

*HIGHLIGHTS*  
AMERICAN TRANSPLANT CONGRESS  
**ATC2023**  
JUNIO 3-7, 2023

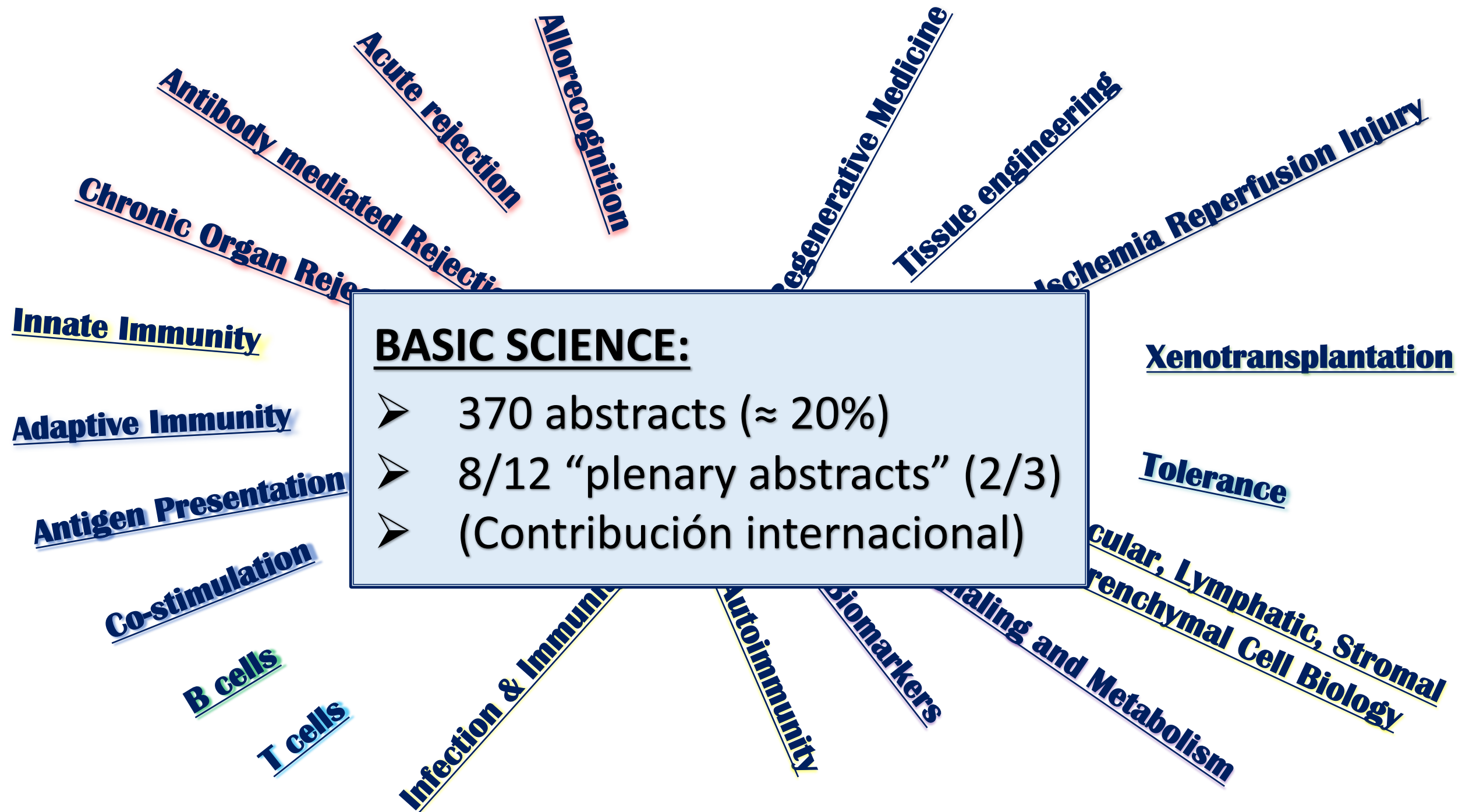
En esta presentación puede haber mención a datos científicos que no están aprobados en el registro. Por favor, consulte la ficha técnica. Las opiniones expresadas en esta presentación corresponden únicamente a quienes las emiten y no representan necesariamente las opiniones de Chiesi España S.A.U.

# Highlights ATC 2023

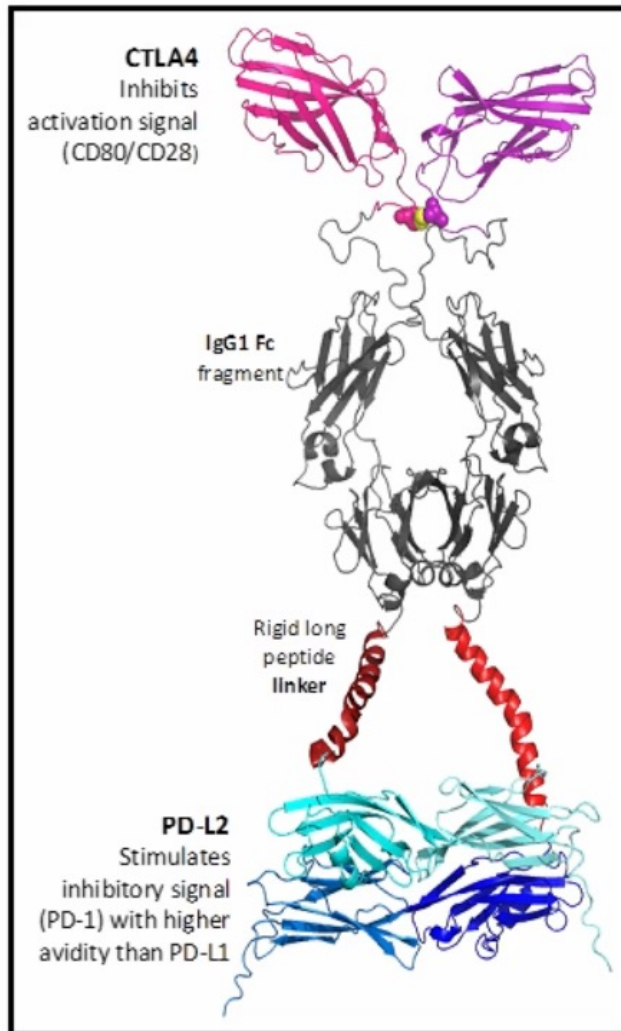
– **BASIC SCIENCE** –

## BASIC SCIENCE:

- 370 abstracts ( $\approx 20\%$ )
- 8/12 “plenary abstracts” (2/3)
- (Contribución internacional)

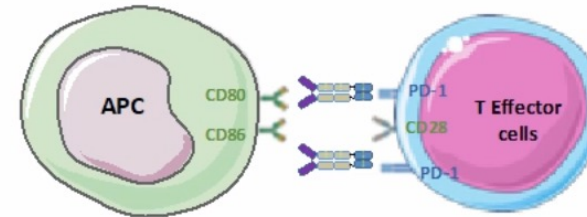


## A novel Bi-specific Fusion Protein With CTLA4-Ig/PD-L2 as a New Immunosuppressive Molecule (Duaccept) Modulating Alloimmune Responses

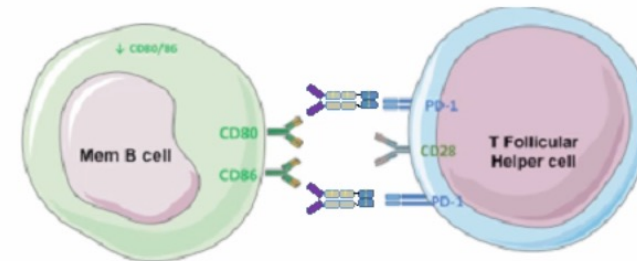


### Dual and opposite costimulatory targeting

- Inhibits a T-cell activation signal (CTLA4-CD80)
- Stimulates a T-cell Inactivation signal (PD-L2-PD-1)



↓ T Effector responses



↓ IgG Ab production

proliferation of CD3+ cells (x10<sup>4</sup>)

0

50

100

150

200

250

300

350

400

450

500

550

600

650

700

750

800

850

900

950

1000

1050

1100

1150

1200

1250

1300

1350

1400

1450

1500

1550

1600

1650

1700

1750

1800

1850

1900

1950

2000

2050

2100

2150

2200

2250

2300

2350

2400

2450

2500

2550

2600

2650

2700

2750

2800

2850

2900

2950

3000

3050

3100

3150

3200

3250

3300

3350

3400

3450

3500

3550

3600

3650

3700

3750

3800

3850

3900

3950

4000

4050

4100

4150

4200

4250

4300

4350

4400

4450

4500

4550

4600

4650

4700

4750

4800

4850

4900

4950

5000

5050

5100

5150

5200

5250

5300

5350

5400

5450

5500

5550

5600

5650

5700

5750

5800

5850

5900

5950

6000

6050

6100

6150

6200

6250

6300

6350

6400

6450

6500

6550

6600

6650

6700

6750

6800

6850

6900

6950

7000

7050

7100

7150

7200

7250

7300

7350

7400

7450

7500

7550

7600

7650

7700

7750

7800

7850

7900

7950

8000

8050

8100

8150

8200

8250

8300

8350

8400

8450

8500

8550

8600

8650

8700

8750

8800

8850

8900

8950

9000

9050

9100

9150

9200

9250

9300

9350

9400

9450

9500

9550

9600

9650

9700

9750

9800

9850

9900

9950

10000

10050

10100

10150

10200

10250

10300

10350

10400

10450

10500

10550

10600

10650

10700

10750

10800

10850

10900

10950

11000

11050

11100

11150

11200

11250

11300

11350

11400

11450

11500

11550

11600

11650

11700

11750

11800

11850

11900

11950

12000

12050

12100

12150

12200

12250

12300

12350

12400

12450

12500

12550

12600

12650

12700

12750

12800

12850

12900

12950

13000

13050

13100

13150

13200

13250

13300

13350

13400

13450

13500

13550

13600

13650

13700

13750

13800

13850

13900

13950

14000

14050

14100

14150

14200

14250

14300

14350

14400

14450

14500

14550

14600

14650

14700

14750

14800

14850

14900

14950

15000

15050

15100

15150

15200

15250

15300

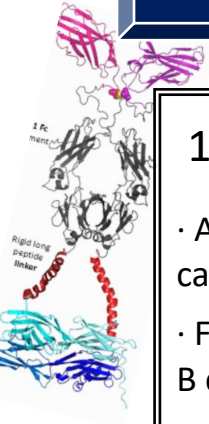
15350

15400

154



## A novel Bi-specific Fusion Protein With CTLA4-Ig/PD-L2 as a New Immunosuppressive Molecule (Duaccept) Modulating Alloimmune Responses



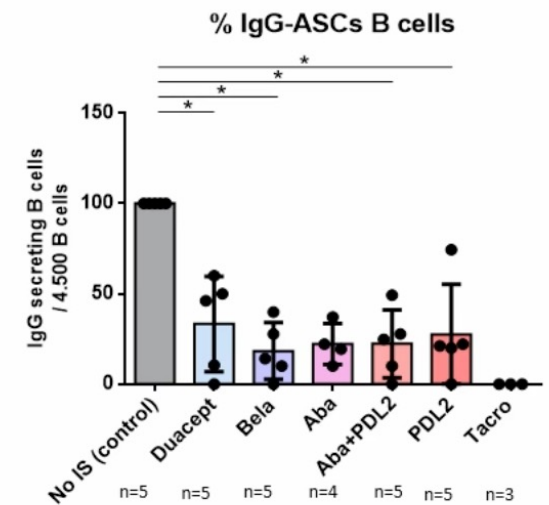
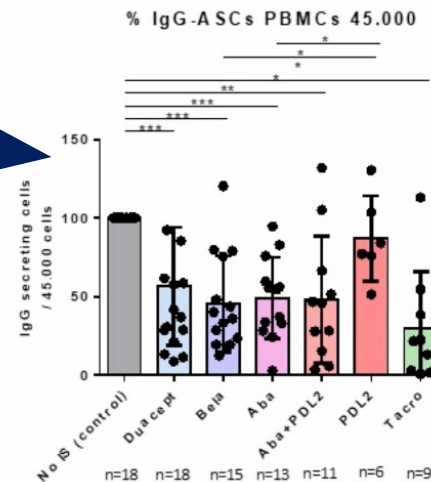
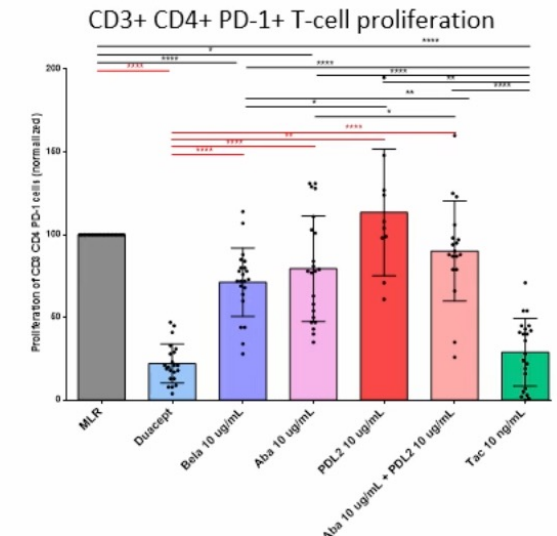
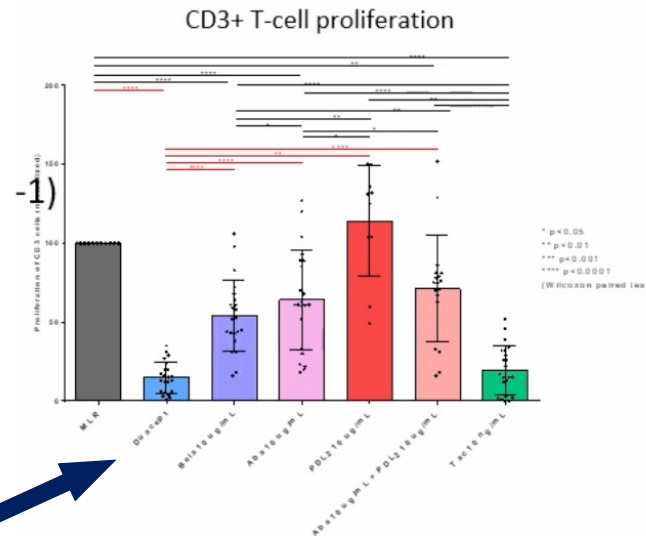
### 16 Sensitized kidney transplant candidates

- Allogeneic mixed lymphocyte reactions (MLR) to assess the capacity to inhibit T-cell proliferation *in vitro*
- FluorSpot assay to assess its capacity to hinder memory B cell differentiation to IgG-secreting plasmablasts.

(Duaccept – Bela – Aba – ABA+PDL2 – PDL2 – Tacro)

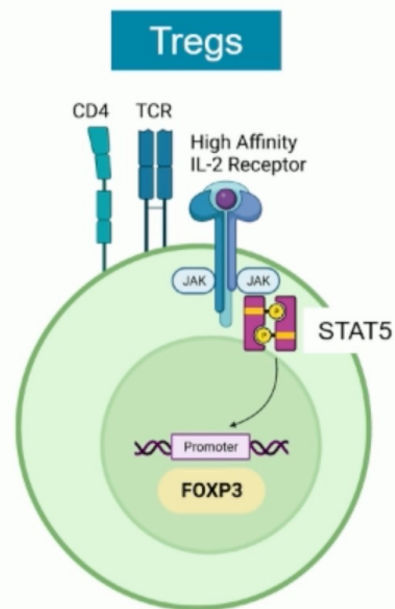
- \* Abrogates T cell proliferation in allogeneic MLR (more potently than CTLA4-Ig)
- \* Reduces allogeneic memory B cells activation and IgG producing plasma cell frequencies

**CONCLUSION: (CTLA4Ig/PDL2) shows high potential to effectively abrogate T and B alloimmune responses.**

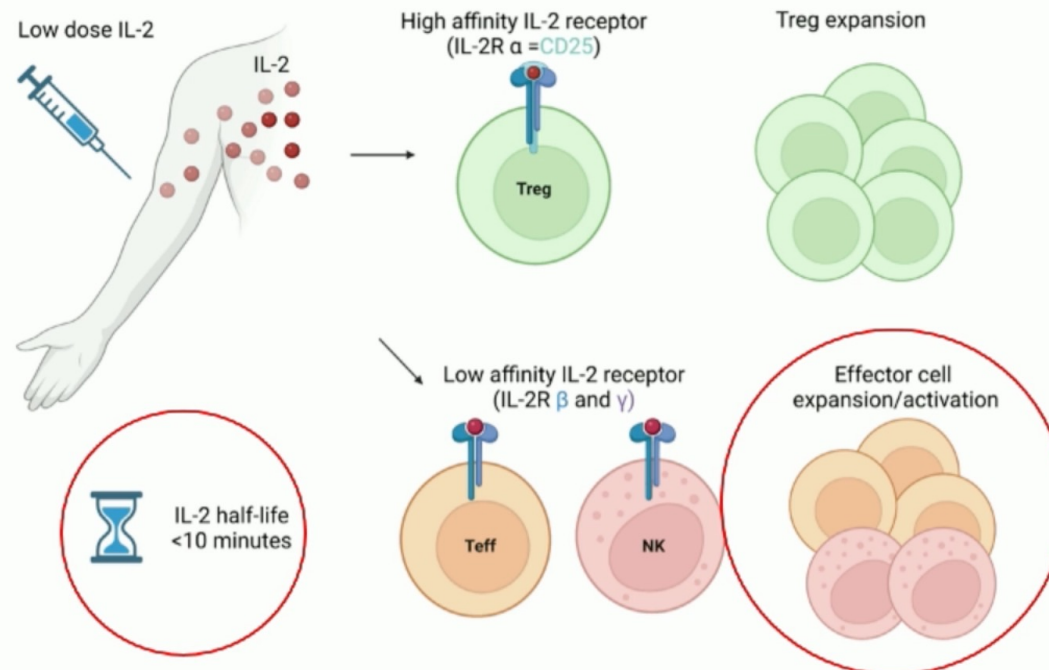


## Selective Regulatory T-cell Expansion By A Novel IL-2 Mutein In Murine Transplant And Humanized NSG Model

### Regulatory T cells (Treg) Expansion in Transplantation

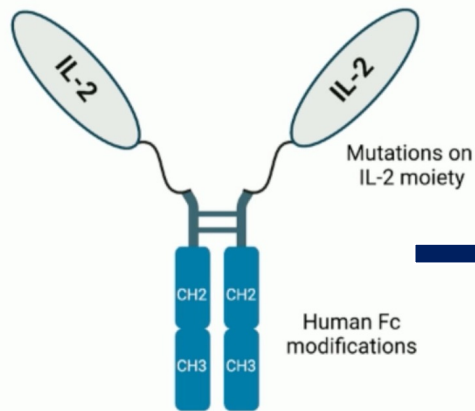


### Treg expansion with low dose IL-2 in transplantation

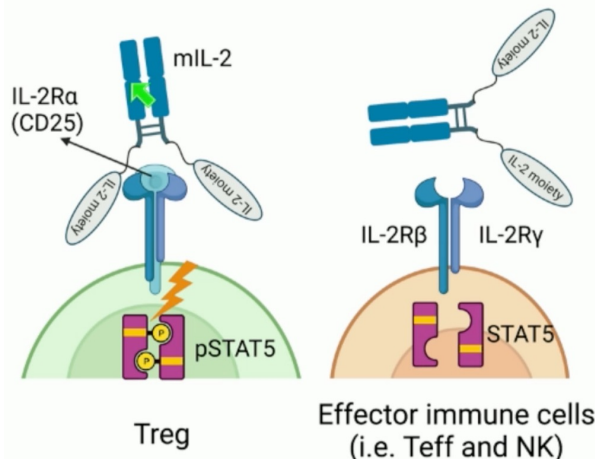


## Selective Regulatory T-cell Expansion By A Novel IL-2 Mutein In Murine Transplant And Humanized NSG Model

### Novel IL-2 Mutein (mIL-2)

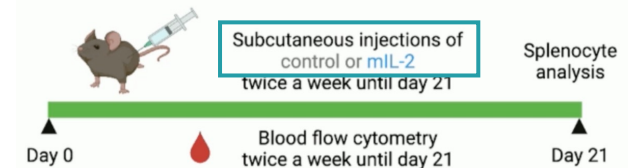


- Selective for Tregs
- Longer plasma half-life
- Decreased IL-2R $\beta$  and IL-2R $\gamma$  but maintained IL-2R $\alpha$  affinity
- Engineered for molecular stability and developability
- Fusion of human IgG1 Fc



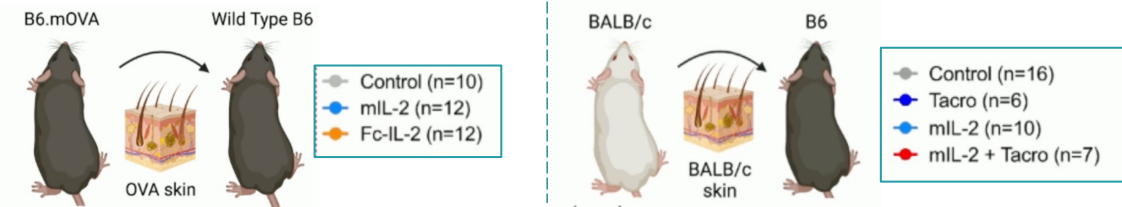
1

### Mouse splenocytes and human PBMCs *in vitro*



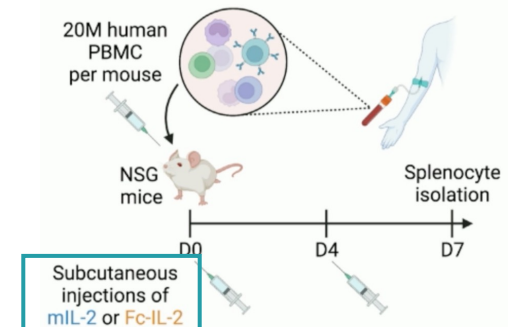
2

### Minor mismatched (OVA to B6) and Major mismatched (BALB/c to B6) Skin Transplant models



3

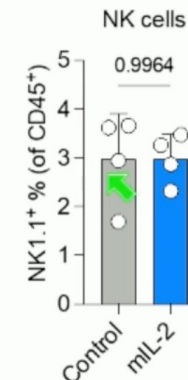
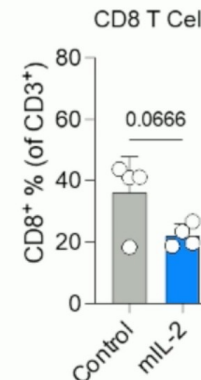
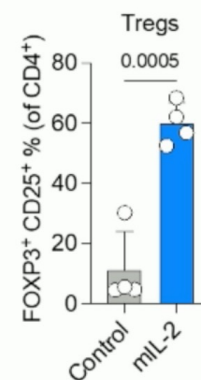
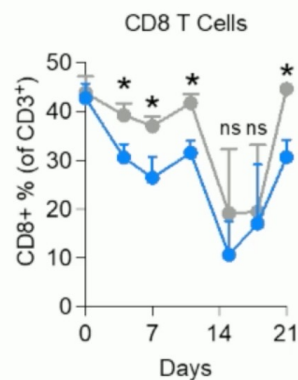
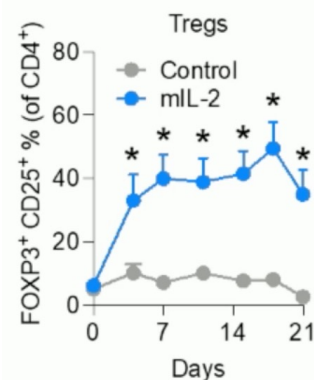
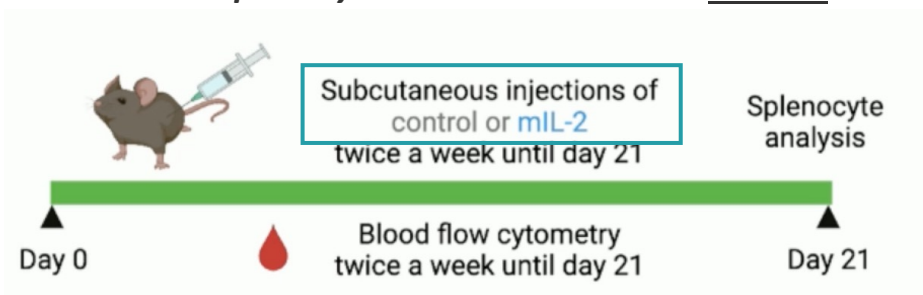
### NSG mouse model (human PBMCs transferred to NSG recipients)



## Selective Regulatory T-cell Expansion By A Novel IL-2 Mutein In Murine Transplant And Humanized NSG Model

1

*Mouse splenocytes and human PBMCs in vitro*



**Blood**

**Spleen, day 21**

Unpublished Data

\* mIL-2 induces selective and sustainable Treg expansion and has a prolonged half life

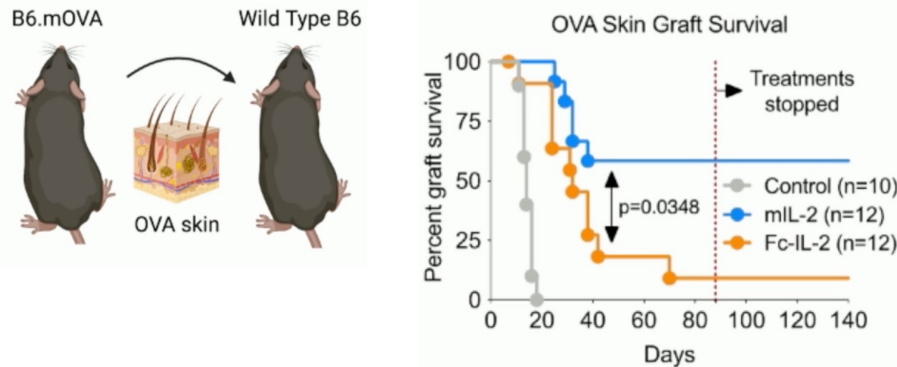


## Selective Regulatory T-cell Expansion By A Novel IL-2 Mutein In Murine Transplant And Humanized NSG Model

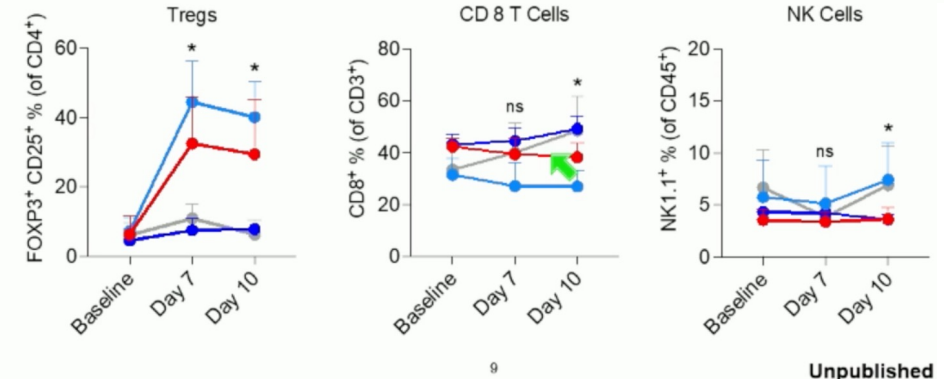
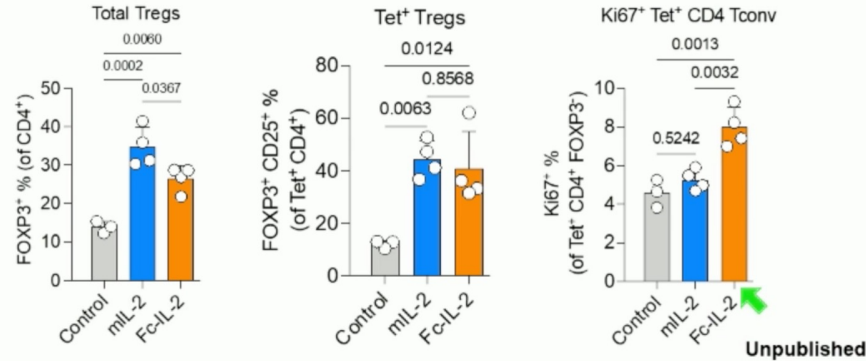
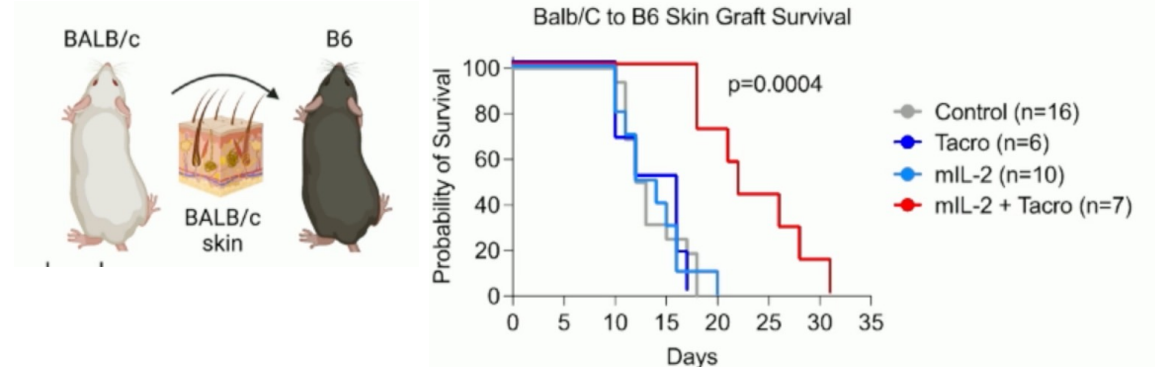
### 2

### Skin transplant models

#### Minor mismatched (OVA to B6)



#### Major mismatched (BALB/c to B6)



\* mIL-2 prolongs survival in both (minor & major mismatched) skin transplantation

\* mIL-2 promotes antigen-specific tolerance

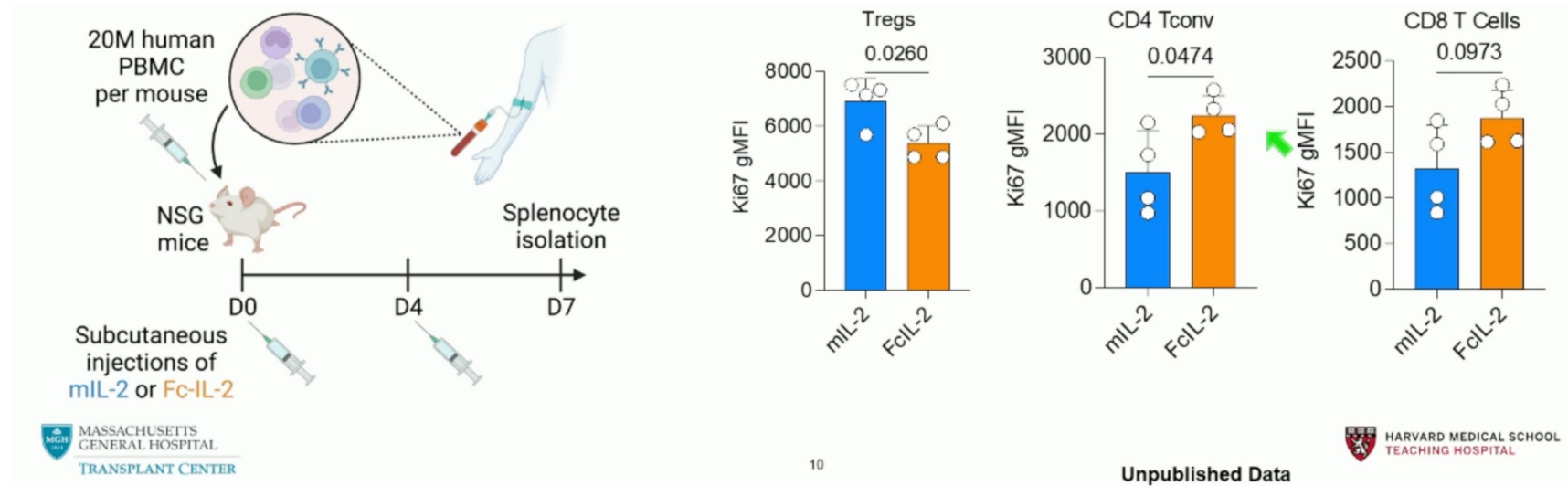


## Selective Regulatory T-cell Expansion By A Novel IL-2 Mutein In Murine Transplant And Humanized NSG Model

### 3

### NSG mouse model

(human PBMCs transferred to NSG recipients)



\* mIL-2 selectively expands human Tregs

**CONCLUSION: mIL-2 selectively expands both mouse and human Tregs and prolongs skin graft survival, inducing antigen-specific tolerance.**

## AXL Inhibition Suppresses Monocyte-to Macrophage Differentiation and Prolongs Allograft Survival

### BACKGROUND

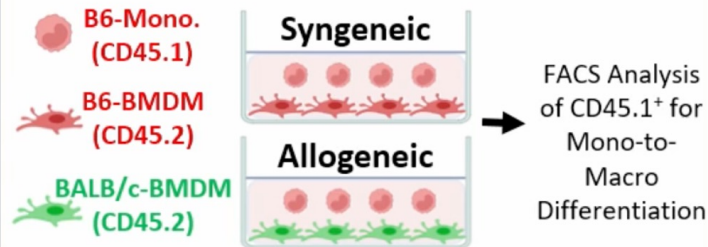
Early intragraft monocyte and macrophage accumulation, and subsequent inflammation correlates with poor transplantation outcomes. (Kirk, AJT 2008)

Single cell transcriptomic data implicates **AXL kinase** as a unique molecule of interest in trajectory analysis of intragraft monocyte-derived macrophage differentiation. (Luo, JCI Insight 2020)

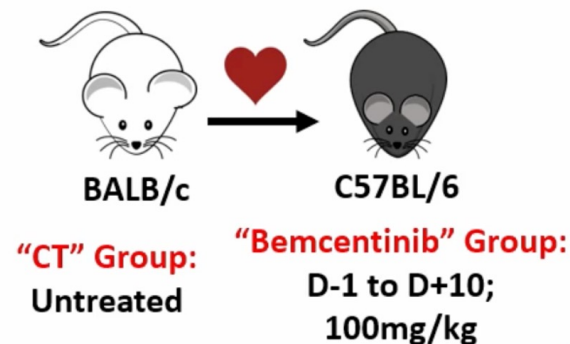
**Bemcentinib** (small molecule AXL inhibitor) would serve as a novel therapeutic approach in transplantation to selectively target the monocyte-macrophage lineage.

### METHODS

#### Model 1: D/R in-vitro innate cell co-culture



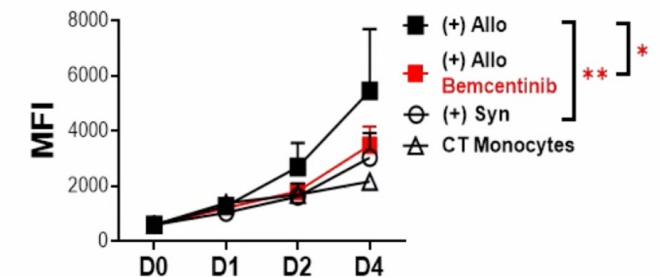
#### Model 2: Mouse heterotopic heart transplant



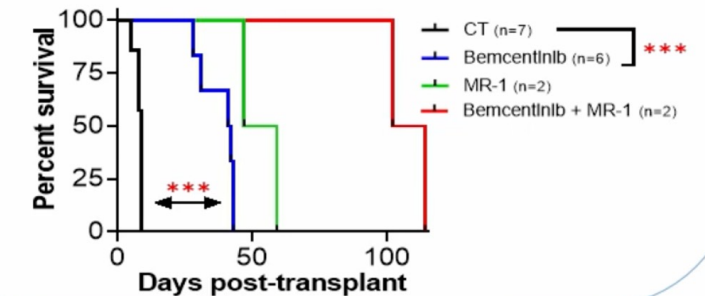
### FINDINGS

#### Result 1: AXL inhibition attenuates monocyte-derived macrophage differentiation.

##### F4/80 Expression of CD45.1<sup>+</sup> cells



#### Result 2: Bemcentinib therapy prolongs cardiac allograft survival.



### CONCLUSION:

- **AXL kinase**: master regulator of intragraft monocyte-derived macrophage differentiation.
- **Blockade of AXL kinase with bemcentinib** prolongs cardiac allograft survival.



# Bac-Transgenic Mice Show a Novel T-Cell Intrinsic Excitatory Role for Apol1 Risk-Alleles

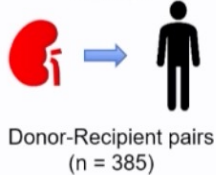
**Systems Biology – Biomarkers**

JCI The Journal of Clinical Investigation

Recipient APOL1 risk alleles associate with death-censored renal allograft survival and rejection episodes

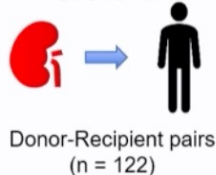
Zhongyang Zhang, ... , Barbara Murphy, Madhav C. Menon

Discovery cohort  
GOCAR



Donor-Recipient pairs  
(n = 385)

Validation cohort  
CTOT01/17



Donor-Recipient pairs  
(n = 122)

APOL1 genotyping

G1: S342G & I384M      G2: NY388-389deletion

Recipient APOL1 genotype:

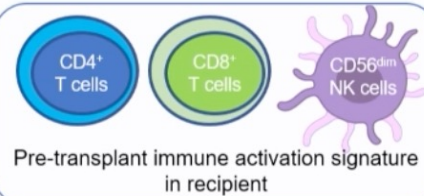
- 1 risk allele (intermediate risk): G0/G1, G0/G2  
- 2 risk alleles (high risk): G1/G1, G2/G2, G1/G2

VS

- 0 risk allele (low risk): G0/G0

independent of

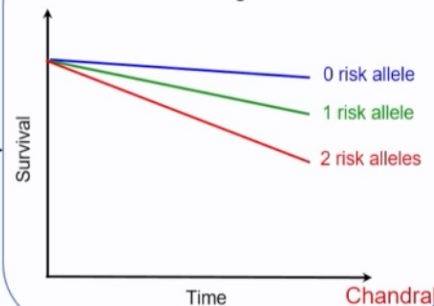
Donor APOL1 genotype



Pre-transplant immune activation signature in recipient

Increased post-transplant  
T cell-mediated rejection

Reduced allograft survival



Chandraker

BAC transgenic mice to study non-podocyte role of G1, G2-APOL1

BAC-transgenic APOL1 mouse models used



Bac TG – APOL1 overexpression model  
APOL1 Promoter responds to Endogenous Cytokines  
Yale O'Brien mouse phenotyping core : Dr Somlo



TRE-IFNG/ Rosa-m26-rTTA  
Shuta Ishibe



TRE-IFNG/Bac TG – APOL1 overexpression model  
APOL1 Promoter responds to DOX+ IFNG expression

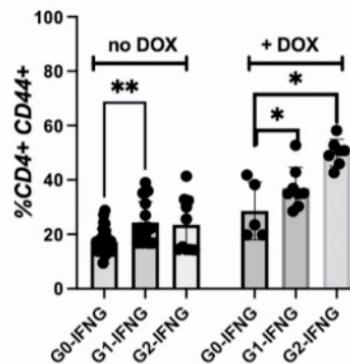
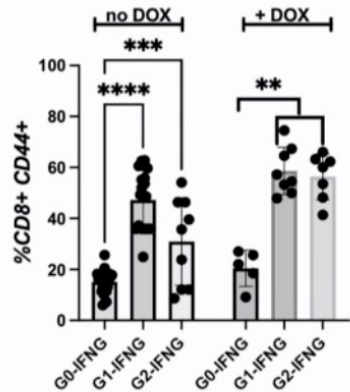


+ / – DOX

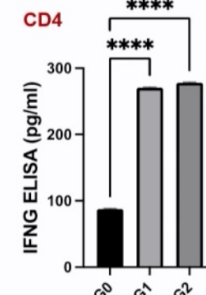
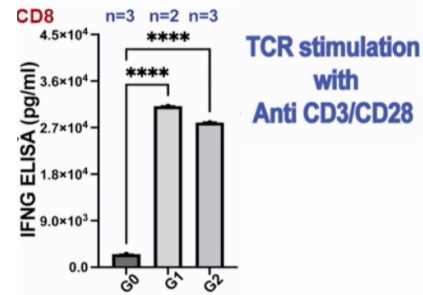
McCarthy G,...Pollak M, et al; 2021  
Olabisi et al; JCI: 2022

# Bac-Transgenic Mice Show a Novel T-Cell Intrinsic Excitatory Role for Apol1 Risk-Alleles

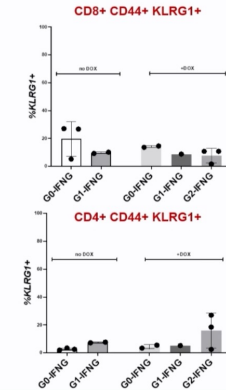
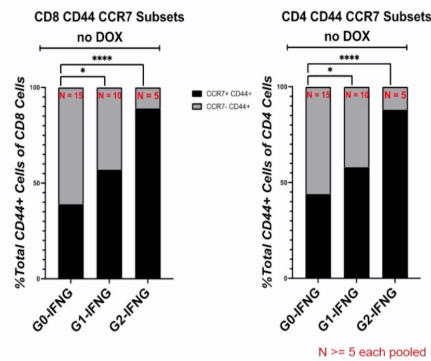
## CD8+ and CD4+ Activation in G1, G2



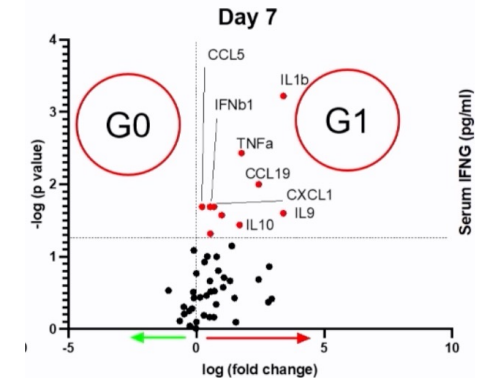
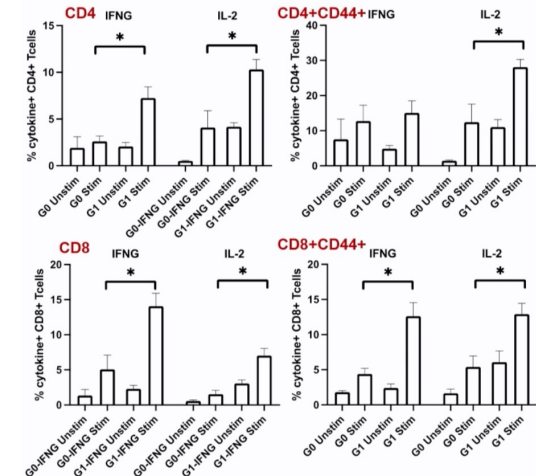
## G1-CD8+ T cells: increased proliferation/IFNg production



## APOL1 variant CD44+ T cell phenotype: increased CCR7+ expression



## Cytokine production after Poly (I:C) in Bac-Tg mice



CONCLUSION: A novel T-cell intrinsic role for APOL1-exonic variants increasing activation, proliferation and cytokine production.



## Genome-Wide Survival Study Identifies Three Novel Associations between Non-HLA Donor-Recipient Mismatches and Kidney Graft Failure

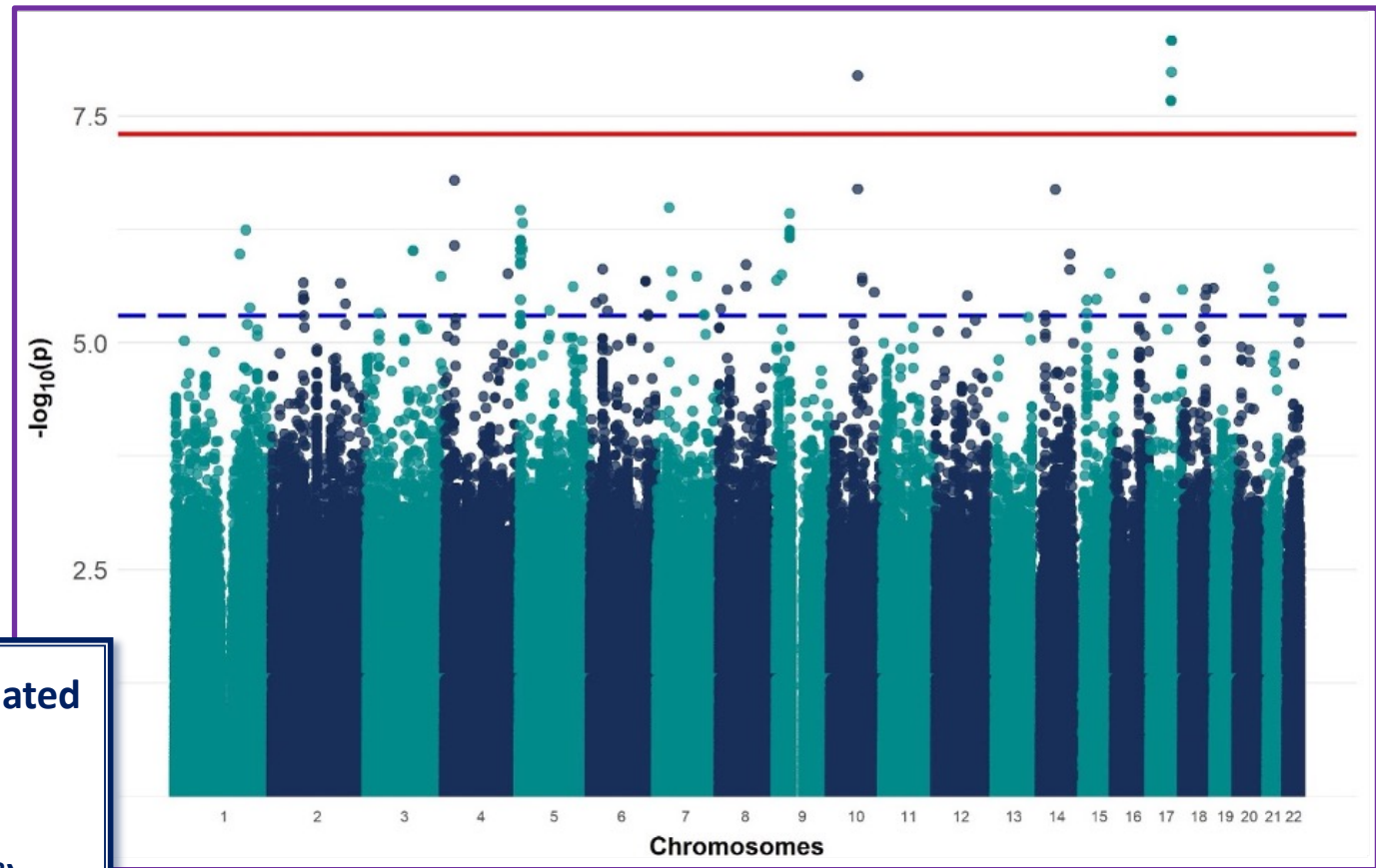
Non-HLA donor-recipient mismatches could be associated with kidney graft survival.

Analysis using genome-wide data in a large French monocentric cohort (KiT-GENIE):

- Transplanted in Nantes 2002-2018
- 1,482 complete European pairs
- Mean follow-up = 6,7 years
- 303 graft failure events

Three D-R mismatches outside of the HLA region were associated with kidney graft survival:

- Chromosome 10 intronic mismatch (HR=3.5,  $p=3.1 \times 10^{-8}$ )
- Chromosome 17 intergenic mismatch (HR=4.0,  $p=1.0 \times 10^{-8}$ )
- Chromosome 21 intronic mismatch (HR=5.2,  $p=2.0 \times 10^{-8}$ )



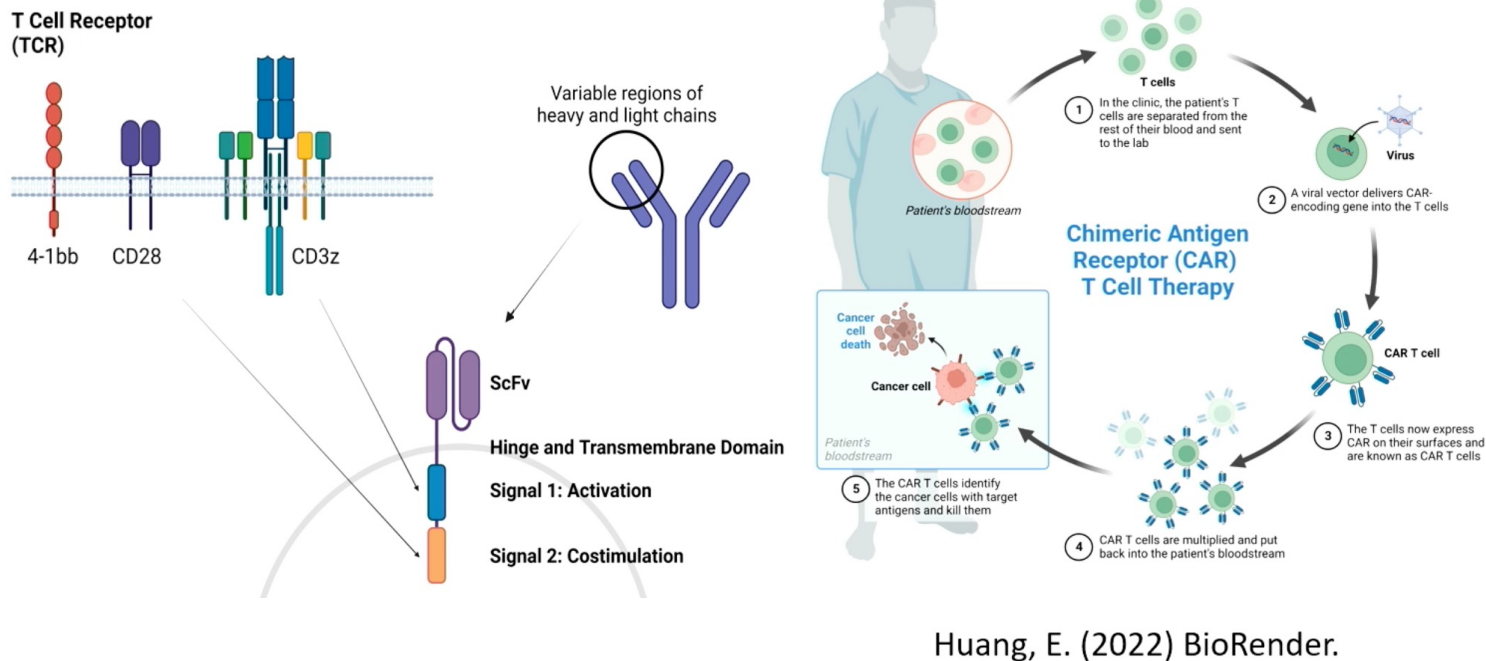
GWSS results for D-R mismatches in European pairs for time-to-Kidney graft failure (significance threshold in red)

(External validation pending)



## Chimeric HLA Antibody Receptor T Cells for Targeted Therapy of Antibody Mediated Rejection in Transplantation

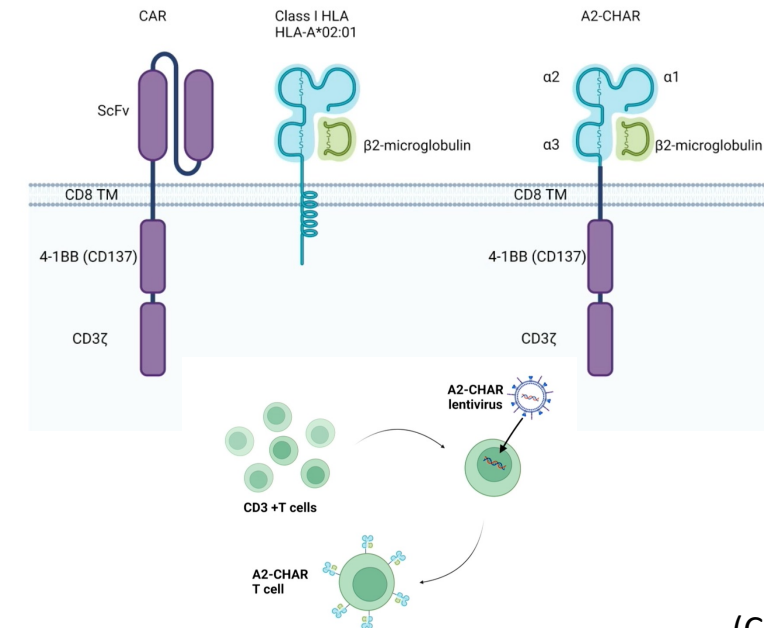
### Chimeric Antigen Receptor (CAR) structure and CAR-T cell therapy



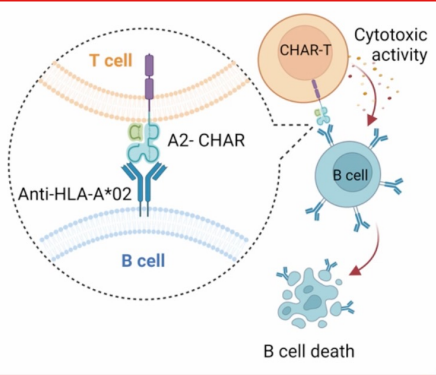
**Hypothesis:** Transduction of cytotoxic T cells with a CAR may be used to develop a targeted therapy for DSA desensitization and ABMR.

**Objective:** To generate T cells with chimeric anti-HLA antibody receptor (CHAR-T cells) that specifically eliminate donor-specific HLA class I antibody-producing B cells.

### Chimeric HLA Antibody Receptor (CHAR) design

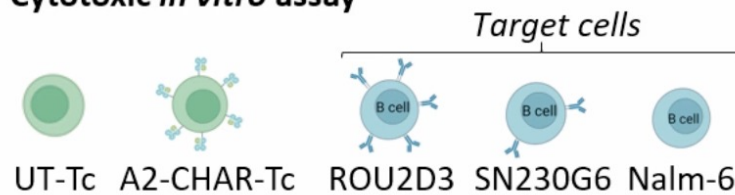


## Chimeric HLA Antibody Receptor T Cells for Targeted Therapy of Antibody Mediated Rejection in Transplantation

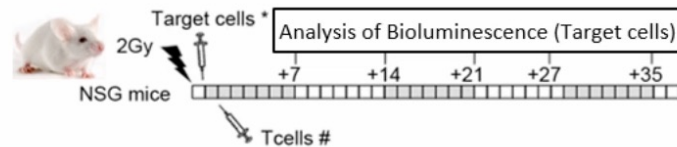


**ROU2D3 and SN230G6:** B cells that express antibodies against HLA-A2 on their membrane

### Cytotoxic *in vitro* assay



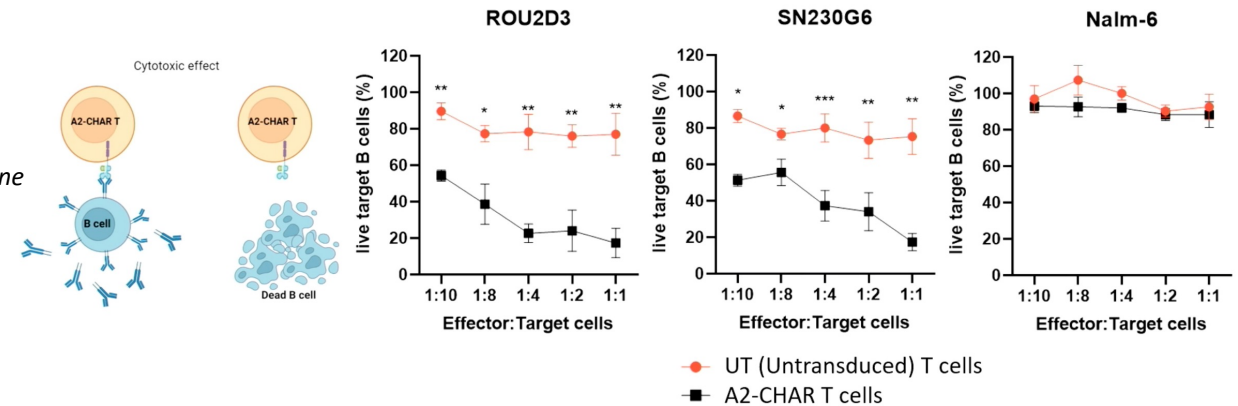
### Cytotoxic *in vivo* assay



### CONCLUSION:

- CHAR technology can kill, *in vitro* and *in vivo*, anti-HLA antibody producing B cells.
- CHAR-T cells could be useful in DSA desensitization protocols and ABMR therapy, by selectively eliminating donor-specific anti-HLA antibody producing B cells.

### A2-CHAR-T Lymphocytes: *in vitro* cytotoxic activity



### A2-CHAR-T Lymphocytes: *in vivo* cytotoxic activity

