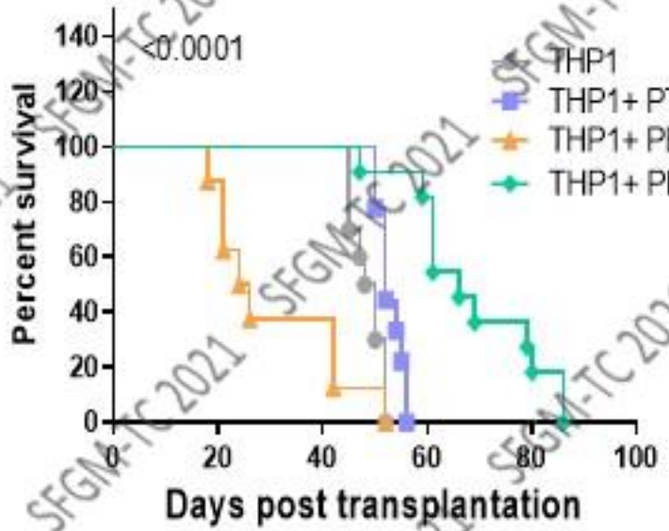
D0: 20x10^6 human PBMCs + 3x106 THP1-Luc+ cells i.v.

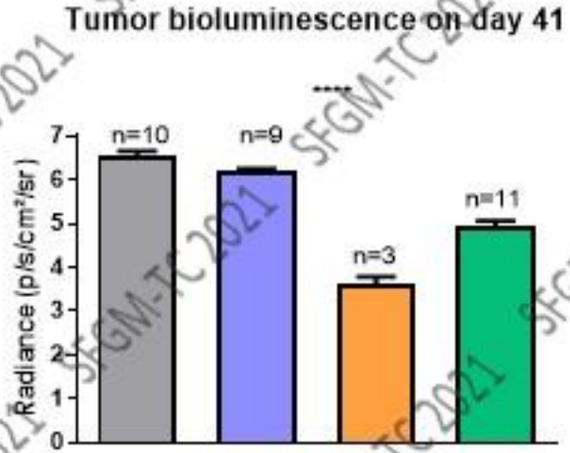
D3: cyclophosphamide 100 mg/kg I.p injection

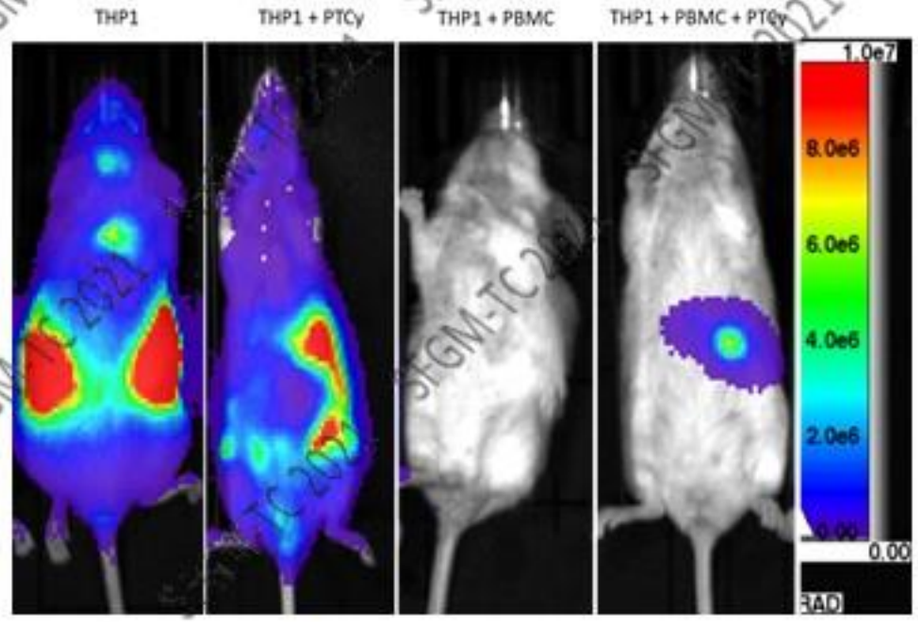
D5: 2x106 THP1-Luc+ cells i.v.

Bioluminescence imaging and survival + scoring

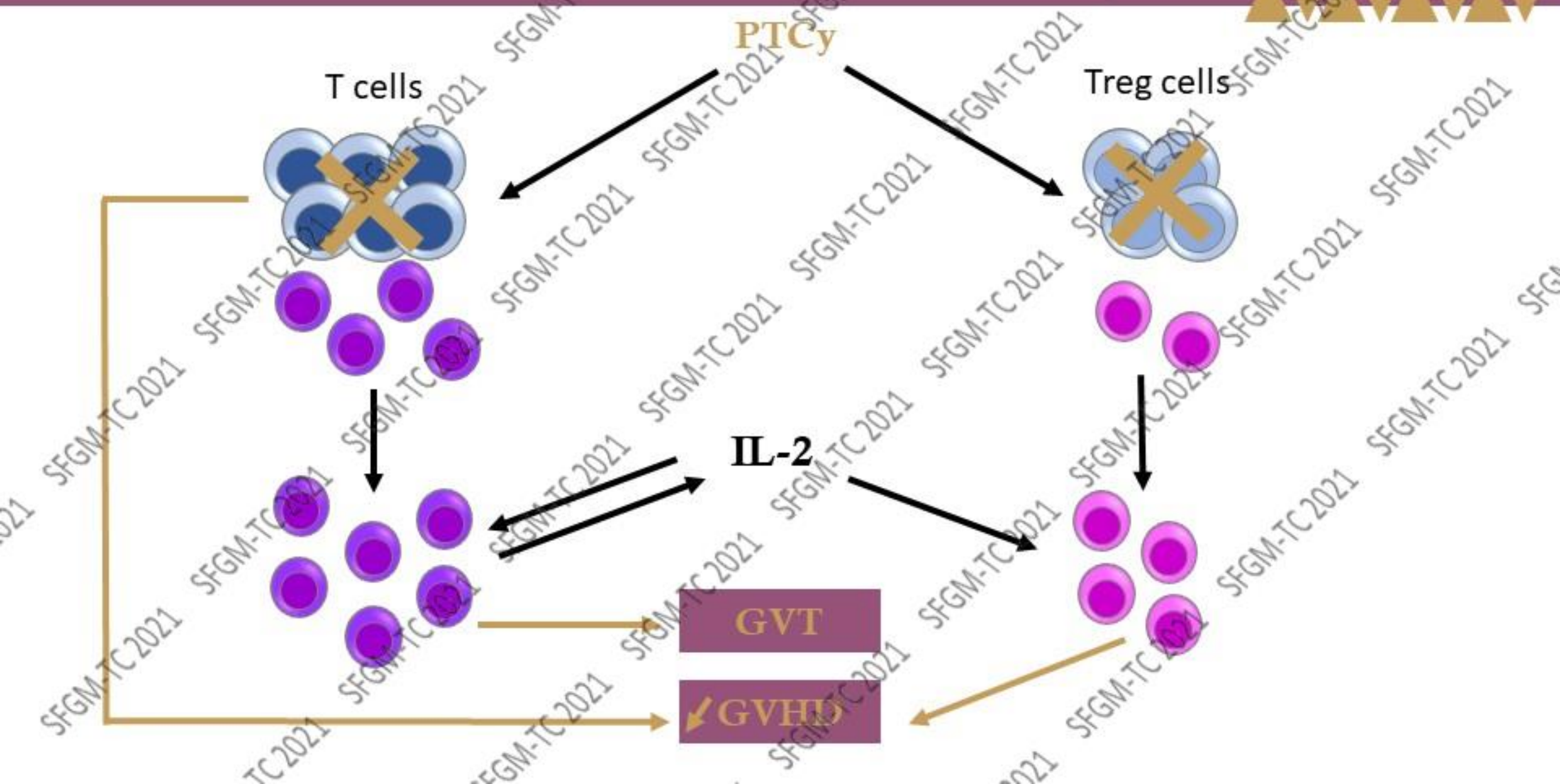
NSG HLA-A2 mice







Conclusions



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