

Daratumumab and belatacept dual targeting impacts on B-cell immunity in highly sensitized patients

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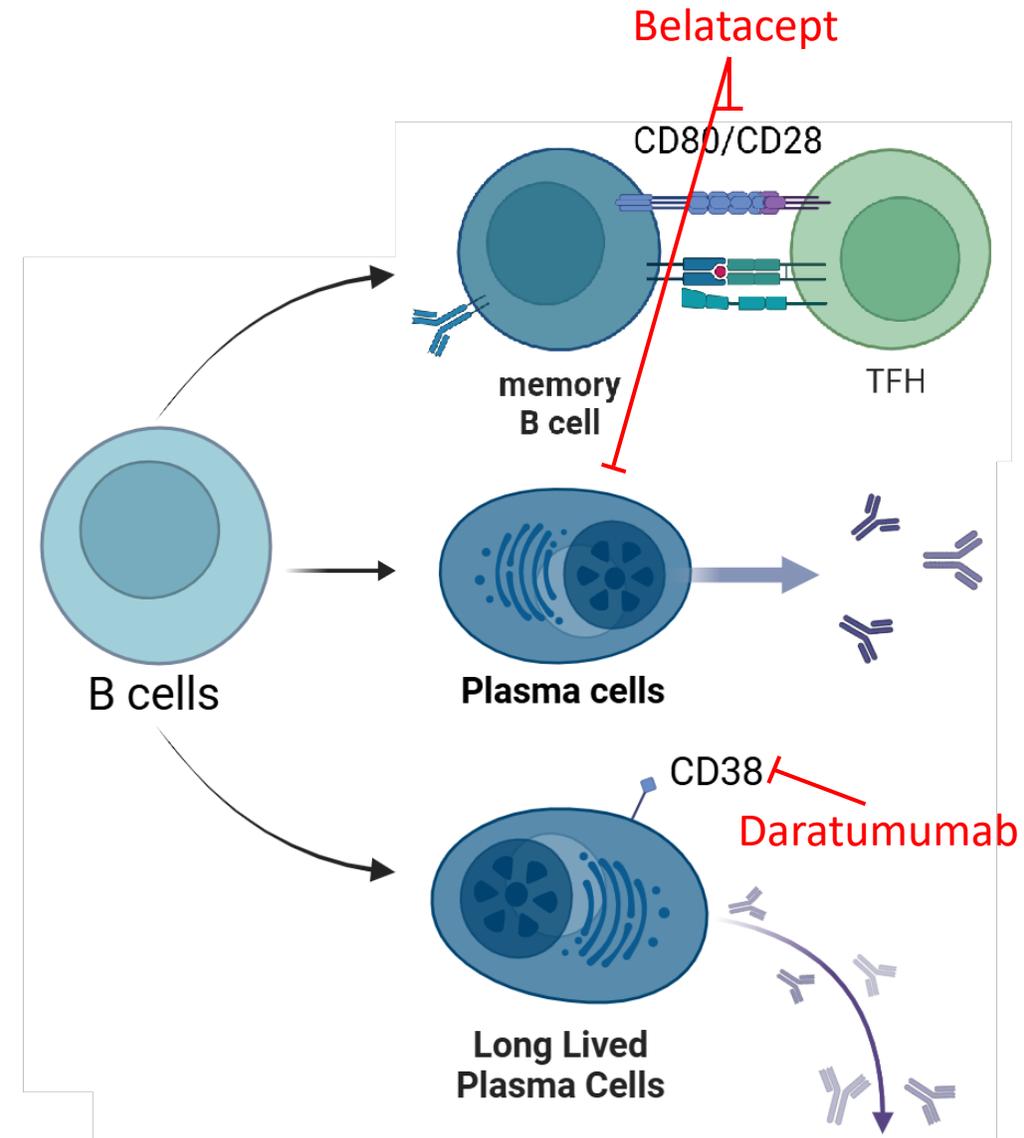
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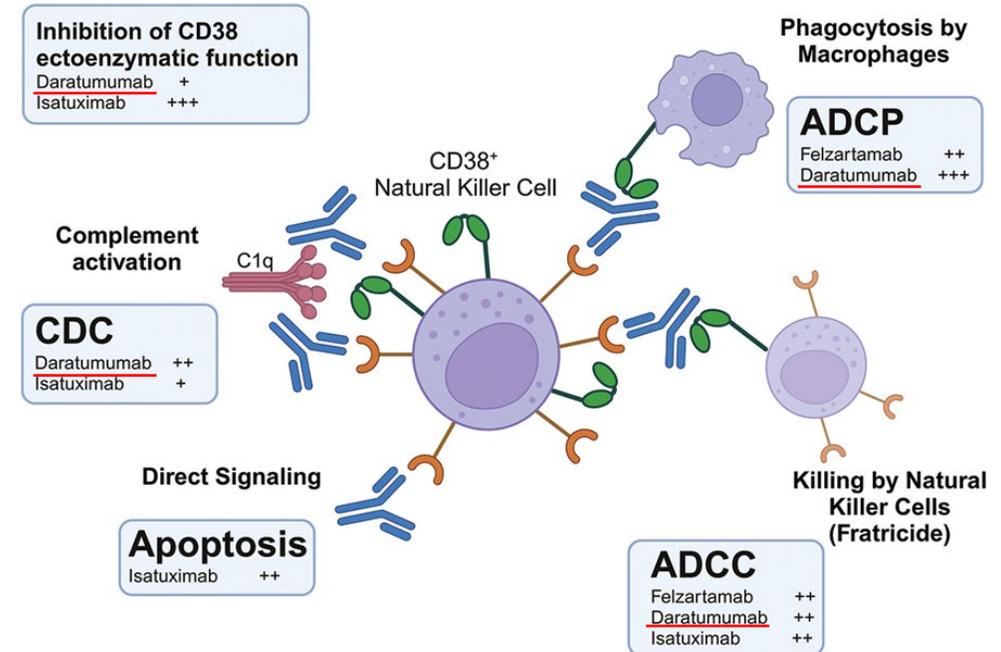
Background

- Current desensitization therapies have shown very poor success to decrease anti-HLA antibodies in **highly sensitized patients**.
- Besides central bone marrow-residing **long-lived plasma cells (LLPC)**, peripheral memory B cells (mBC) with their **T Follicular Helper Cell (TFH)** counterparts contribute to antibody formation.
- We designed a prospective, phase-I, exploratory pilot trial (COMBAT; NCT05145296) based on a dual-target approach with co-stimulation blockade with **belatacept** and the anti-CD38 mAb **daratumumab** to abrogate these alloimmune compartments.



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- The anti-CD38 mAb daratumumab covers **the broadest spectrum of modes of action** targeting CD38-expressing plasma cells



Hypothesis and objectives

Combining a dual target immunosuppressive approach would lead to a sustained inhibition of anti-HLA antibody formation, allowing highly sensitized kidney transplant (KT) candidates on the waiting list to have access to an HLA compatible kidney transplant.

OBJECTIVES:

- To evaluate the **safety** of this dual combination therapy in highly sensitized patients waiting for a compatible kidney donor.
- Assess the **biological effect** of the dual combination of belatacept and daratumumab on anti-HLA antibody reduction using single antigen beads (SAB), and circulating HLA-specific mBc, TFH cells and LLPC in bone marrow.

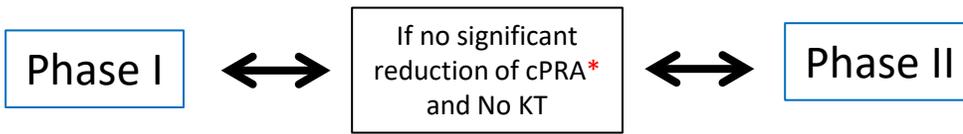
Study design and Patient characteristics

- Phase Ia/II single arm, pilot study
- **Primary endpoint:** Safety
- **Secondary Clinical endpoint:** Efficacy
 - a) significant decrease in Ab MFI ($\geq 80\%$ or to a value < 2000 MFI) of ≥ 10 HLA antibodies class I and II or
 - b) decrease in cPRA to $< 98\%$.
- **Secondary Mechanistic endpoint:**
 - Decrease in bone-marrow residing HLA-producing LLPC
 - Decrease in peripheral HLA-specific mBc
- **Patients of the study**
 - 5 highly sensitized KT candidates, with cPRA $\geq 99.5\%$ waiting for a compatible HLA donor offer for > 3 years



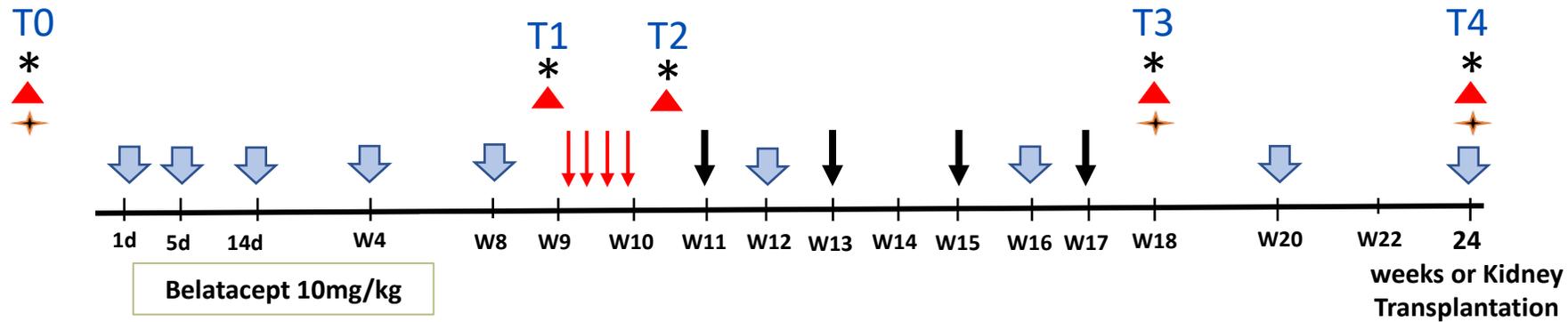
Median age , years (min-max)	41 (28-68)
Sex , n (%)	
Male	2 (40.0)
Female	3 (60.0)
Dialysis time , median years (min-max)	9 (5-17)
Waitlist time , median years (min-max)	6 (5-16)
Cause of renal failure , n (%)	
Congenital	1 (20.0)
Glomerular disease	3 (60.0)
Unknown	1 (20.0)
No. of prior kidney transplants , n (%)	
1	5 (100.0)
Prior sensitizing events at screening , n (%)	
Pregnancy	1 (20.0)
Transfusion	2 (40.0)
Transplant	5 (100.0)

Methods: Immunosuppressive Protocols



- * Sera:
 - Anti-HLA Ab
- ✦ Bone Marrow Aspirate
 - Anti-HLA LLPC and mBc
- ▲ PB assessment:
 - Anti-HLA mBc

Low dose protocol: 3 patients

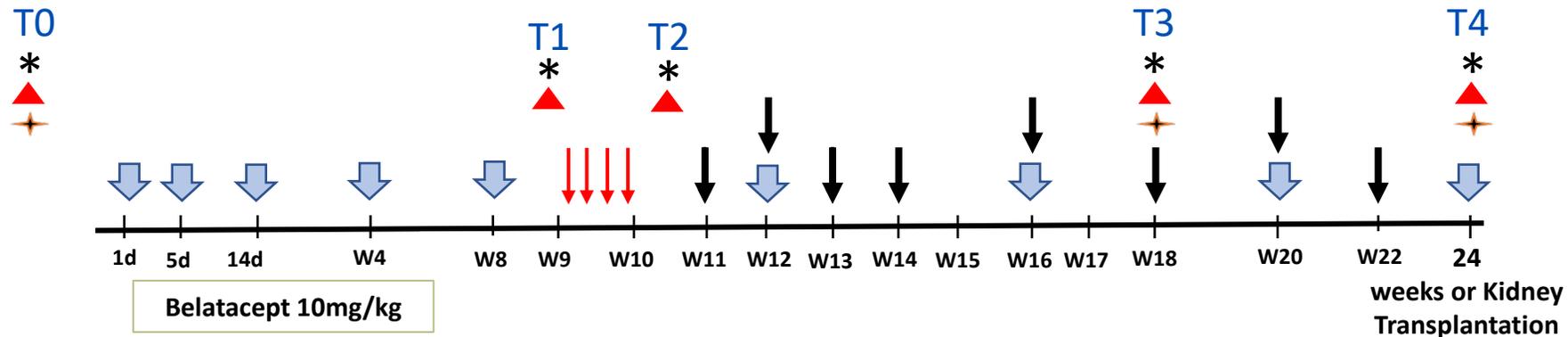


PE/IA /48h ↓

Belatacept 5mg/kg ↓

Daratumumab 8mg/kg ↓

Extended High dose protocol: 2 patients



PE/IA /48h ↓

Belatacept 5mg/kg ↓

Daratumumab 16mg/kg ↓

RESULTS: Safety

	All (N=5)	
	All Grades	Grade ≥3
Any event	4 (80.0)	1 (20.0)
Colitis	1 (20.0)	1 (20.0)
Infusion reaction	1 (20.0)	0
Hypertension	1 (20.0)	0
Nausea	1 (20.0)	0
Sigmoiditis	1 (20.0)	0
Hypotension	1 (20.0)	0
Trombosis	1 (20.0)	0



4 out of 5 patients completed the study

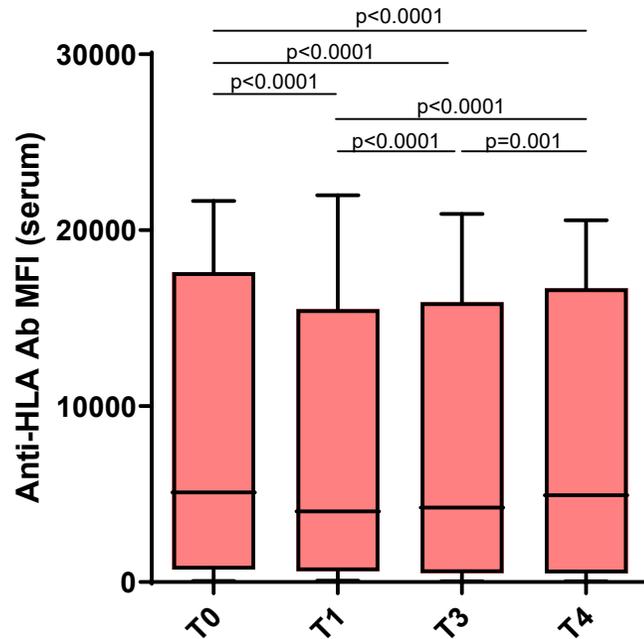
1 patient withdrew the study after the 4 initial doses of belatacept because of acute colitis that resolved with pharmacological therapy.

Overall, the study has showed a safe profile

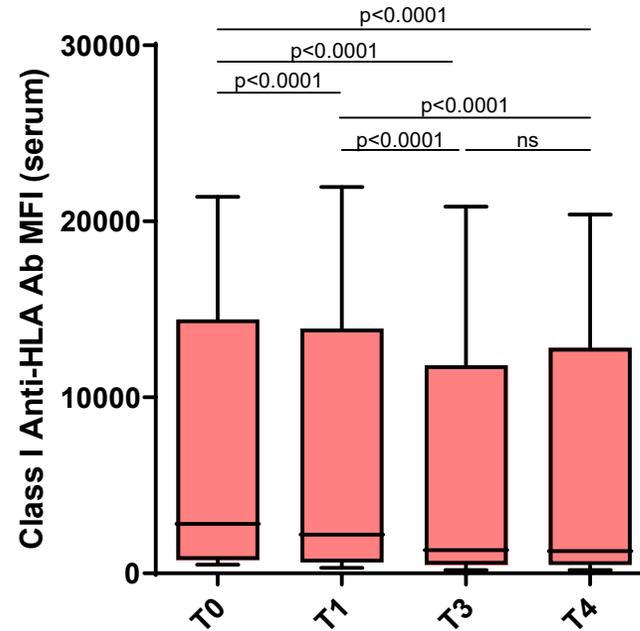
RESULTS (I): CLINICAL EFFICACY

A) Global impact on anti-HLA antibodies MFI levels over time

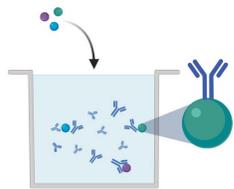
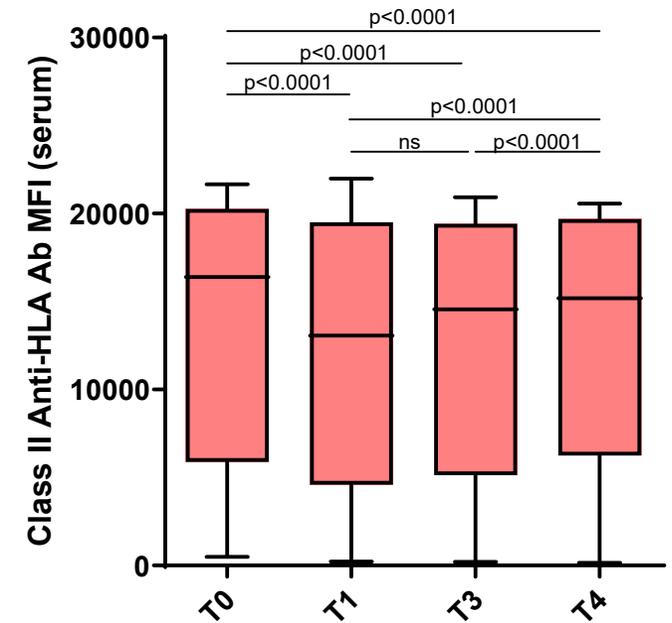
All anti-HLA Ab



Class I anti-HLA Ab



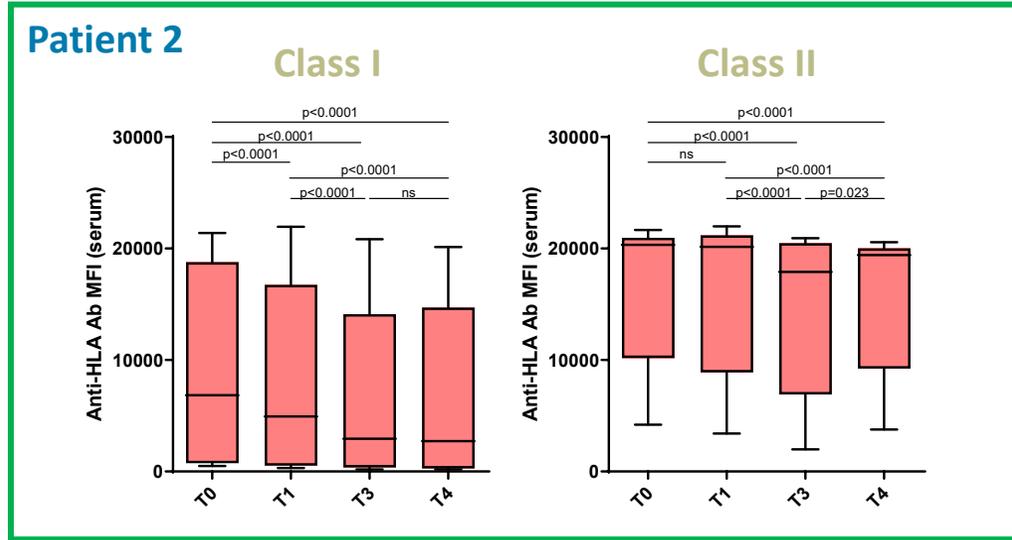
Class II anti-HLA Ab



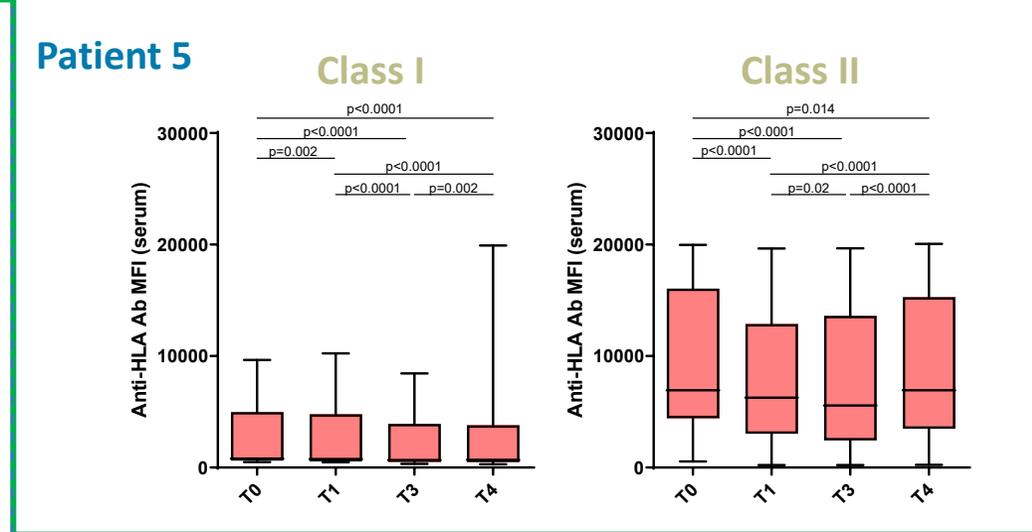
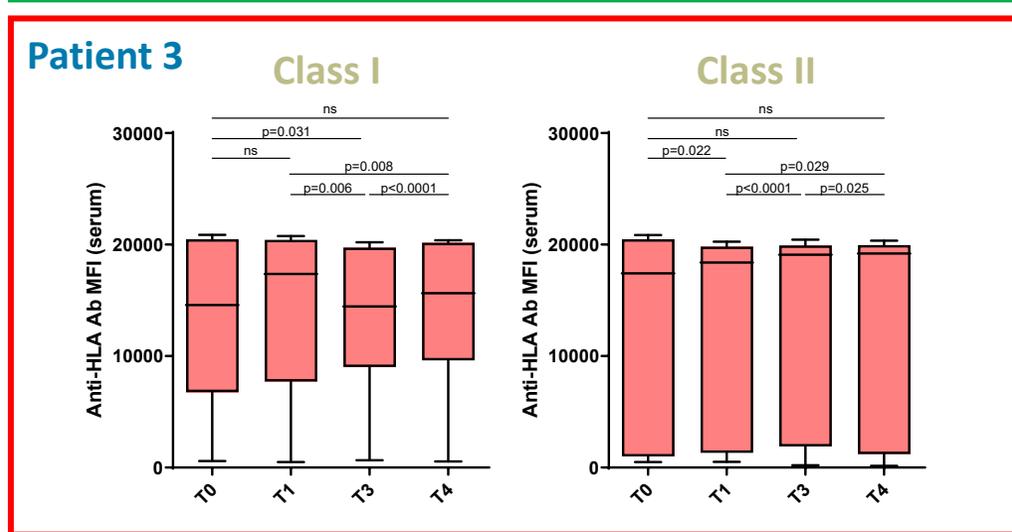
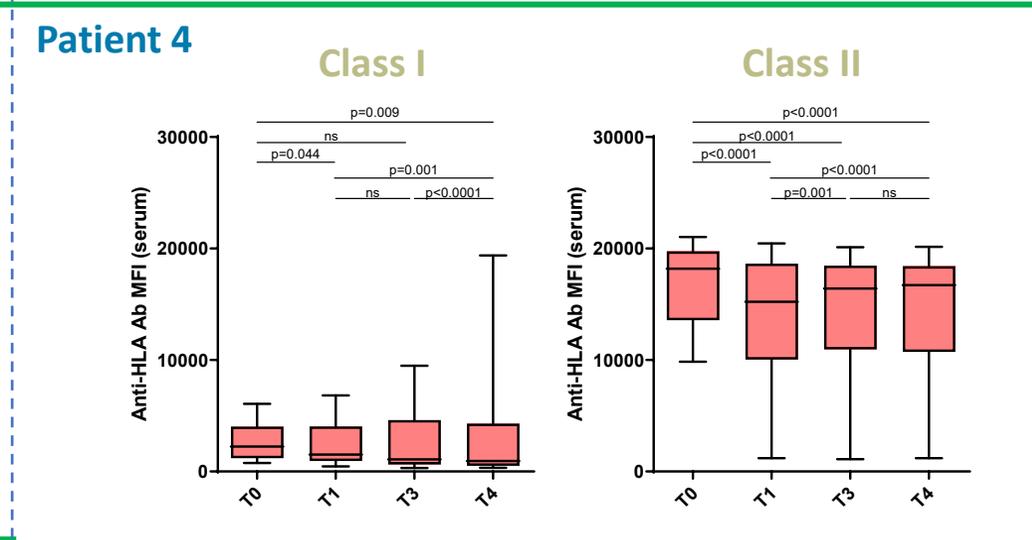
Single Antigen Beads Assays

Individual impact on anti-HLA antibody MFI levels over time

Low dose Daratumumab



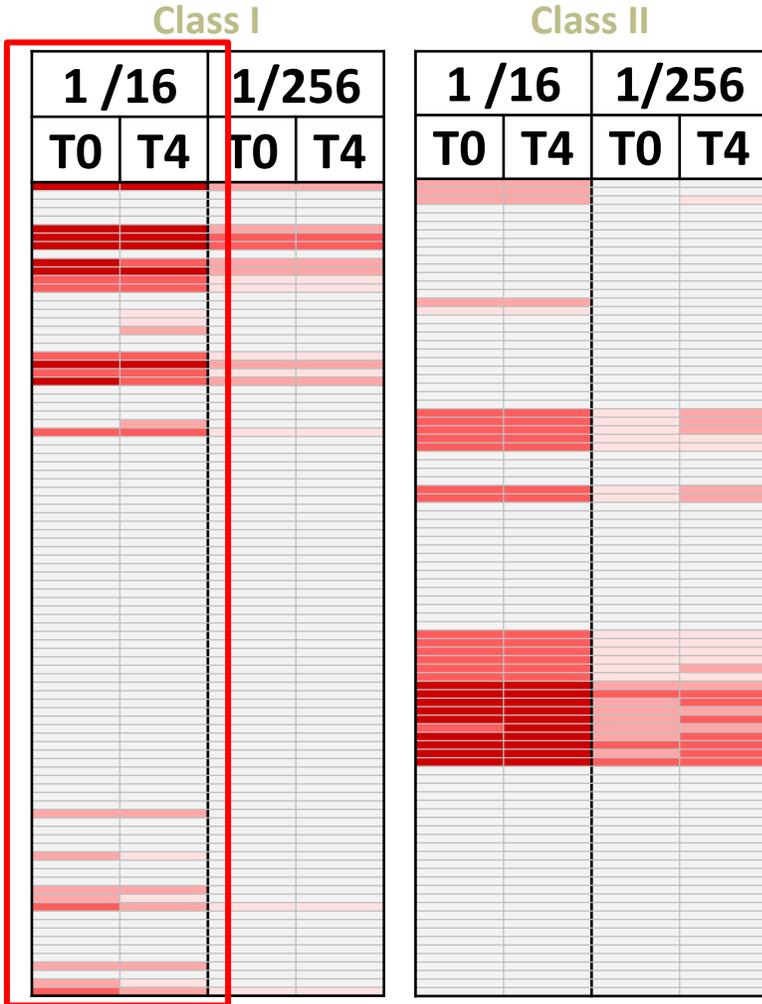
Extended High dose protocol



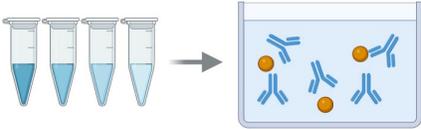
B) Global impact on anti-HLA antibody Titers over time

Responder patient

Non-Responder patient



Ab dilutions

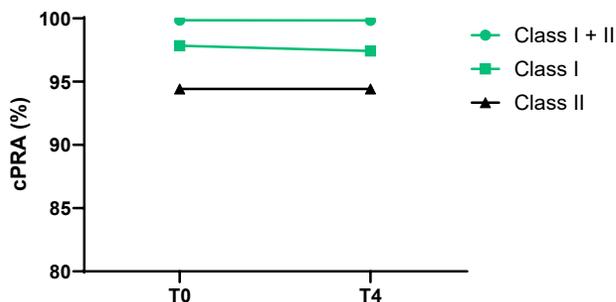


C) Impact on cPRA and anti-HLA antibodies

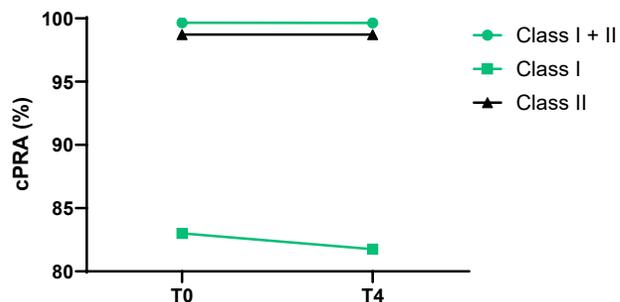
cPRA

Anti-HLA Ab elimination

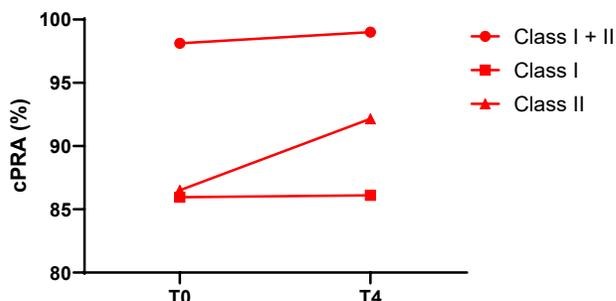
Patient 2



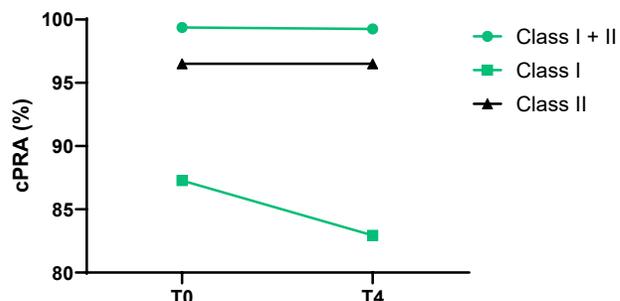
Patient 4



Patient 3

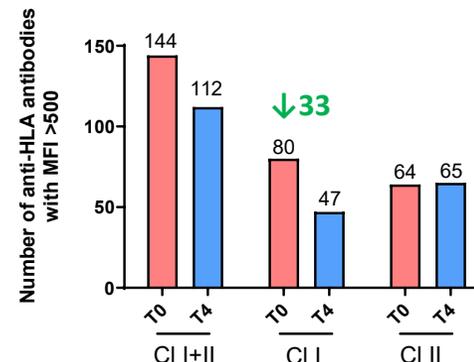


Patient 5

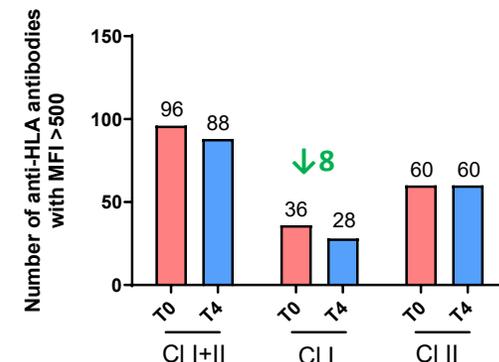


3 out of 4 patients showed significant, albeit low reduction on cPRA, especially in class I

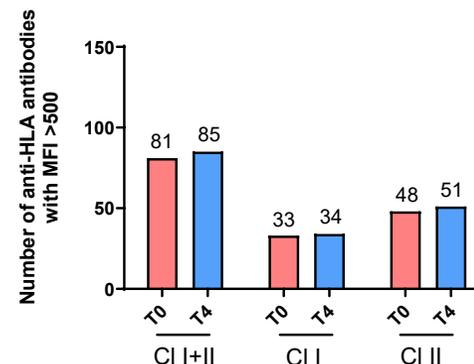
Patient 2



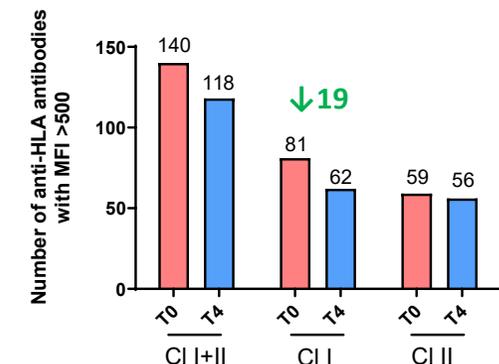
Patient 4



Patient 3



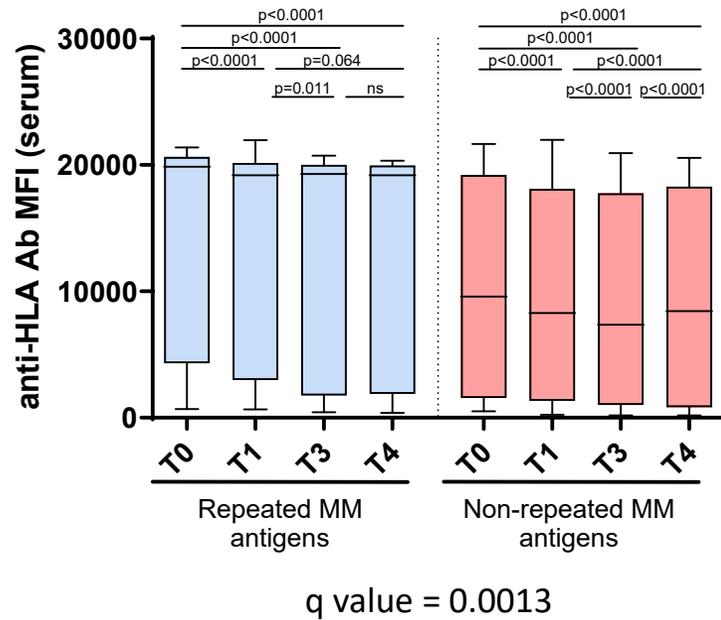
Patient 5



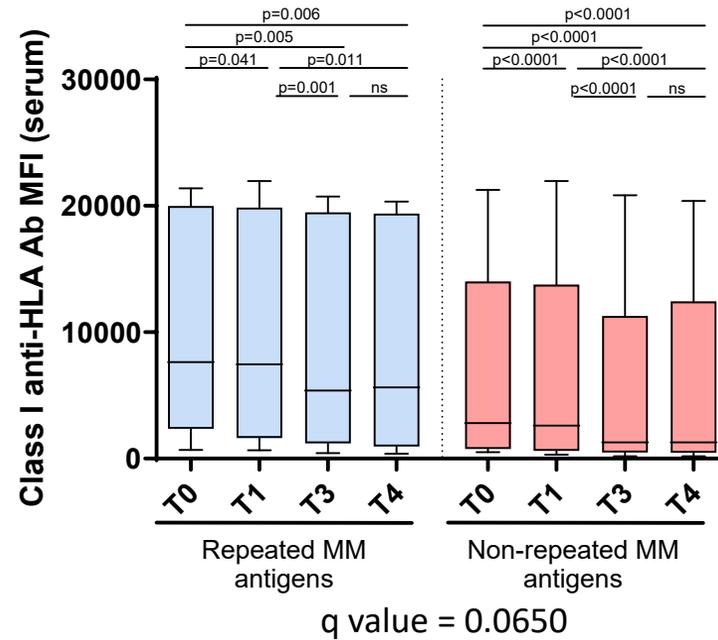
3 out of 4 patients displayed a complete elimination of class I antibodies, whereas class II remained unchanged

Differences between anti-HLA Ab against repeated and non-repeated MM Ag

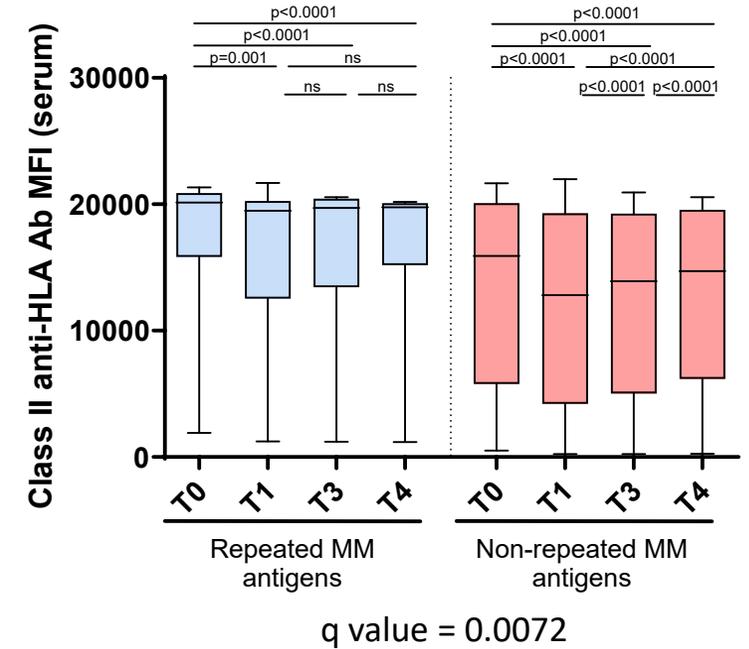
All anti-HLA Ab



Class I anti-HLA Ab



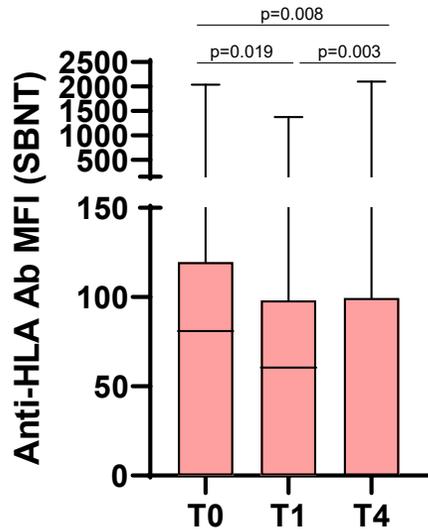
Class II anti-HLA Ab



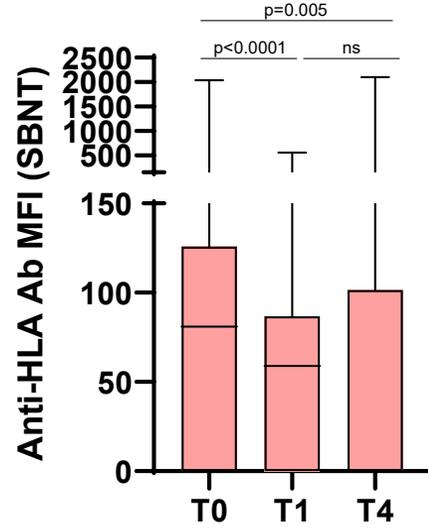
RESULTS (II): MECHANISTIC EFFICACY

A) Impact on circulating HLA-specific Ab-producing memory B cells

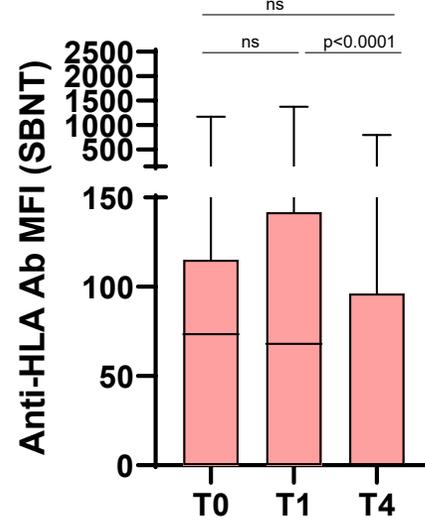
All anti-HLA Ab



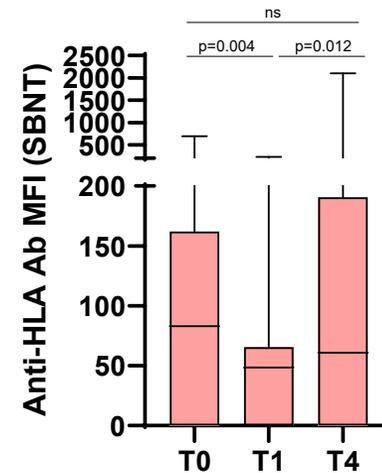
Class I anti-HLA Ab



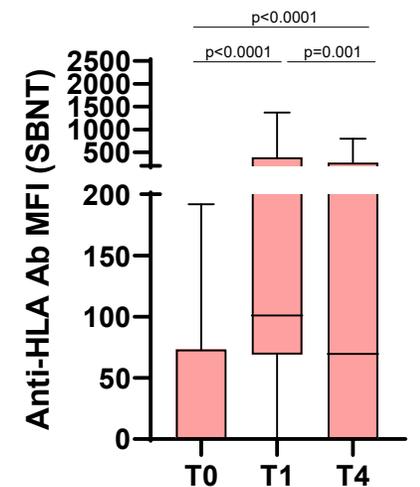
Class II anti-HLA Ab



Class I anti-HLA Ab



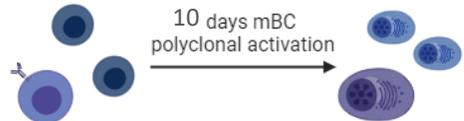
Class II anti-HLA Ab



Patient 3 (NON-RESPONDER)

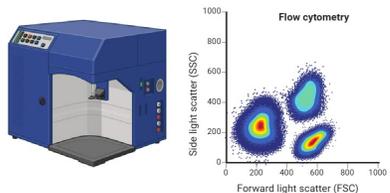
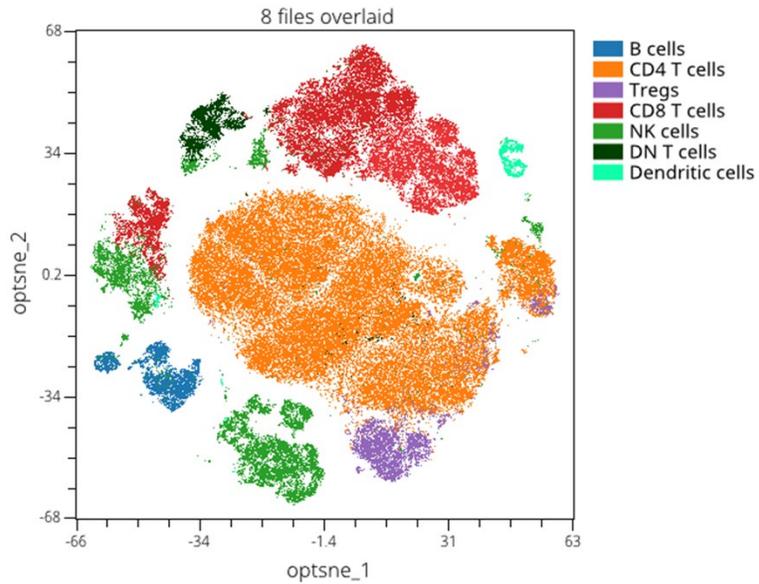
Recipient lymphocytes

Antibody-secreting cells



B) Multidimensional analyses of circulating cellular subset populations

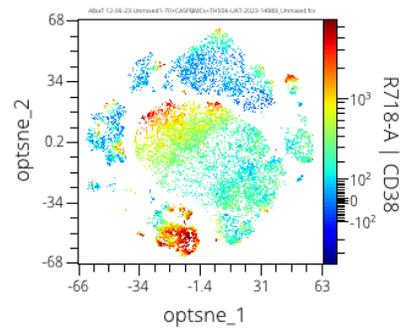
Depletion of CD38+ cell populations using Spectral Flow Cytometry



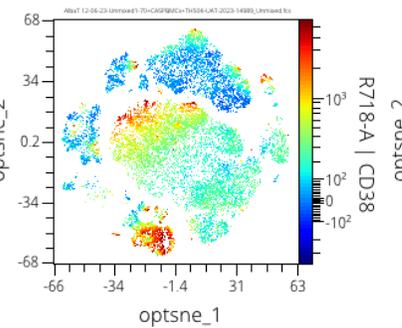
Multidimensional Spectral flow cytometry

T0

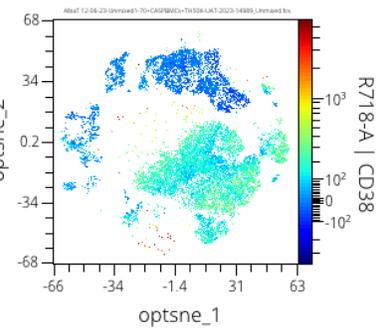
Responder patients



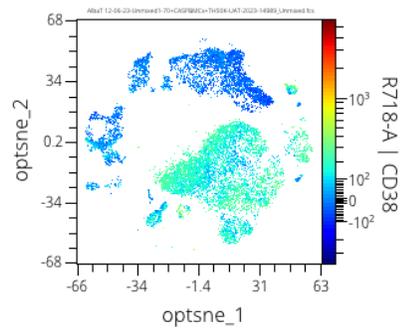
T1



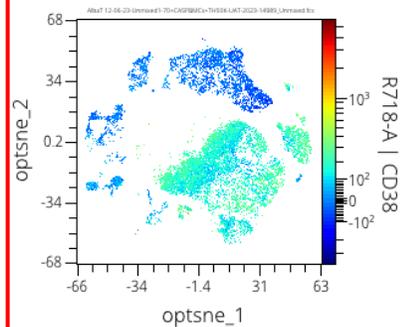
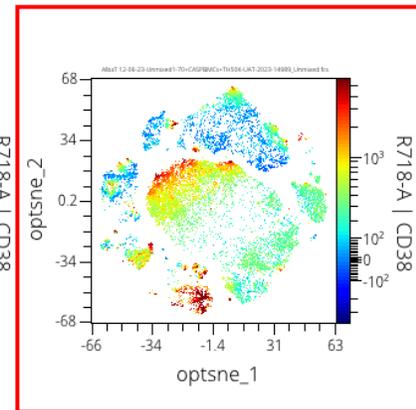
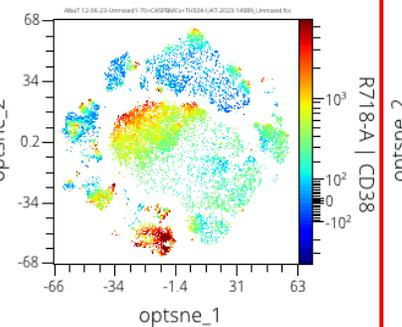
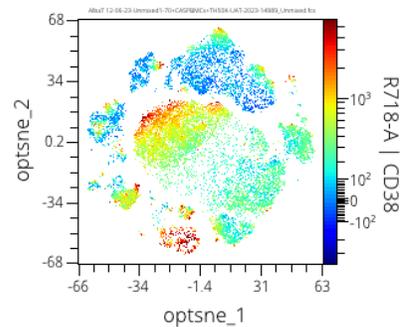
T3



T4



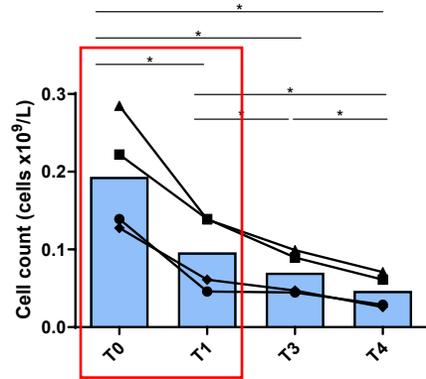
Non-responder patient



Fast depletion of peripheral memory B-cell compartments, but CD38- Sw-mBc

B cells

(CD3- CD19+)



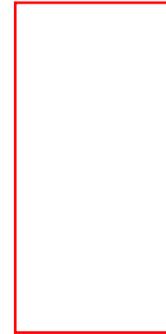
Plasma cells

(CD19+ CD20- CD38+)



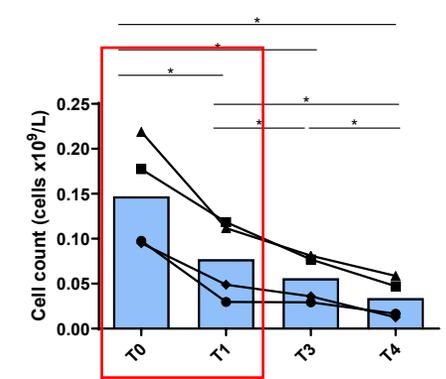
Plasmablasts

(CD19+ CD27+ IgD- CD38+ CD24-)



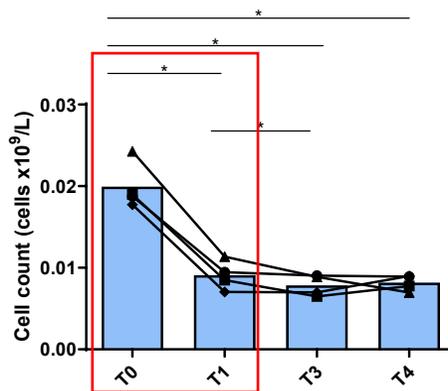
Naive B cells

(CD19+ CD27- IgD+)



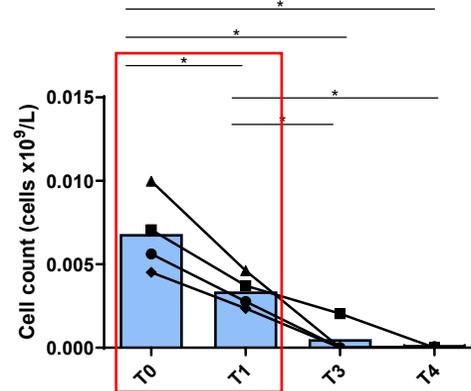
Switched-mBCs

(CD19+ CD27+ IgD+ CD24+)



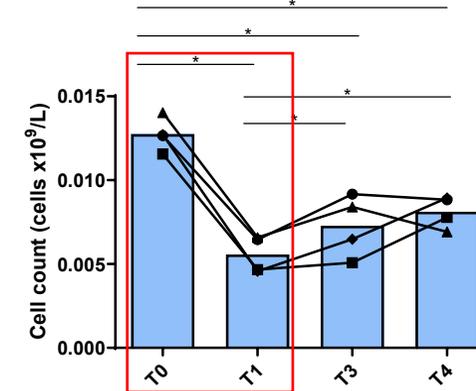
CD38+ Switched-mBCs

(CD19+ CD27+ IgD+ CD24+ CD38+)



CD38- Switched-mBCs

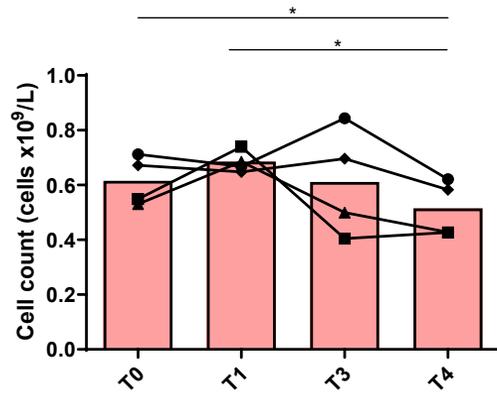
(CD19+ CD27+ IgD+ CD24+ CD38-)



Impact on peripheral T and NK cell subsets

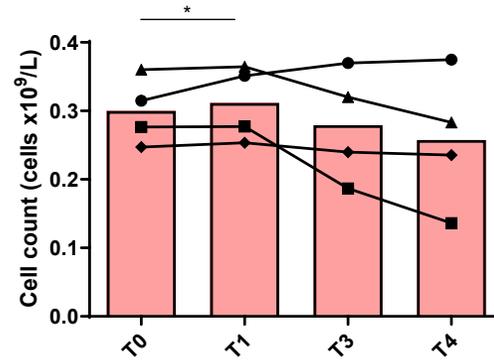
CD4 T cells

(CD3+ CD4+)



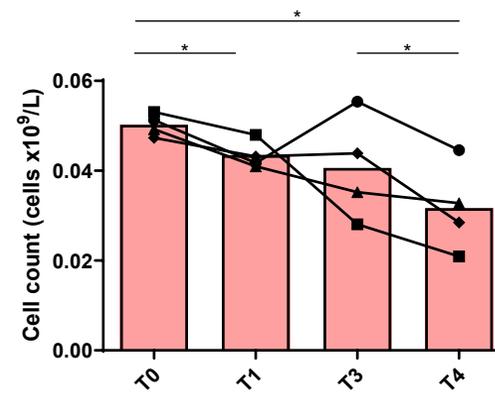
CD8 T cells

(CD3+ CD8+)



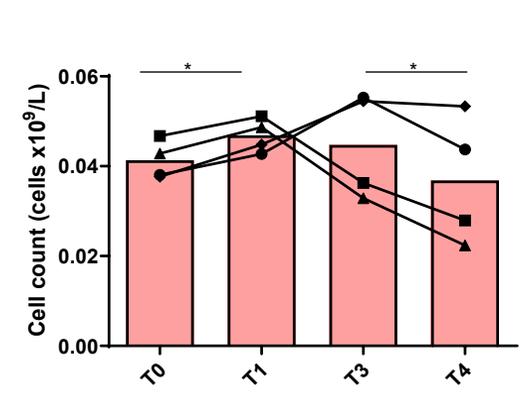
Tregs

(CD3+ CD4+ CD25+ CD127-)



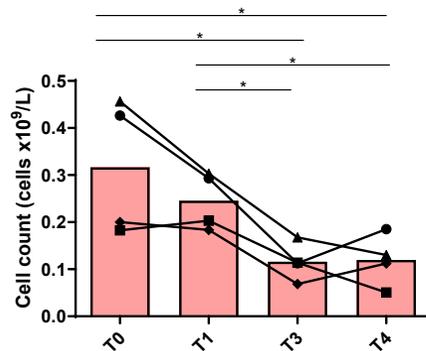
TFH

(CD3+ CD4+ CXCR5+ PD1+)



NK cells

(CD3- CD56+ CD16±)

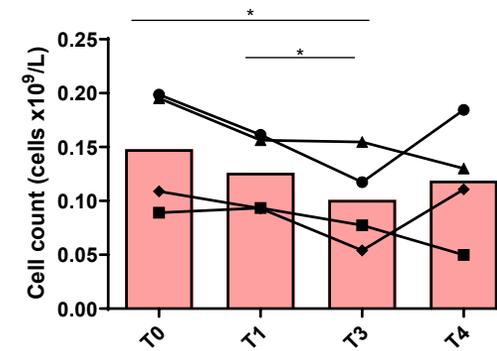


NK cells CD38+

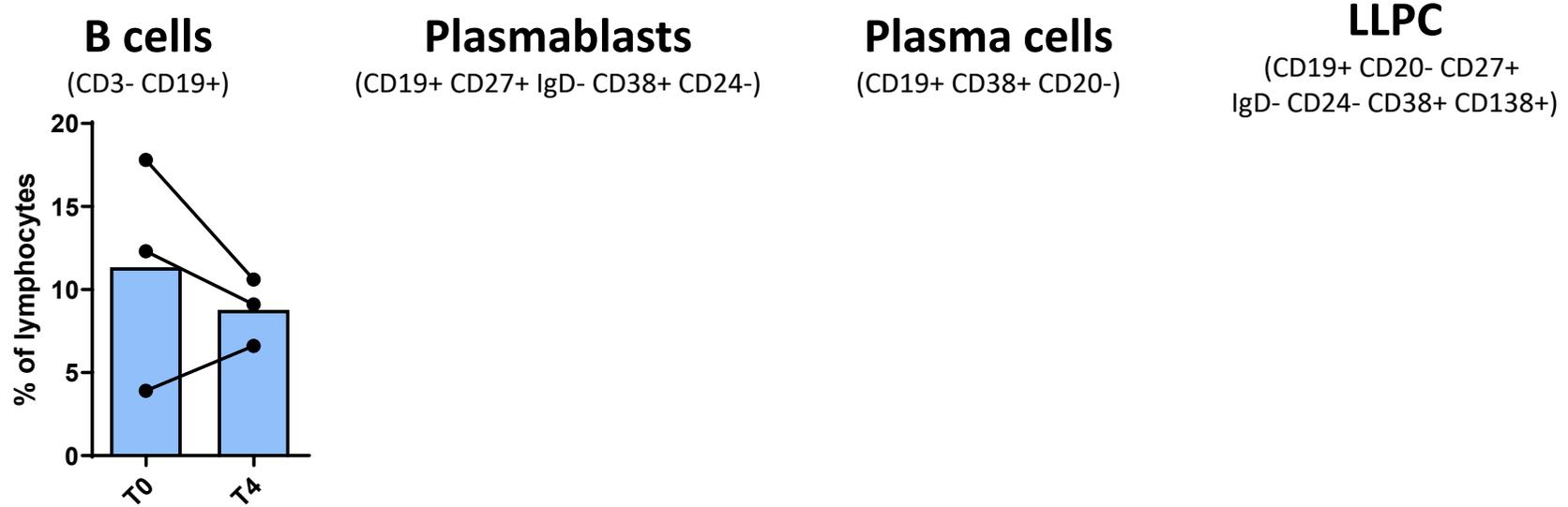
(CD3- CD56+ CD16± CD38+)

NK cells CD38-

(CD3- CD56+ CD16± CD38-)

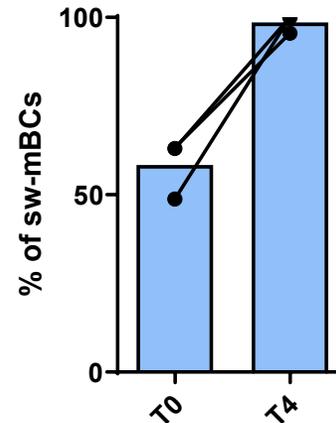


Depletion of Bone Marrow-residing Plasma cells



CD38+ Switched-mBCs (CD19+ CD27+ IgD+ CD24+ CD38+)

CD38- Switched-mBCs (CD19+ CD27+ IgD+ CD24+ CD38-)



Summary

- The combination of Belatacept and Daratumumab as desensitization therapy shows a safety profile
- This treatment results in a significant reduction of anti-HLA antibody MFI and titers, especially against class I Ag
- However, such effect translates into a relatively poor clinical impact, as depicted by the mild reduction on total cPRA.
- There is a significant reduction of Ab against repeated and non-repeated MM Ag, with a more pronounced impact on non-repeated MM Ab.
- Belatacept shows a rapid effect on multiple circulating mBc subsets, also those not targeted by daratumumab (CD38- sw mBc)
- Daratumumab therapy effectively depletes BM-residing Plasmablast and LLPC and further reduces circulating CD38+ B cells and Plasma cells.
- Maintenance of this combined therapy for a longer period could further improve anti-HLA antibody reduction, by avoiding the refilling of novel cell subsets responsible of Ab production

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