# Daratumumab Prevents Experimental Xenogeneic Graft-Versus-Host Disease by Skewing Proportions of T Cell Functional Subsets and Inhibiting T Cell Activation and Migration

Gao et al., Front. Immunol., 2021

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# Daratumumab

- monoclonal antibody
- humanized
- targets CD38 epitope
- killing ability against tumor cells through
  - complement dependent cytotoxicity
  - antibody dependent cellular cytotoxicity
  - antibody-dependent cellular phagocytosis
  - Fc-mediated crosslinking

**Indication**

- Relapsed and newly diagnosed multiple myeloma

# Multiple myeloma

## Overview

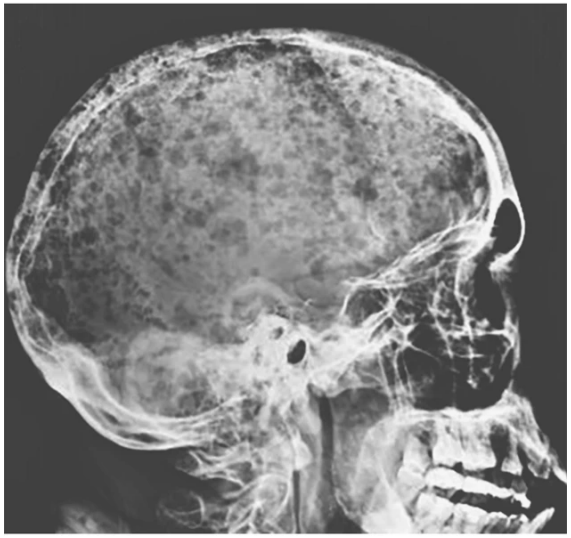
- 1 % of cancer deaths in Western countries
- peak age of incidence of 65 to 70 y
- genetically heterogenous

## Pathogenesis

- neoplastic plasma cells
- dependent on IL-6
- accumulation in bone marrow

  → cell mediated bone destruction

  lesions of 1 to 4 cm diameter

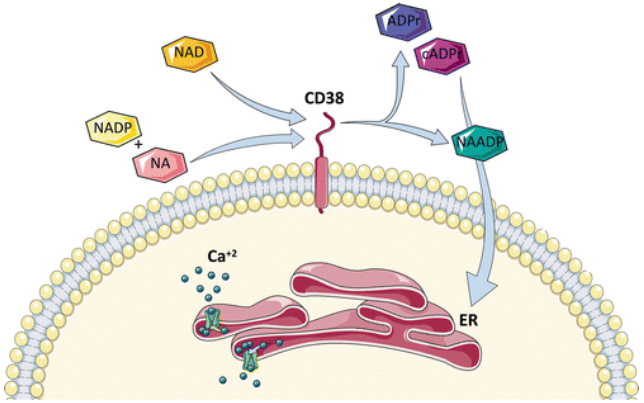


Holzgreve, H. „Schrotschussschädel“ zeigt multiples Myelom an. *MMW - Fortschritte der Medizin* **160**, 34 (2018).

# CD38

- type II transmembrane glycoprotein
- activation marker of T lymphocytes
- expressed on T and B lymphocytes and macrophages
- function as
  - enzyme
  - cell adhesion molecule
  - cell surface receptor
- Regulation of extracellular  adenosine
- metabolizes $NAD^+$ to adenosine 59-diphosphate-ribose (ADPR) and cyclic ADP-ribose (cADPR)

  → mobilization of $Ca^{2+}$




**May function as an immune checkpoint molecule!**



Santos-Argumedo, L. CD38. in *Encyclopedia of Signaling Molecules* (ed. Choi, S.) 869–877 (Springer International Publishing, 2018). doi:10.1007/978-3-319-67199-4_278.

# Previous studies

- CD38 is emerging therapeutic target in autoimmune diseases
  - rheumathoid arthritis
  - systemic lupus erythematosus
  - asthma
  - neurodegeneration
  - inflammatory bowel disease

- incidence of GVHD in relapsed multiple myeloma patients after allogeneic hematopoietic cell transplantation (allo-HCT) only 15 % in patients treated with Daratumumab

**CD38 as potential target against alloreactive T cells in GVHD**

# Graft-versus-host disease (GVHD)

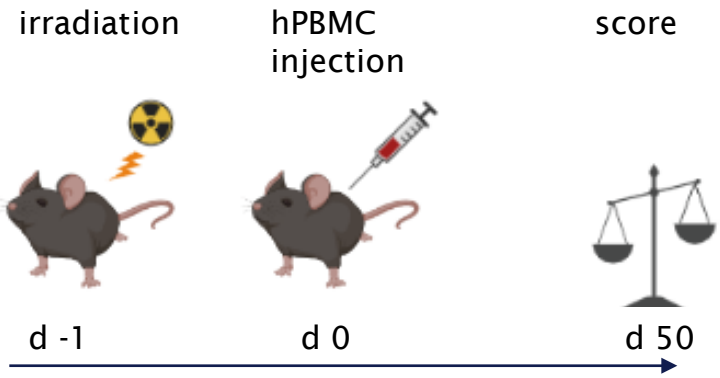
- most common complication after allo-HCT
- major cause of mortality and morbidity in patients
- 30 - 50 % of allo-HCT develop acute GVHD
- attack of recipient host cells by allo-reactive donor cells
  - inflammatory cytokines → acute or chronic tissue damage

**state-of-the-art therapy**

- suppressive calcineurin inhibitors combined with
  - methotrexate
  - sirolimus
  - mycophenolate mofetil
- graft-versus-leukemia effects impaired
- corticosteroids first-line treatment of acute GVHD
- 35 - 50 % of patients refractory

# Xenogenic model of GVHD (1)

- NOD-Prkdc$^{scid}$/IL2rg$^{tm1}$/Bcgen (B-NSG/B-NDG) mice
  - T and B cell deficiency (interruption of commom gamma-chain)
  - lacks NK cells
  - deficiency in cytokine signalling
- sublethal irradiation on day -1
- i.v. injection of 1 x $10^7$ hPBMCs from healthy volunteers on day 0




- engraftment of human cells was detected in peripheral blood samples 7, 14 and 21 days after injection

# Xenogenic model of GVHD (2)

- i.p. injection of Daratumumab (5 mg/kg) once a week from day 0 for two weeks post transplantation
- control group: human IgG control antibody
- six to eight mice; three experiments

## Score

- weight loss
- posture
- activity
- skin integrity
- fur ruffing

- Scale of 0 to 2
- euthanized at score of 6 or loss of 25 % body weight

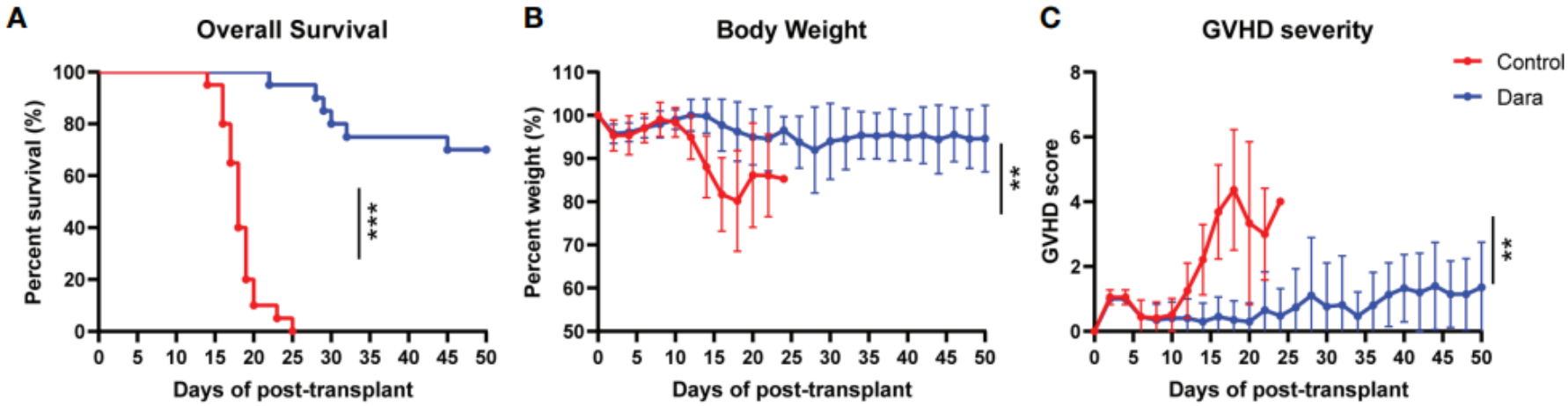
https://www.envigo.com/model/nodcb17-prkdcscid-il2rgtm1-bcgenhsd

# Survival was significantly longer in mice treated with Daratumumab

**median survival**

- control group: 18 days
- Dara: 70 % over 50 days
  - less weight loss
  - lower GVHD scores

- irradiation
- 1 x $10^7$ hPBMCs
- 5 mg/kg Dara
- weekly administration



# Control group mice had significantly enlarged spleens and evident tissue damage

**euthanized 14 days post transplantation**

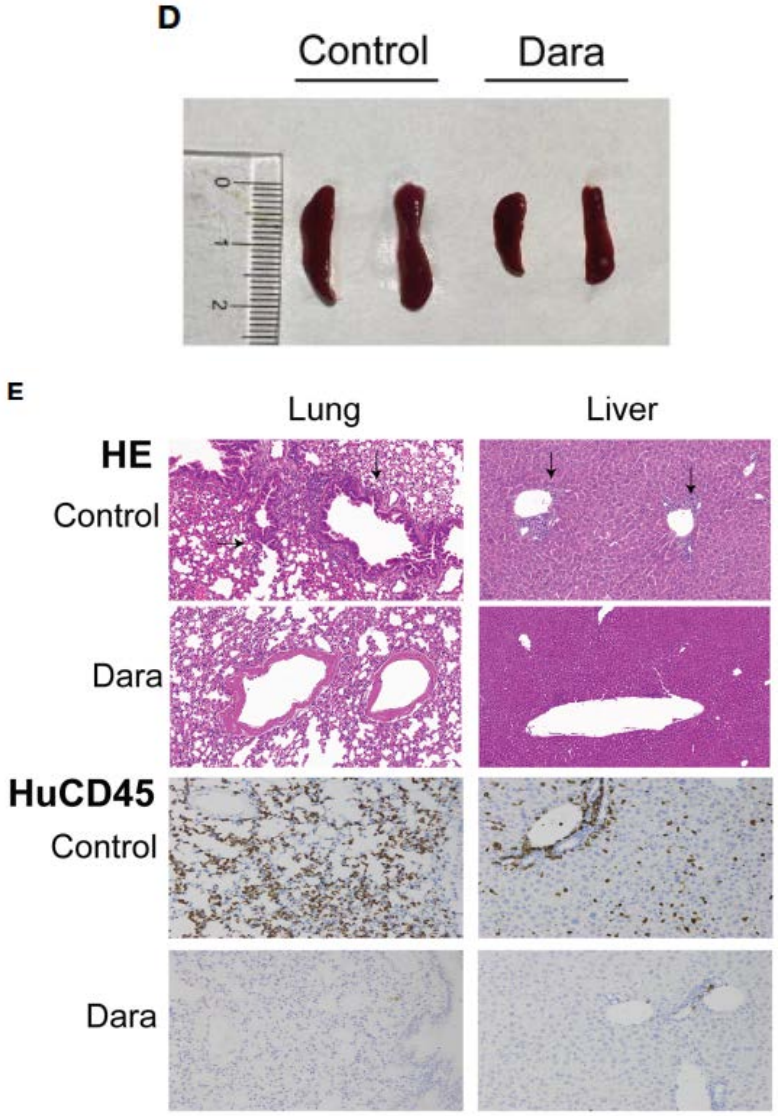
- histological evaluations of lung and liver
- HE and HuCD45 staining

**control group**

- lymphoid infiltration
- endotheliitis
- parenchymal alterations
- hepatocyte apoptosis

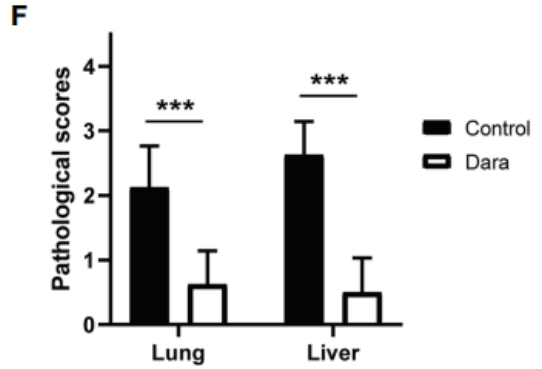
**Daratumumab treatment**

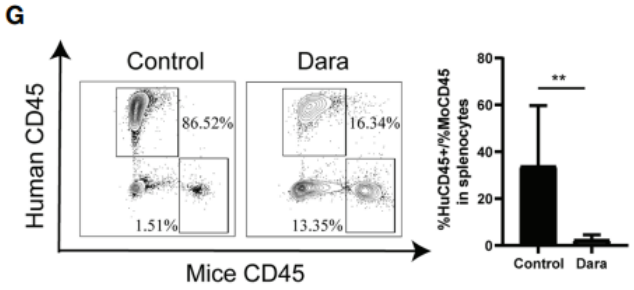
- subnormal histology

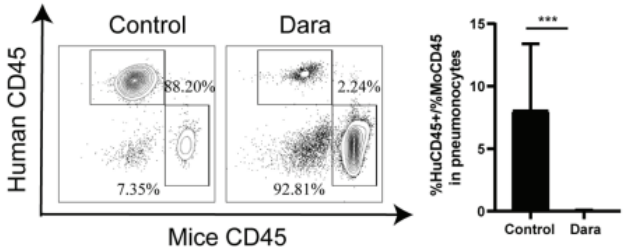


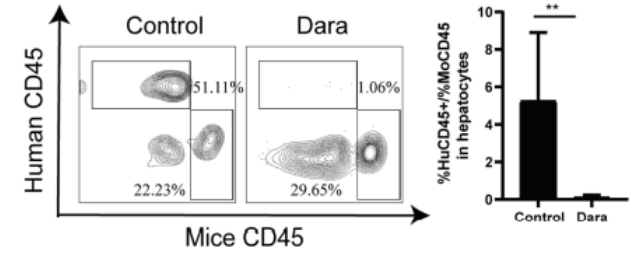
# Confirmation by blinded evaluation of histopathological scores for lung and liver and flow cytometry analysis

- blinded evaluation 14 d post transplant
- Analysis of splenocytes, lung and liver
- rate of human/mouse leukocytes significantly lower in Dara-treated group (n = 8)



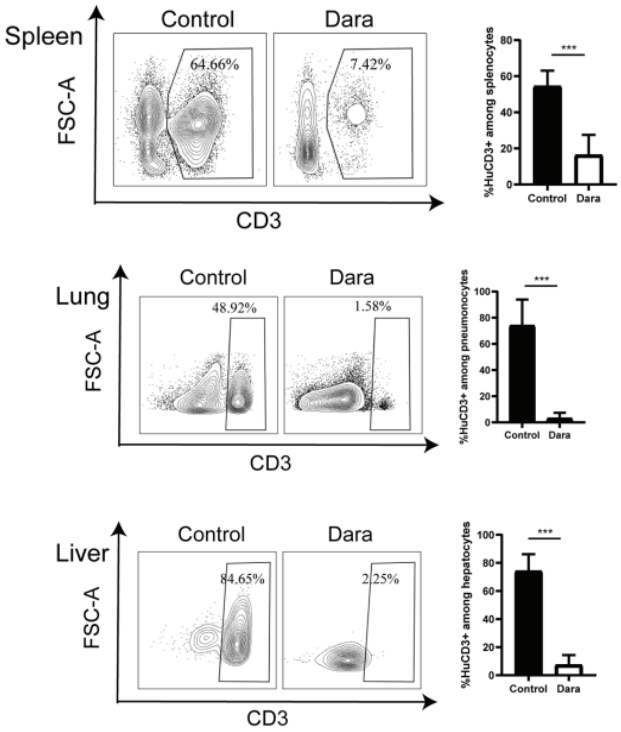



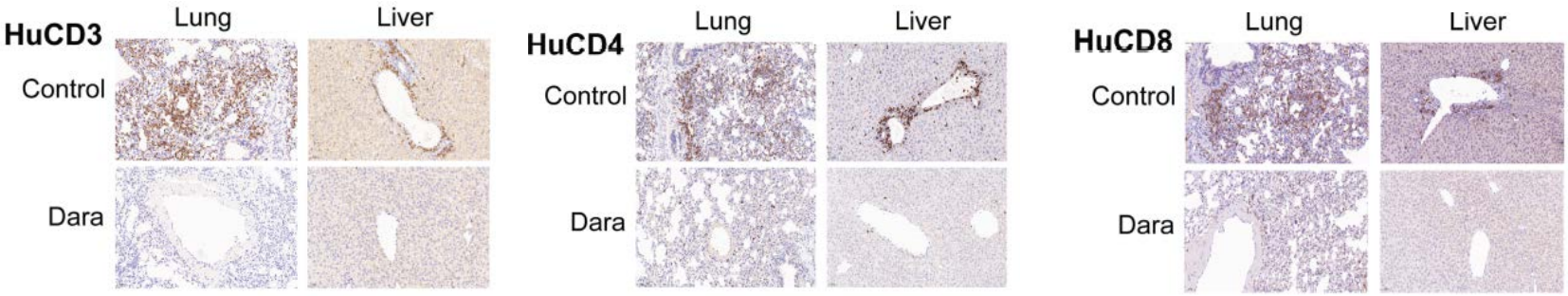



# Dara reduced human T cell infiltration in GVHD targeted organs lung and liver

- proportion of $CD3^+$ cells in splenocytes reduced in Dara-treated mice
- reduced inflitration of $CD3^+$, $CD4^+$ and $CD8^+$ T cells in lung and liver

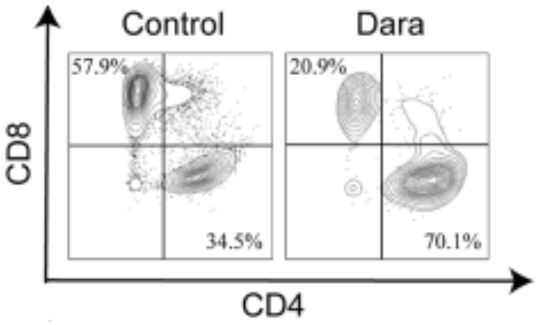


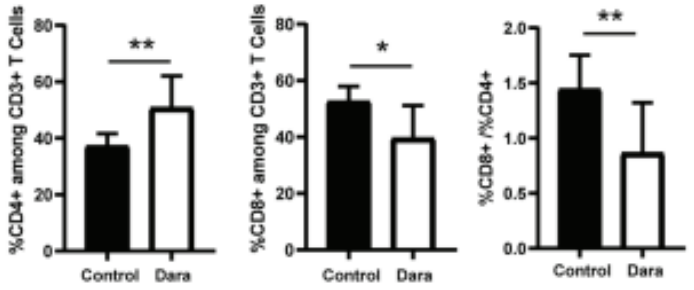



# Dara-treatment influenced proportion of T cell subsets (1)

- reduced frequency of $CD8^+$ T cells
- higher frequency of $CD4^+$ T cells

  → lower ratio of $CD8^+$ / $CD4^+$ cells

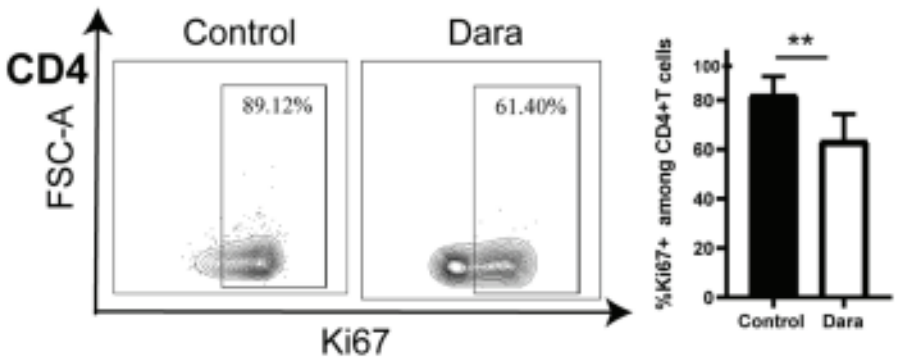


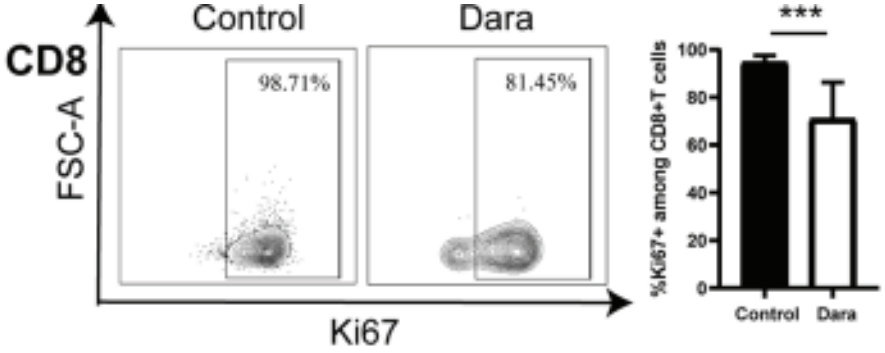



# Dara-treatment influenced proportion of T cell subsets (2)

- only due to anti-proliferative effect of drug?
- KI67 staining
- reduction of KI67$^{+}$ cells in both subsets

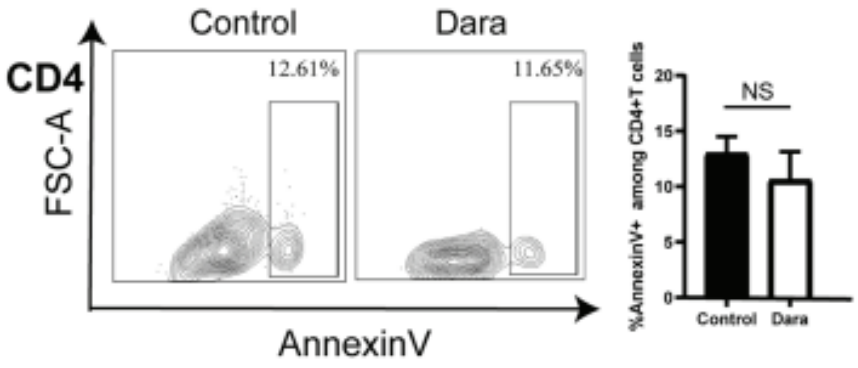
  → proliferation reduced in CD4$^{+}$ and CD8$^{+}$ cells

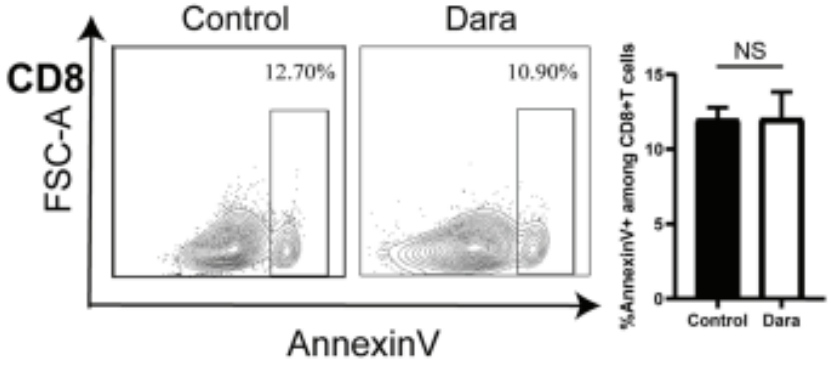


# Only caused by Dara-mediated apoptosis of $CD8^+$ T cells?

- Dara had no significant effect of induction of apoptosis in T cells

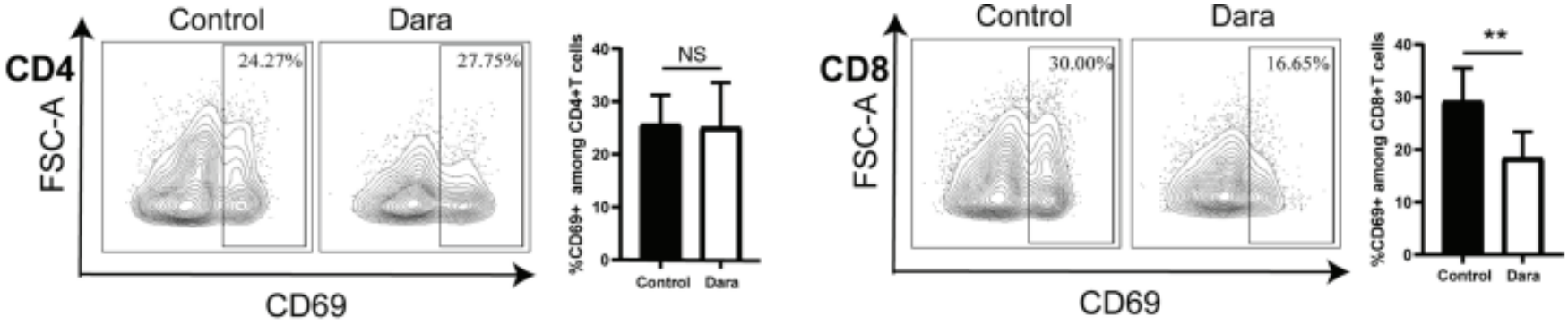


# Evaluation of activation status of Dara-treated T cells

- CD38 is a commonly used activation marker of T cells
- influence of Daratumumab on expression of CD69?

  →significantly decreased frequency of $CD69^+$ cells in $CD8^+$ cells

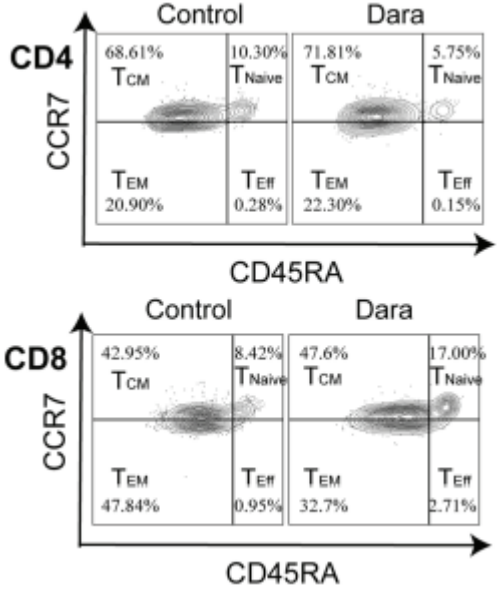


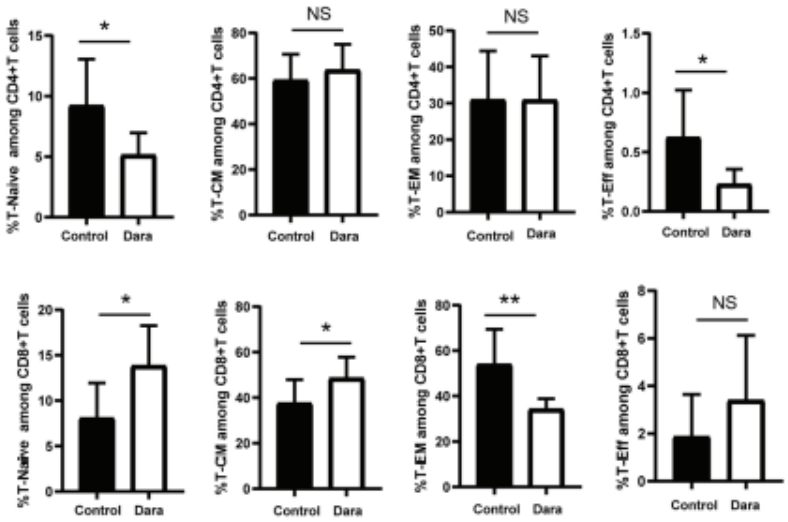

**Daratumumab mainly inhibits $CD8^+$ T cell activation**

# Daratumumab showed impact on CD8+ T cell differentiation

- Analysis of differentiation from naive to memory/effector phenotypes
- evaluation of markers CD45RA/CCR7
- naive and central memory (Tcm) CD8+ cell count enhanced
- decreased effector memory T cells (Tem)
- no change in effector T cells (Teff)

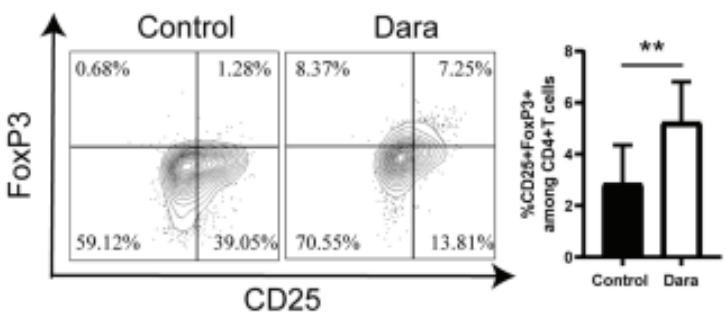
- no inhibitory effect on CD4+ T cell differentiation

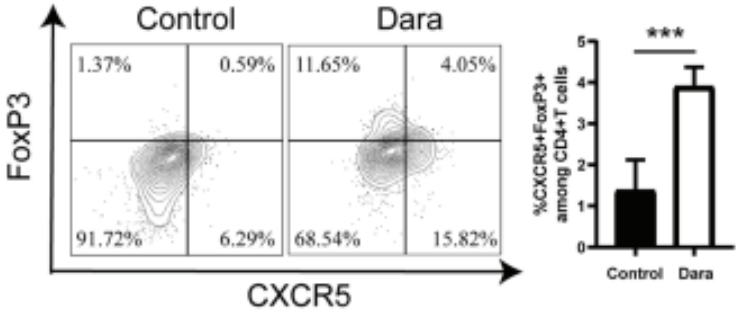


# Regulatory T cells (Tregs) play an important role in GVHD

- Tregs have immunosuppressive funtion in GVHD
- analysis of proportions of Tregs and follicular regulatory T cells (Tfr)
  - Tregs CD4+ CD25+ FoxP3+
  - Tfr CD4+ CXCR5+ FoxP3+

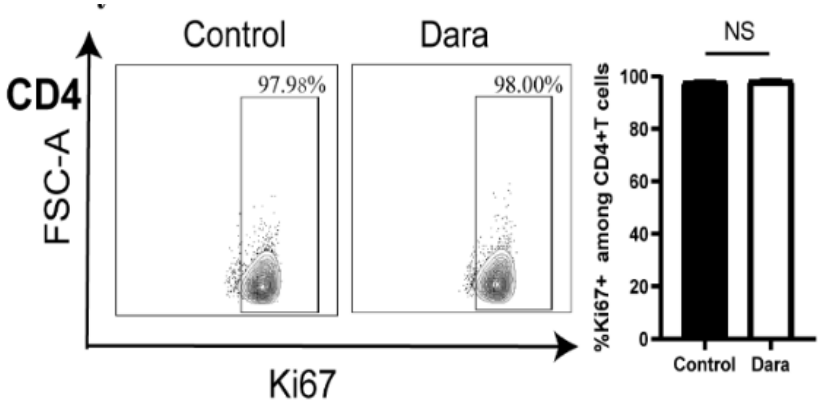


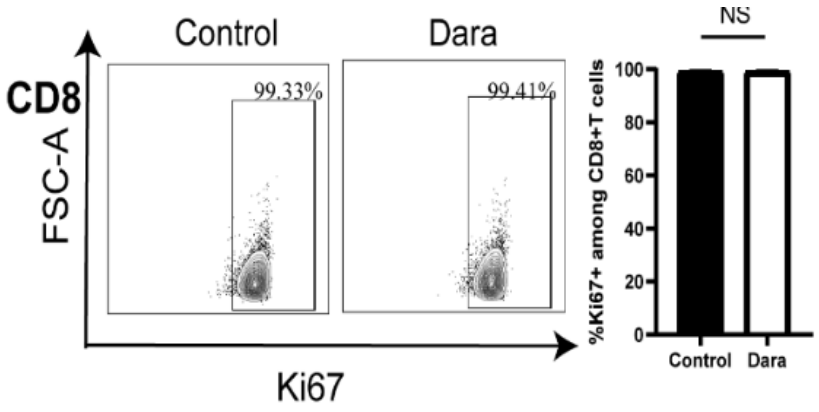




**Daratumumab may alleviate xeno-GVHD by increasing frequency of immunosuppressive cells**

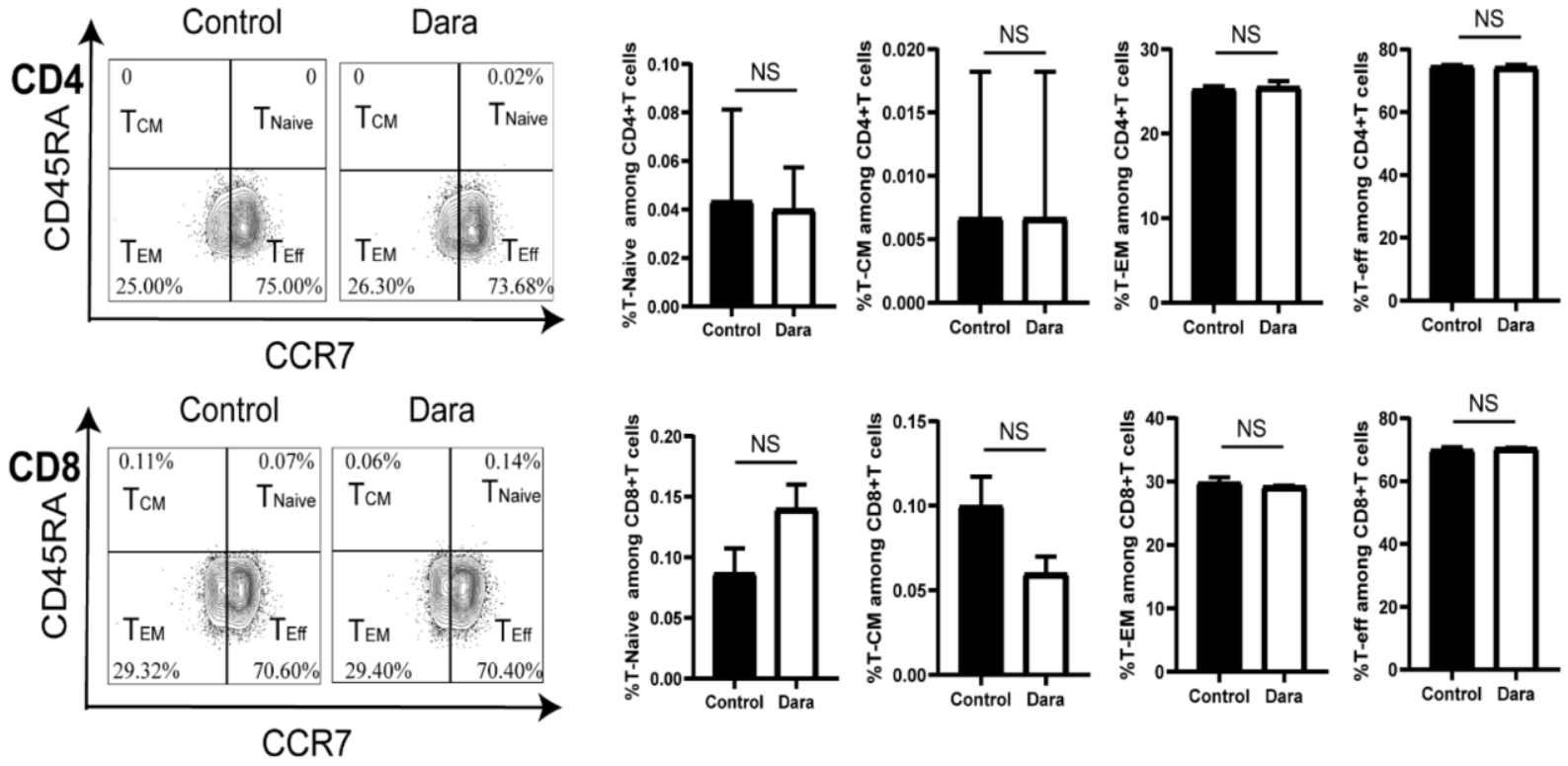
# Validation of *in vivo* findings in human *in vitro* experiments

- cultivation of human T cells for 48 h at 50 µg/mL Daratumumab
- no effect on apoptosis
- no inhibition of proliferation
- KI67 expression similar in Dara and control group
- decreased frequencies of $CD69^+$ cells
- T cell differentiation not influenced
- significantly higher frequencies of Tregs



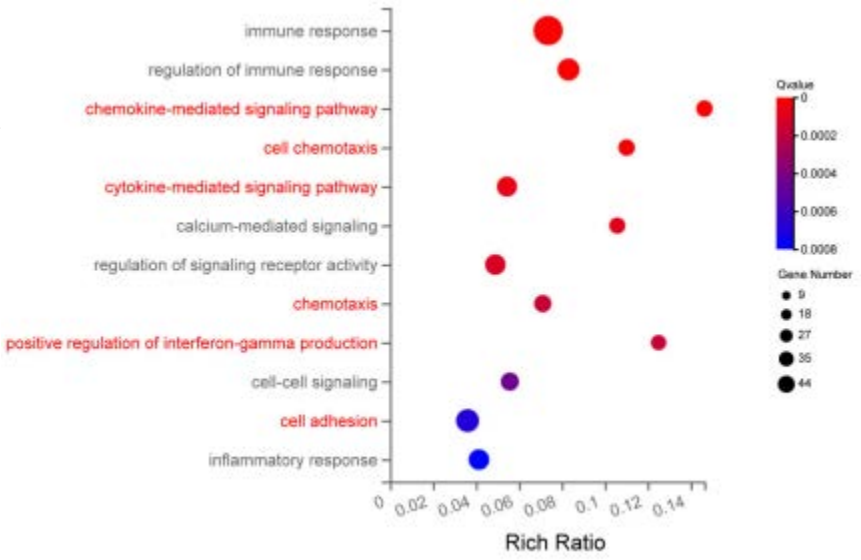



# Validation of *in vivo* findings in human *in vitro* experiments



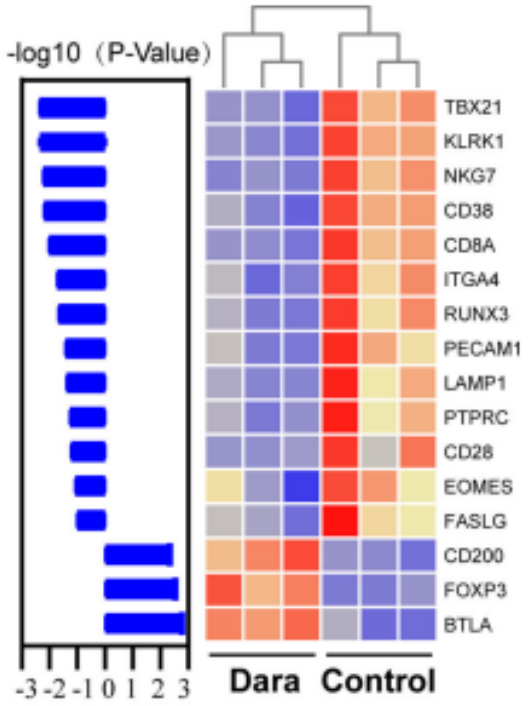
# Impact of Daratumumab on cellular pathways

- Transcriptome sequencing of sorted human $CD3^+$ T cells engrafted into spleens (n = 3)
- 358 genes significantly differentially expressed (log2 ratio ≥ 1, q < 0.005)
- pathway analysis
  - immune response
  - chemokine-mediated singnalling pathway
  - cytokine-mediated singnalling pathway
  - chemotaxis
  - cell adhesion
  - positive regulation of IFNy production



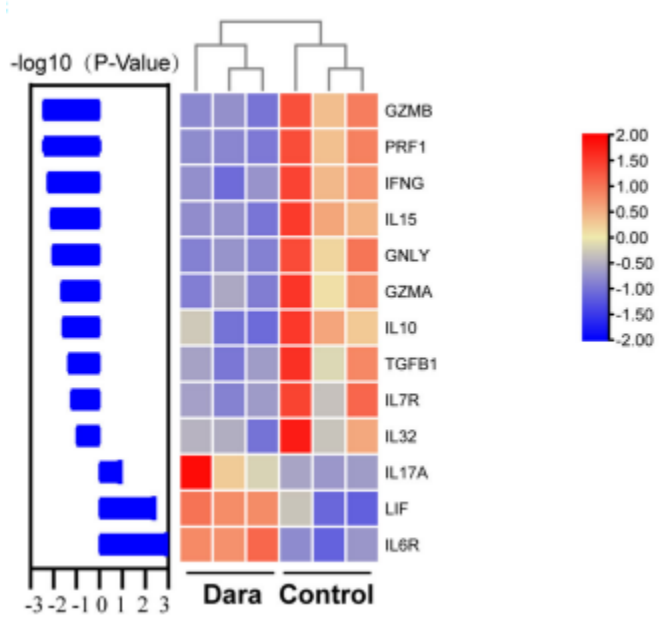
# Daratumumab alters T cell immune reaction at gene expression level (1)

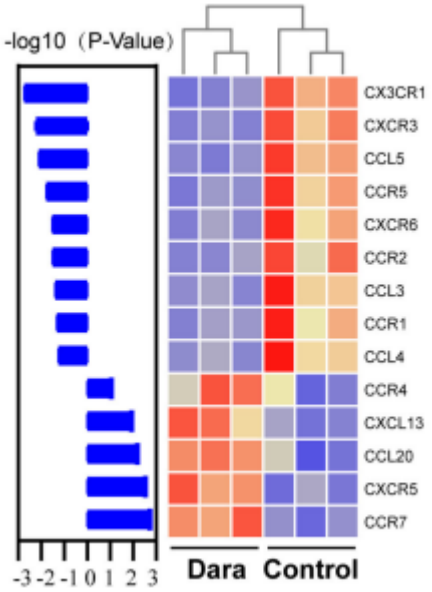
- T cell immune reaction most altered at gene expression level
- decreased expression of TF involved in:
  - T cell cytotoxic reaction
  - Type 1 T cell differentiation
    - T-bet
    - RUNX3
  - activation/immune response markers
    - CD28
  - increased expression of TF of Treg and Tfr
    - FOXP3



# Daratumumab alters T cell immune reaction at gene expression level (2)

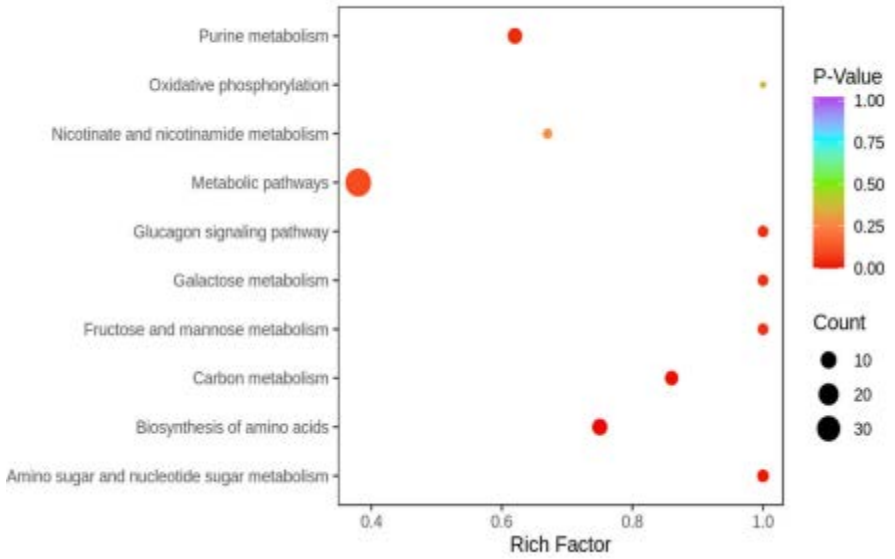
- decreased expression
  - cytotoxic effector molecules
  - cytokines
  - chemokines
  - chemoattractant receptors
- increased expression
  - IL17
  - CXCR5 → Tfr cells



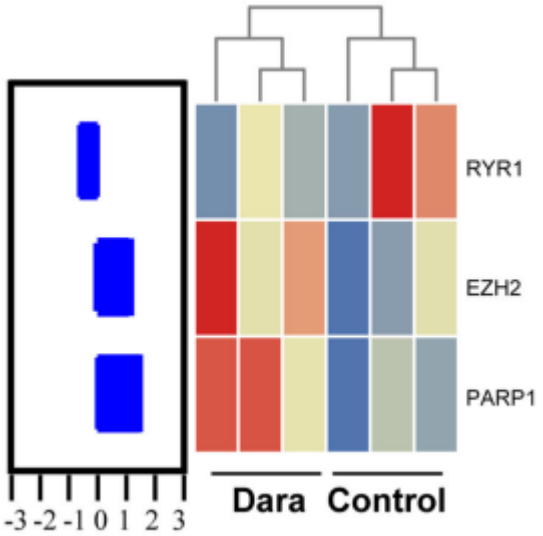
# Daratumumab modulates metabolic pathways of T cells

- Human T cells cultured *in vitro*
- CD38 determines intracellular levels of $NAD^+$
- Dara modulates
  - purine metabolism
  - oxidative phosphorylation
  - nicotinate and nicotinamide metabolism



# Daratumumab influences transcription of enzymes involved in CD38 mediated regulation of metabolic pathways and chromatin modification *in vivo*

- analysis of engrafted human T cells
- changed transcription levels
  - ryanodine receptor 1 (RYR1)
  - poly (ADP-ribose) polymerase (PARP)
  - enhancer of zeste homolog 2 (EZH2)



# Flow cytometry analysis of T cell functional subsets

- Evaluation of
  - IL17A
  - IFNy
  - Granzyme A
  - Granzyme B

**Reduced proportion of Th1 and Tc1 cells**

| Parameter* | Method | Control (n=8) | | Dara (n=8) | | p-value |
|---|---|---|---|---|---|---|
| | | Median | SD | Median | SD | |
| **Sacrifice at day14, spleen** | | | | | | |
| Functional subsets **among CD4$^+$ T cells (%)** | FACS* | | | | | |
| IFNγ$^+$ IL17A$^-$ T cells (Th1) | | 76.31 | 3.52 | 60.15 | 1.56 | <0.0001 |
| IFNγ$^-$ IL17A$^+$ T cells (Th17) | | 0.4 | 0.16 | 1.9 | 0.63 | <0.0001 |
| IFNγ$^+$ IL17A$^+$ T cells (Th1/17) | | 4.65 | 1.69 | 9.61 | 1.7 | <0.0001 |
| Functional subsets **among CD8$^+$ T cells (%)** | FACS | | | | | |
| IFNγ$^+$ IL17A$^-$ T cells (Tc1) | | 95.13 | 0.62 | 84.34 | 4.11 | <0.0001 |
| IFNγ$^-$ IL17A$^+$ T cells (Tc17) | | 0.07 | 0.06 | 0.89 | 0.84 | 0.015 |
| IFNγ$^+$ IL17A$^+$ T cells (Tc1/17) | | 1.84 | 0.66 | 4.41 | 1.33 | 0.0002 |
| Granzyme A$^+$ T cells | | 70.05 | 8.01 | 53.05 | 9.07 | 0.0011 |
| Granzyme B$^+$ T cells | | 68.21 | 8.53 | 31.48 | 9.97 | <0.0001 |

# Cytokine concentrations in serum at day 14

- Evaluation of
  - IFNγ
  - IL6
  - IL10
  - IL17A

**Increased IL17 levels in Dara-treated mice**

**→ Dara may induce Th17 subset differentiation**

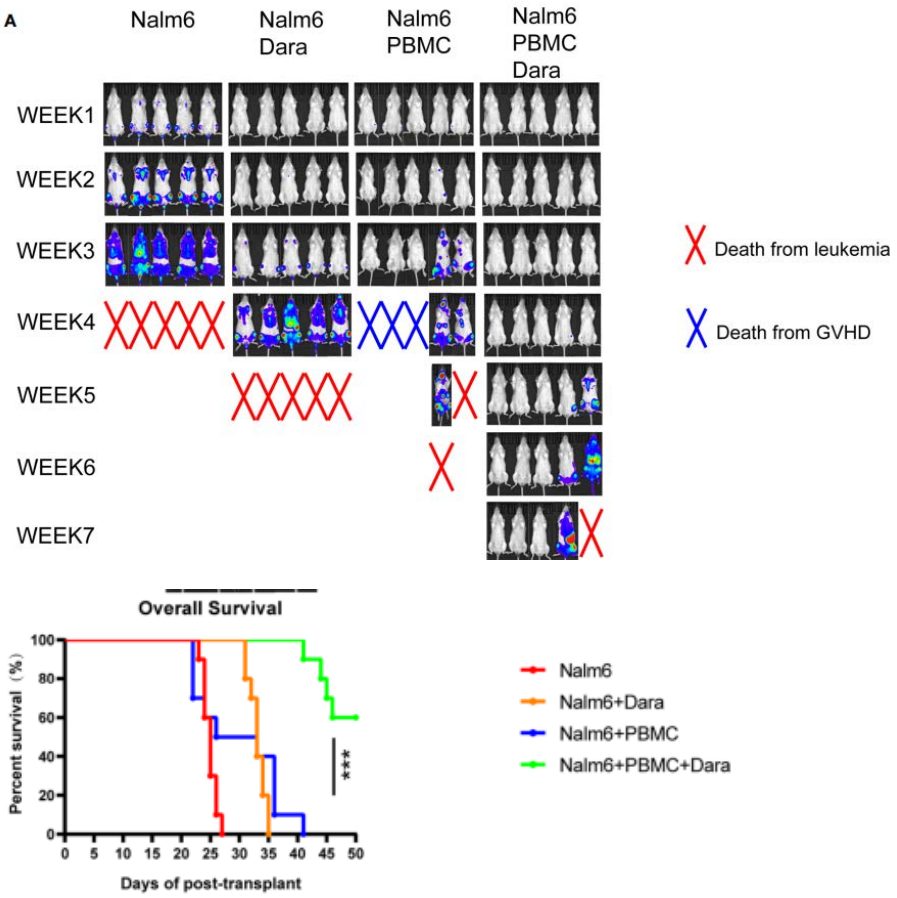
| Parameter* | Method | Control (n=8) | | Dara (n=8) | | p-value |
|---|---|---|---|---|---|---|
| | | Median | SD | Median | SD | |
| **Serum cytokine at day14 (pg/ml)** | CBA* | | | | | |
| IFNγ | | 509.21 | 248.4 | 132.25 | 73.41 | 0.0001 |
| IL6 | | 2.47 | 0.83 | 1.15 | 0.49 | 0.0002 |
| IL10 | | 24.06 | 23.44 | 1.92 | 2.36 | 0.0054 |
| IL17A | | 1.32 | 1.39 | 4.23 | 2.77 | 0.0054 |

# Dara treatment inhibited mRNA expression $CD4^+$ and $CD8^+$ chemokine and receptor genes as well as cytotoxicity-linked genes

| Parameter* | Method | Control (n=8) | | Dara (n=8) | | p-value |
|---|---|---|---|---|---|---|
| | | Median | SD | Median | SD | |
| **Sacrifice at day14, spleen** | | | | | | |
| **Gene expression levels of T cells** | RT-qPCR | | | | | |
| **Pro-inflammatory cytokine gene expression among $CD4^+$ -T cells** | RT-qPCR | | | | | |
| *IFNG* | | 0.83 | 0.16 | 0.47 | 0.18 | 0.0275 |
| *IL17A* | | 0.72 | 0.48 | 8.93 | 6.03 | 0.0348 |
| TBX21/T-bet | | 0.95 | 0.08 | 0.77 | 0.08 | 0.021 |
| **Cytotoxic effector molecule gene expression among $CD8^+$-T cells** | RT-qPCR | | | | | |
| *IFNG* | | 1.32 | 0.28 | 0.89 | 0.2 | 0.04 |
| *IL17A* | | 0.76 | 0.49 | 2.71 | 0.88 | 0.0081 |
| GZMA/Granzyme A | | 1.06 | 0.15 | 0.63 | 0.16 | 0.0069 |
| GZMB/Granzyme B | | 1.15 | 0.26 | 0.55 | 0.28 | 0.0208 |
| GNLY/Granulysin | | 1.17 | 0.2 | 0.23 | 0.11 | 0.0002 |
| PRF1/perforin | | 1.06 | 0.06 | 0.6 | 0.3 | 0.0218 |
| **$CD4^+$-T cell related chemokine and chemoattractant receptor gene expression** | RT-qPCR | | | | | |
| *CCL2* | | 0.86 | 0.18 | 0.45 | 0.2 | 0.0229 |
| *CCL3* | | 1.05 | 0.27 | 0.5 | 0.21 | 0.0176 |
| *CCL5* | | 0.96 | 0.13 | 0.44 | 0.15 | 0.0022 |
| *CCR1* | | 1.11 | 0.33 | 0.62 | 0.18 | 0.0387 |
| *CCR2* | | 0.73 | 0.27 | 0.25 | 0.08 | 0.014 |
| *CCR5* | | 0.92 | 0.13 | 0.58 | 0.25 | 0.0498 |
| **$CD8^+$-T cell related chemokine and chemoattractant receptor gene expression** | RT-qPCR | | | | | |
| *CCL3* | | 0.95 | 0.15 | 0.49 | 0.2 | 0.0098 |
| *CCL4* | | 1.08 | 0.15 | 0.43 | 0.09 | 0.0003 |
| *CCR1* | | 1.42 | 0.32 | 0.76 | 0.3 | 0.0239 |
| *CCR2* | | 0.83 | 0.25 | 0.41 | 0.2 | 0.0413 |

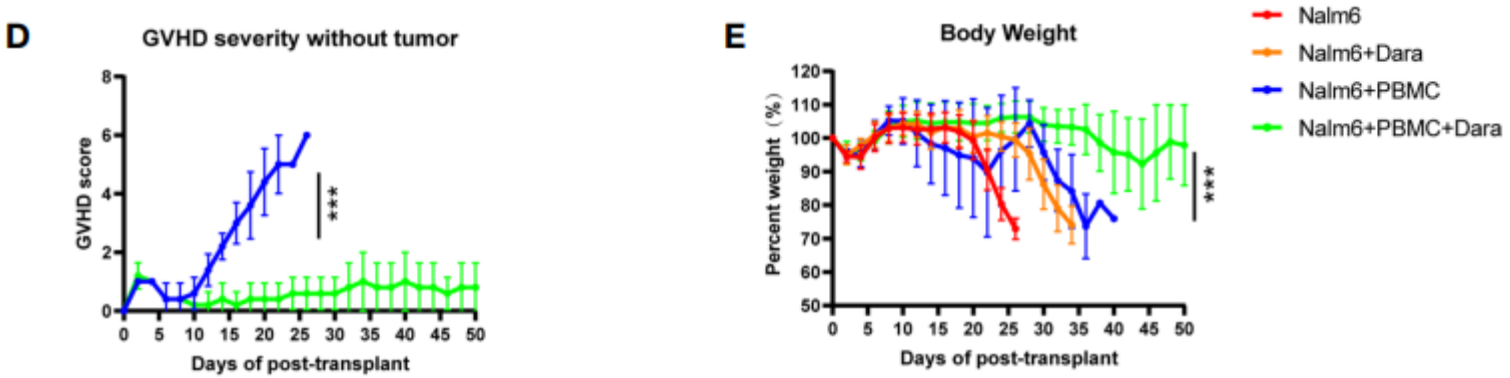
# Daratumumab preserves Graft-versus-leukemia effect (1)

- Human leukemia cell line Nalm6 expressing luciferase
- NSG mice transplanted with 1 x $10^5$ Nalm6.LucGFP cells
- With or without hPBMCs and Daratumumab
- Elimination of leukemia cells evaluated with *in vivo* bioluminescent imaging system




**Treatment with hPBMCs significantly reduced leukemia**
**Mice treated with Daratumumab showed superior survival rates**

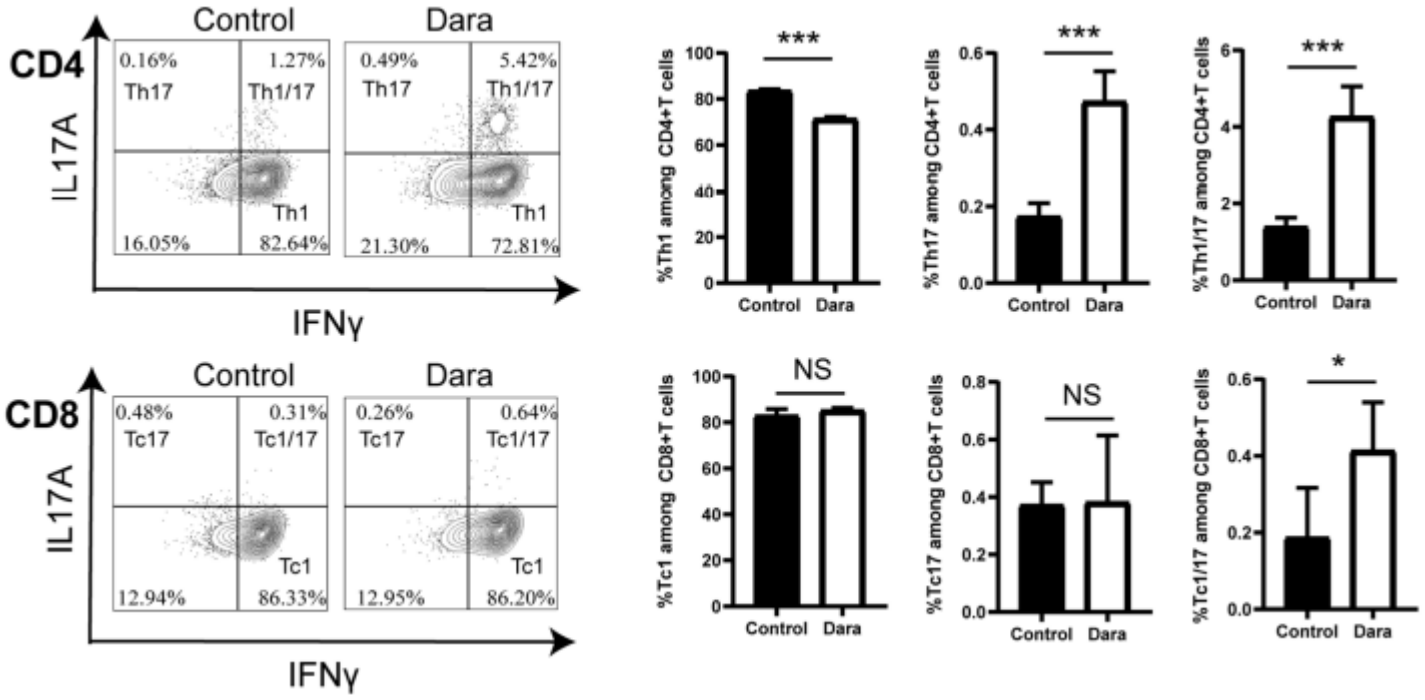
# Daratumumab preserves Graft-versus-leukemia effect (2)




**No significant weight loss in mice receiving hPBMCs and Dara**

**Dara reduced GVHD severity**

# Frequencies of Th17 T cells were significantly higher in engrafted human T cells under Dara treatment



# Conclusion

**xeno-GVHD model supports further clinical use of Daratumumab for GVHD**

- Dara inhibits proliferation, activation and differentiation of $CD8^+$ T cells
- Dara reduces expression of cytotoxic effector molecules, proinflammatory cytokines, chemokines and chemoattractant receptors
- Dara treatment may induce an increase in CD38 negative regulatory T cells
- inhibition of CD38 led to metabolic reprogramming of T cells with superior tumor control

**Daratumumab might be a promising option for separating GVHD from GVL effects in patients with hematopoietic malignancies receiving allo-HCT**

# Thank you for your attention!