Modulation de la différenciation lymphocytaire T par thérapie cellulaire et génique dans le thymus

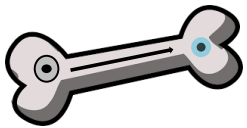
Valérie Zimmermann

Thymopoiesis

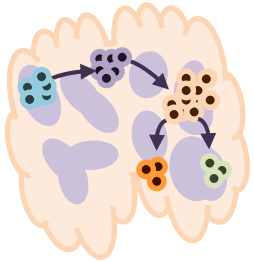
24-28 days

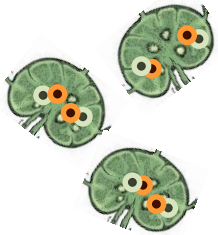
Bone Marrow → Thymus → Periphery

Hematopoietic progenitor

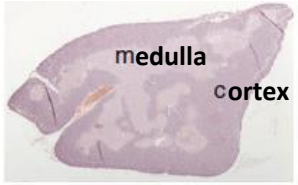


Hematopoietic Stem Cell

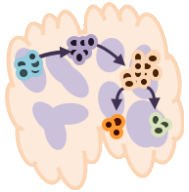




Lymph nodes



Thymopoiesis



24-28 days

Bone Marrow

Thymus

Periphery

Double Negative
DN: CD4-CD8-

Double Positive
DP: CD4+CD8+

Single Positive

Single Positive

CD4

SP4

CD4+ T cells

CD4
CD8

SP8

CD8

CD8+ T cells

Hematopoietic progenitor

WT

CD4

SP4

DP

DN

SP8

CD8

Severe Combined Immunodeficiency Disease (SCID)

- Heterogeneous group of genetic diseases
- T lymphocytes are absent or non-functional
- Abnormal development of other hematopoietic lineages (B, NK, ...)
- Lethal due to opportunistic infections in infancy

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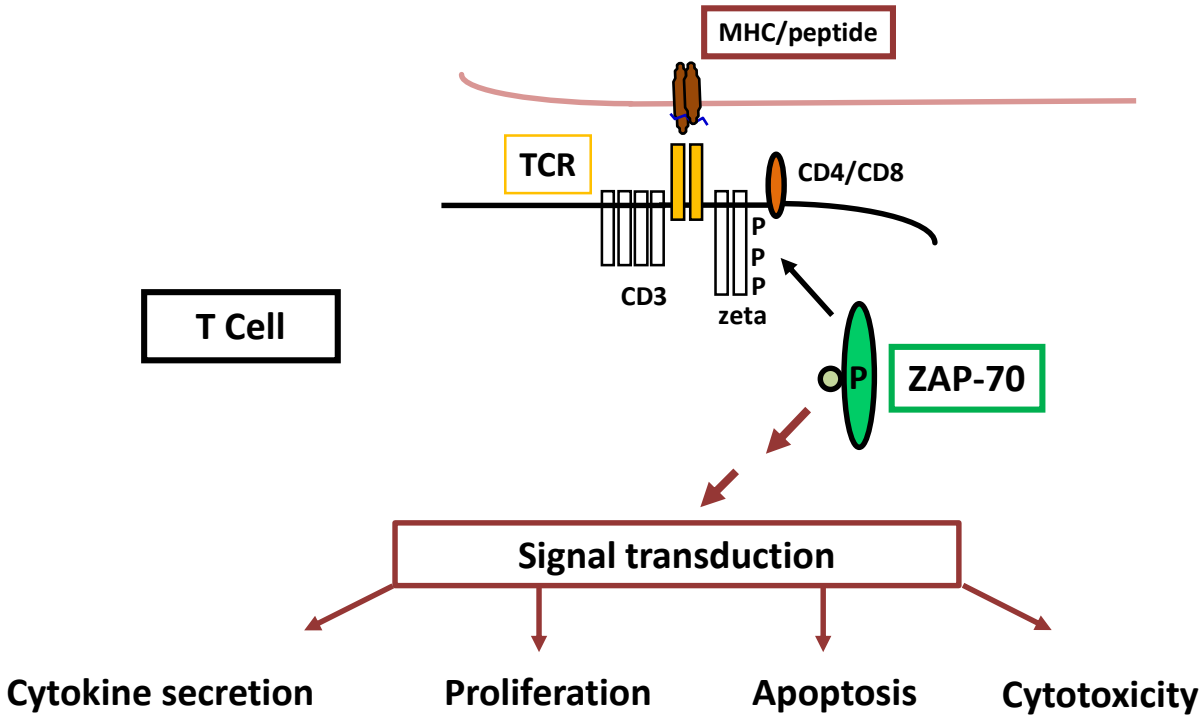
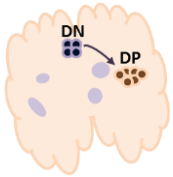
→ ZAP-70 immunodeficiency

ZAP-70: A tyrosine kinase required for T Cell Receptor (TCR) signaling

→ Response to TCR engagement



Altered thymopoiesis in ZAP-70-/- mice



24-28 days

Bone Marrow

Thymus

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Double Negative
DN: CD4-CD8-

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Single Positive

Single Positive

TCR

CD4
CD8

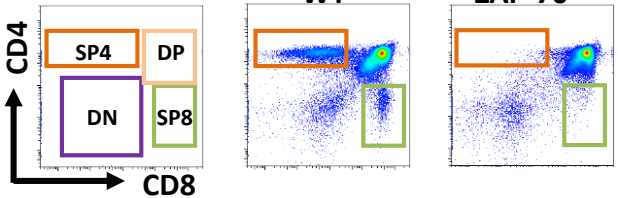
SP4

SP8

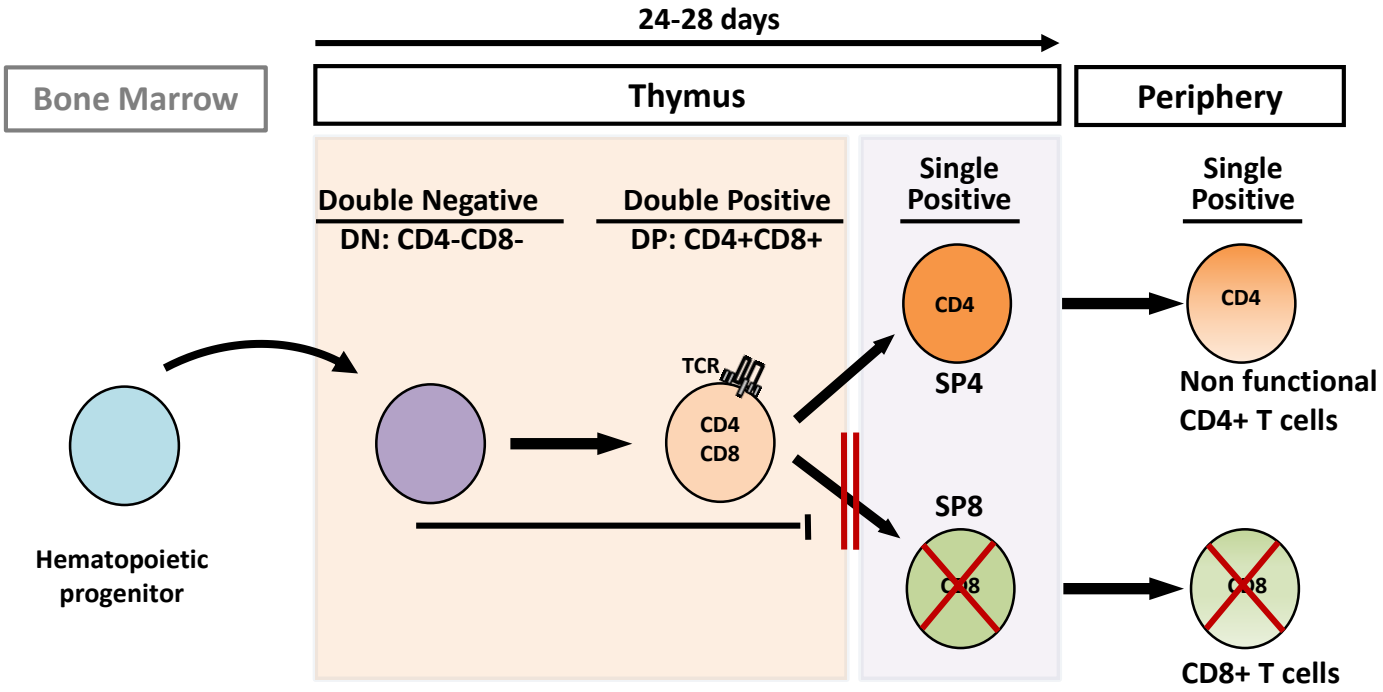
CD4+ T cells

CD8+ T cells

Hematopoietic progenitor

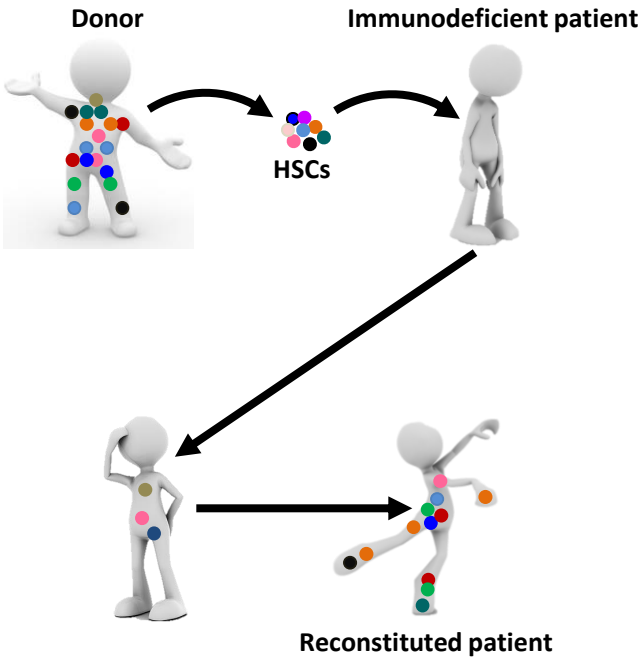


Altered thymopoiesis in ZAP-70-/- patient



SCID treatments

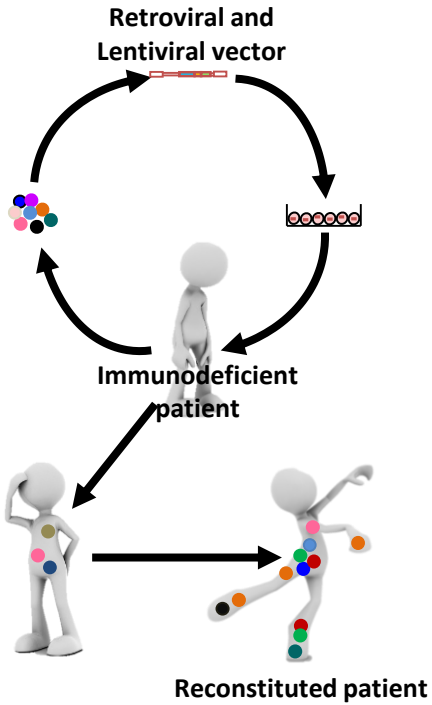
→ Allogeneic HSC transplantation (cell therapy)



- **Benefits:**
  - high success rate
  - long term reconstitution
- **Difficulties:**
  - lack of compatible donors

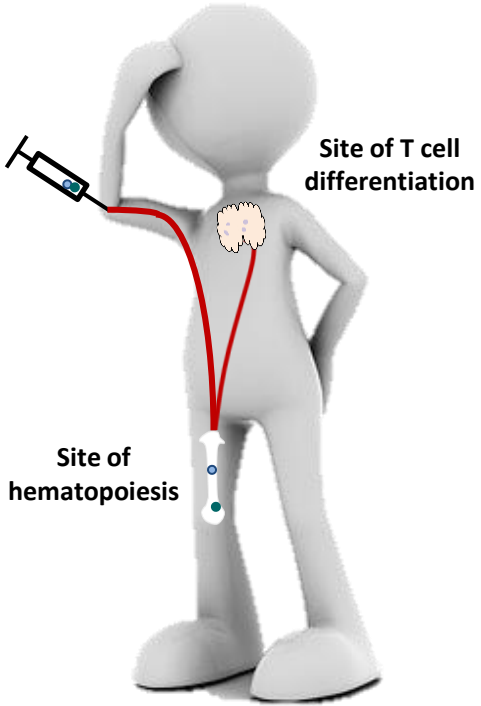
SCID treatments

→ Genetic correction of patient HSCs (gene therapy)



- **Benefits:**
  - no donor required
- **Difficulties:**
  - insertional mutagenesis
  - loss of HSC potential by *ex vivo* manipulation

Common limitations to reconstitution of intravenously injected allogeneic and gene-corrected HSC



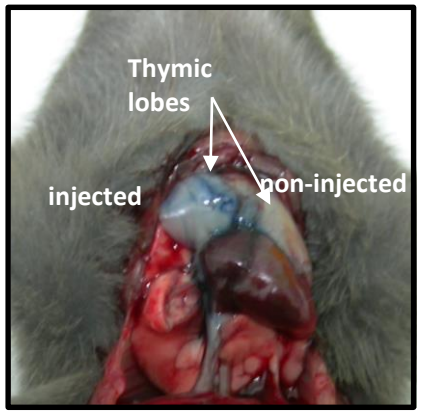
- Time for polyclonal T cell reconstitution >100 days
- Duration of progenitor migration to the thymus

An innovative therapeutic approach: Targeting the thymus

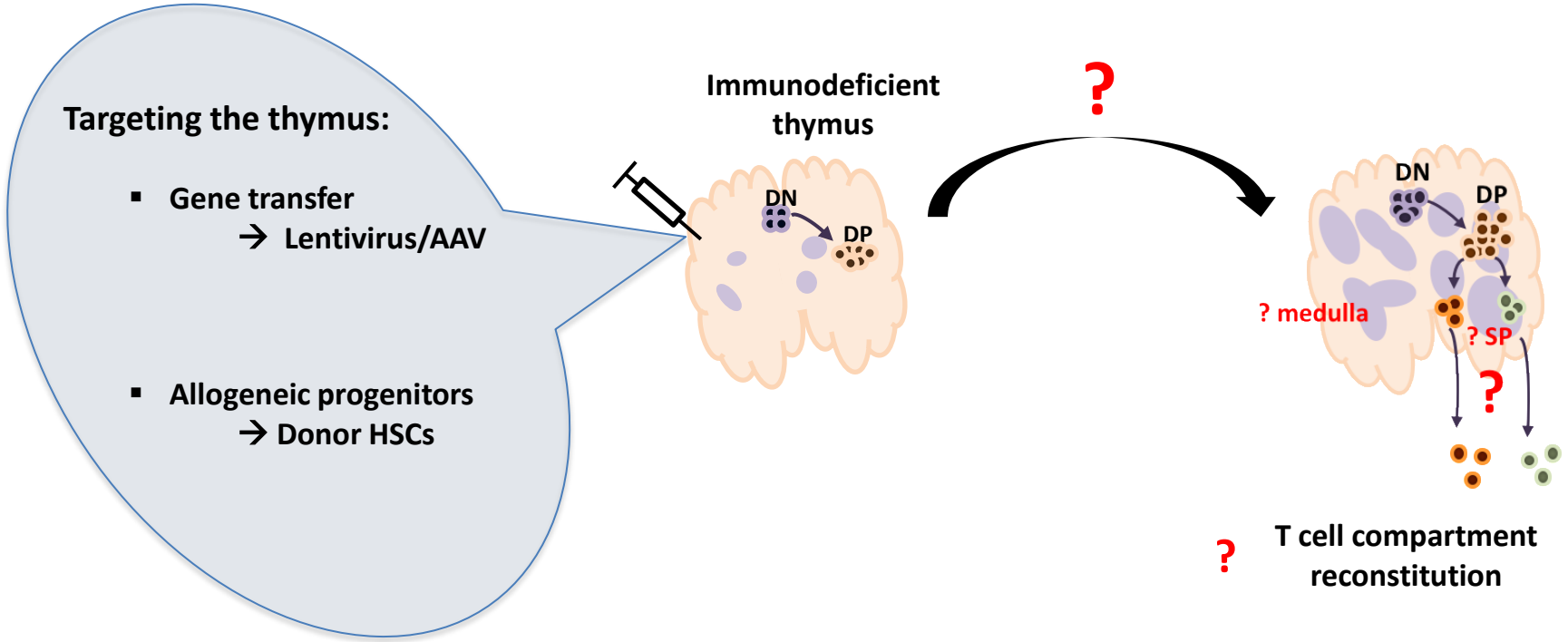
- Site of T cell differentiation
- Site of the block in genetic immunodeficiencies
- Easily accessible under anesthesia; no surgical intervention required
- Intrathymic administration:

→ Minimizes loss of cells/vectors during migration

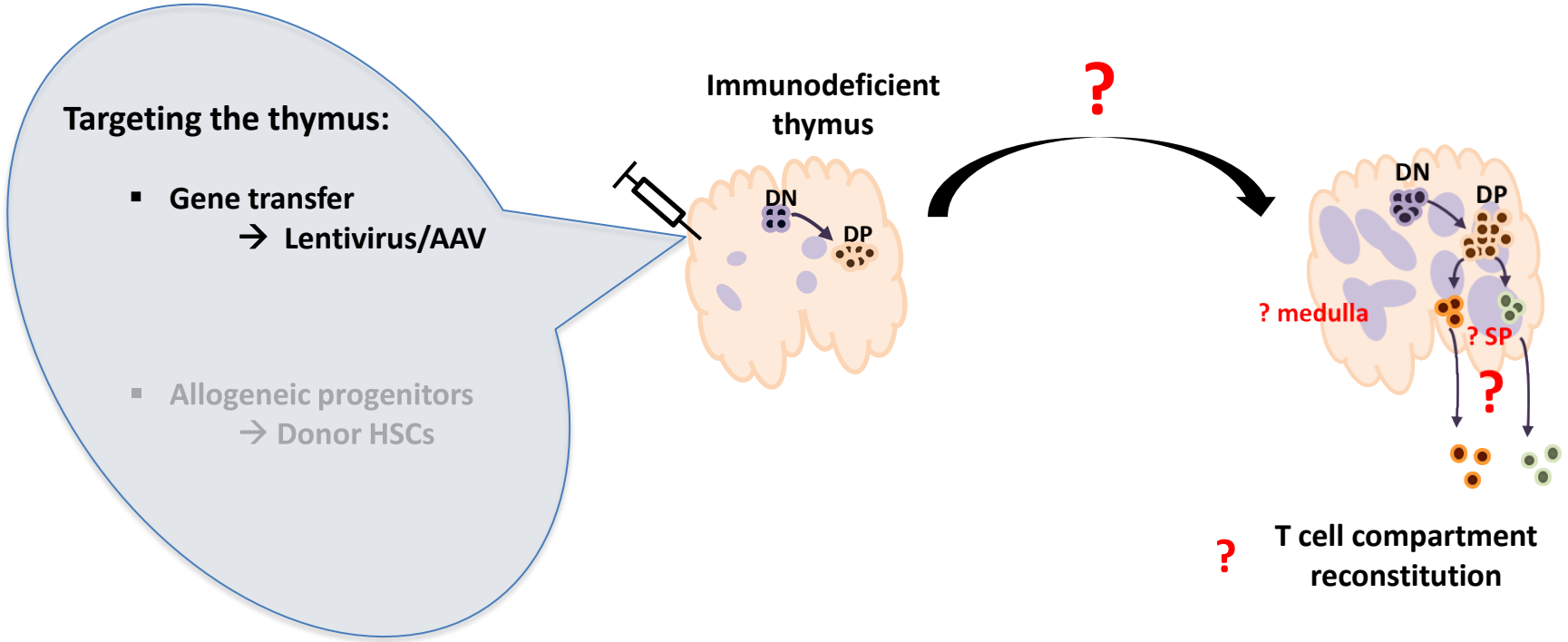
→ Homing «assured»



An innovative therapeutic approach: Targeting the thymus



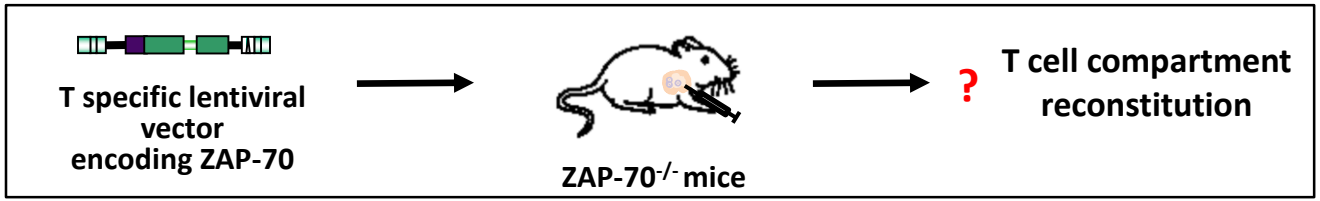
An innovative therapeutic approach: Targeting the thymus



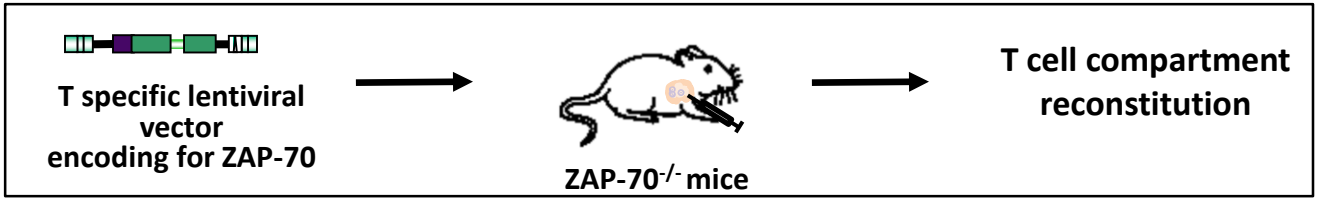
Treatment of ZAP-70 deficiency by intrathymic injection of a T-cell specific lentiviral vector

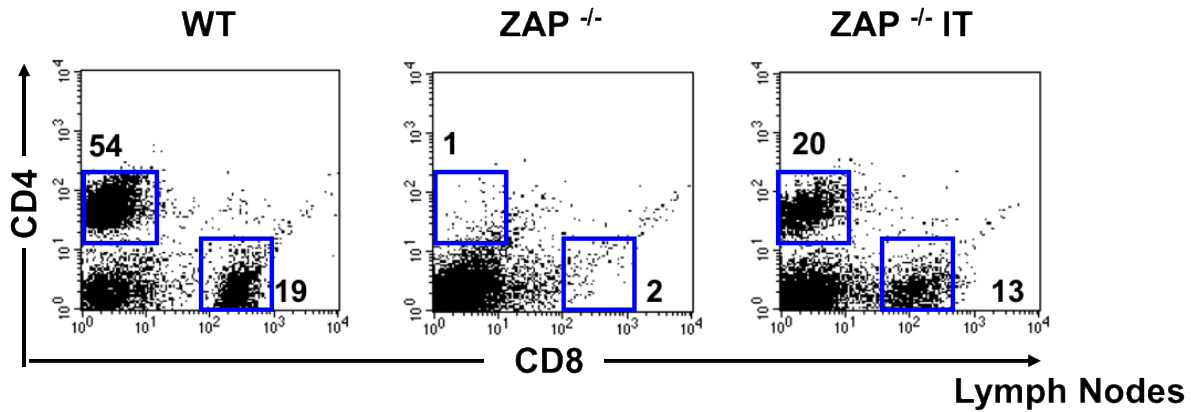
Why lentiviral vectors ?

- Transduction of quiescent and dividing cells
- Long term and stable expression of the transgene
- Lineage-specific transgene expression is feasible

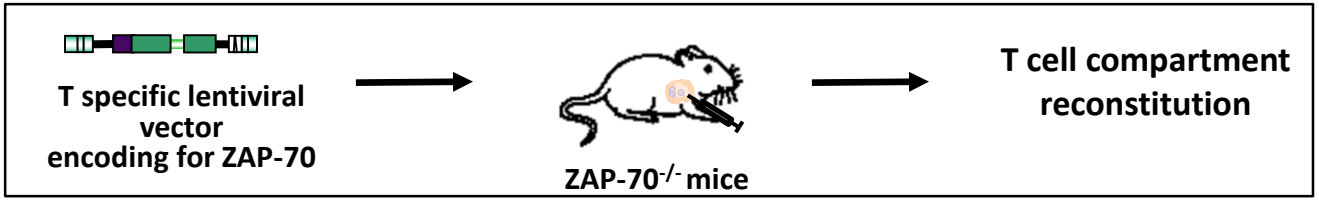


Intrathymic lentiviral transfer in ZAP-70$^{-/-}$ mice



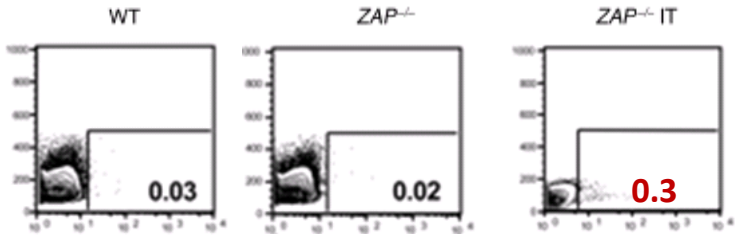


Intrathymic lentiviral transfer in ZAP-70$^{-/-}$ mice



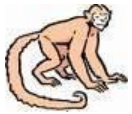
→ **But:**

- Low transduction of thymocytes



- Limited diversity of the T cell repertoire

- Ineffective in macaques



Adjali *et al.* , JCI, 2005

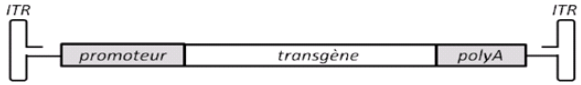
Promising results…

…need to improve thymocyte transduction:

Assessment of other vectors: rAAV ?

Adeno-Associated Virus (AAV) Vectors

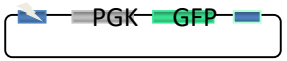
→ **Derived from single-stranded DNA adeno-associated viruses**



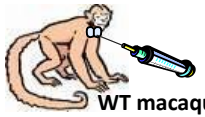
Advantages:
- already used in clinic
- transduction of quiescent and dividing cells
- long term and stable expression of the transgene in non-dividing cells
- broad and flexible tropism: different serotypes available (capside modification)
- predominantly non-integrative

Disadvantages:
- predominantly non-integrative
- minimal published data on hematopoietic cell transduction by AAV

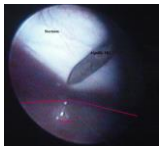
rAAV2/8 promotes efficient intrathymic gene transfer in mice and macaques



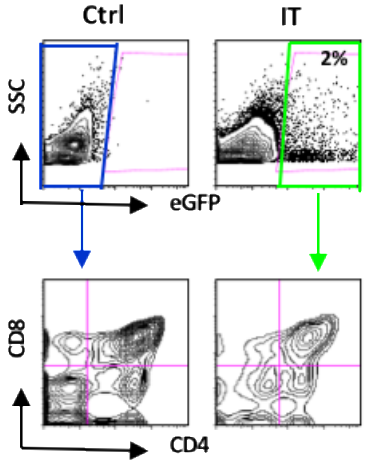
ss + scAAV2/1-PGK-GFP
ss + scAAV2/2-PGK-GFP
ss + scAAV2/4-PGK-GFP
ss + scAAV2/5-PGK-GFP
ss + scAAV2/8-PGK-GFP

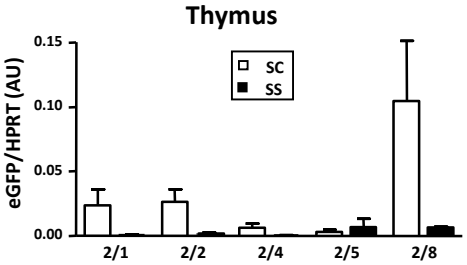


IT injection: endoscopy



Analysis of thymocyte transduction





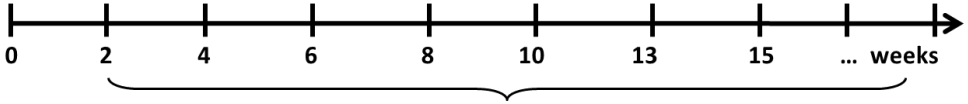
Day 7

Potential use of scAAV2/8 for modulation of thymus function

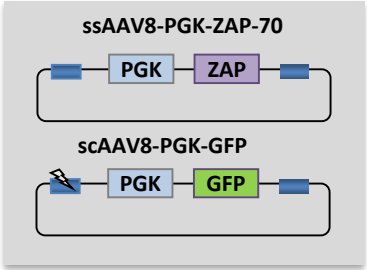
Moreau/Vicente et al, *Mol. Therapy*, 2009

Testing AAV8-ZAP-70 gene therapy: ZAP-70$^{-/-}$ mouse model

ZAP-70$^{-/-}$ mice

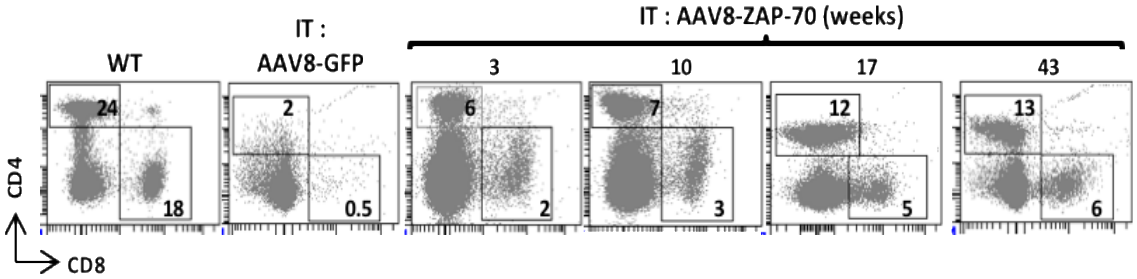


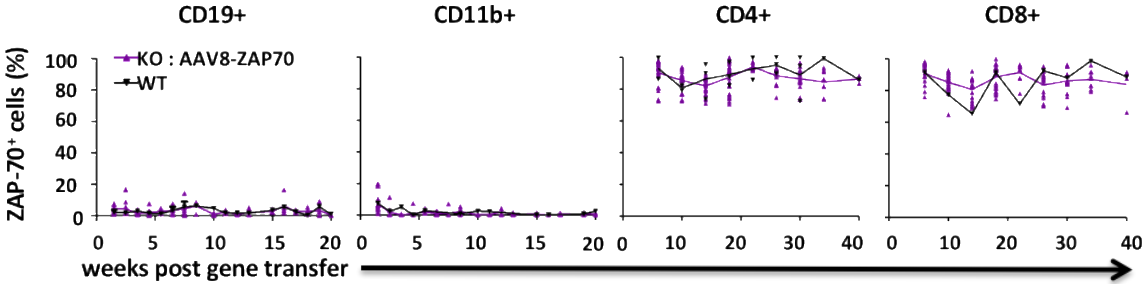
AAV8 IT injection



→ **Following immune reconstitution**

IT AAV8 gene transfer induces long term peripheral T cell reconstitution





Lymph Nodes

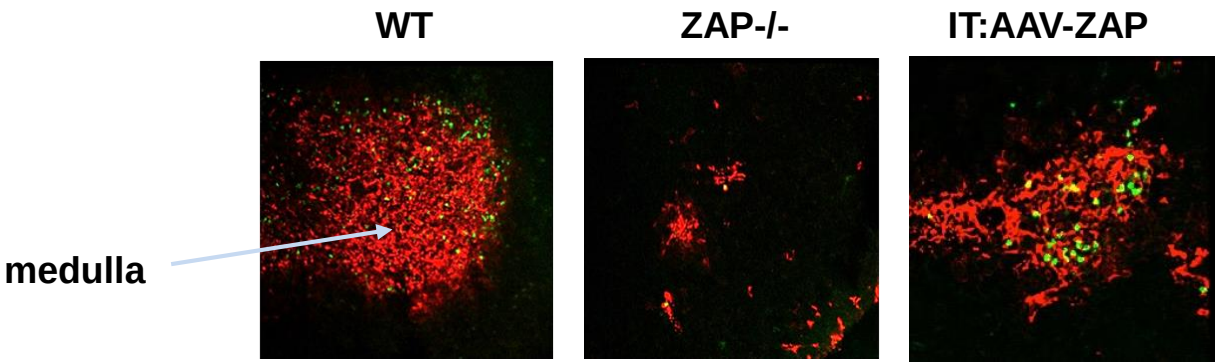
AAV8-ZAP-70 IT transfer:

→ **Peripheral T cell numbers remain elevated for up to 43 weeks post transplantation**

Conclusions

AAV8-ZAP-70 gene transfer:

- **Rapid but transient formation of a thymic medulla**
- **Rapid reconstitution of a functional T cell compartment in ZAP-70$^{-/-}$ mice**
- **Stable expression of ZAP-70-transduced T cells for >40 weeks**



Future Directions…

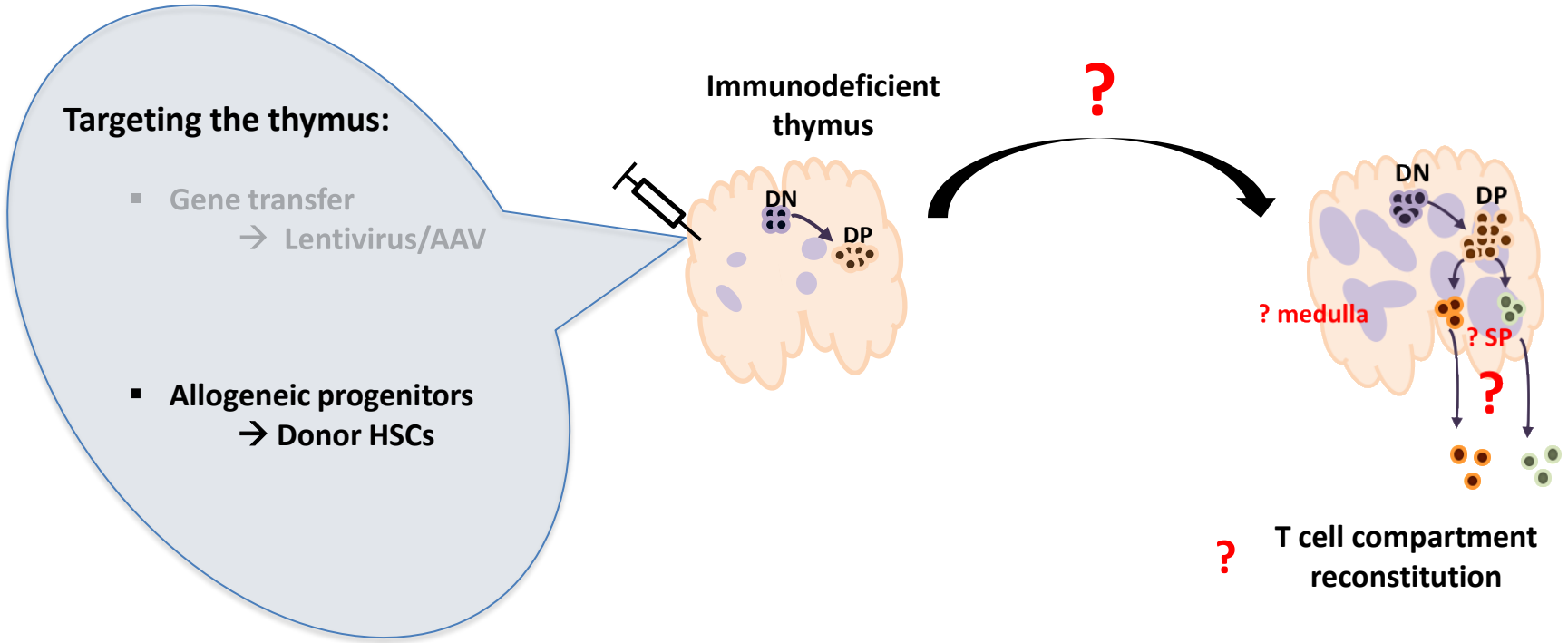
- **Effect of high ectopic ZAP-70 levels on T cell responsiveness**

  → Pathological conditions (infection, tumor, …)

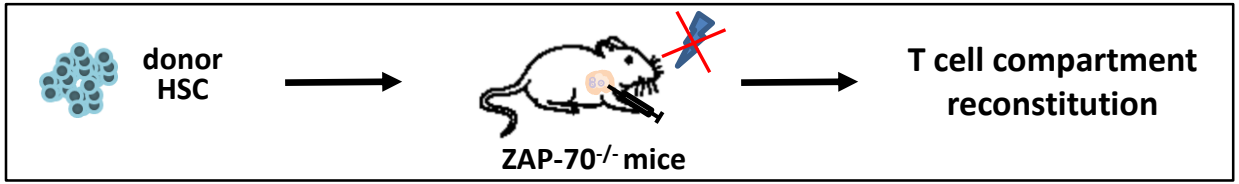
- **Evaluate the mechanisms accounting for the persistence of ZAP-70 in AAV2/8-transduced T cells**

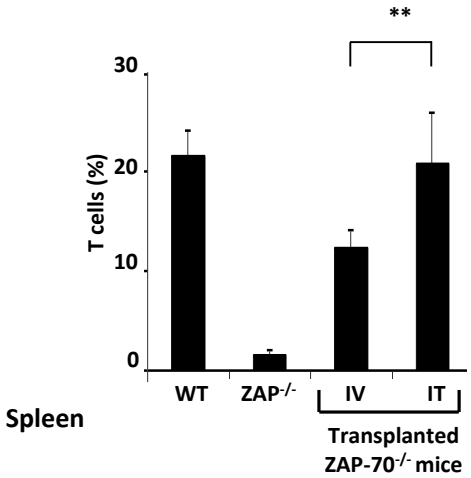
  → vector copy number in different organs
  → vector integration

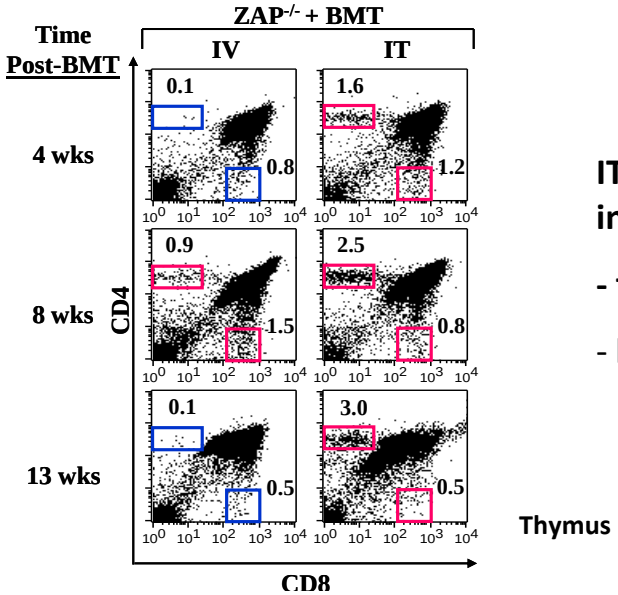
An innovative therapeutic approach: Targeting the thymus



T cell reconstitution in ZAP-70$^{-/-}$ mice following IT administration of BM progenitor cells





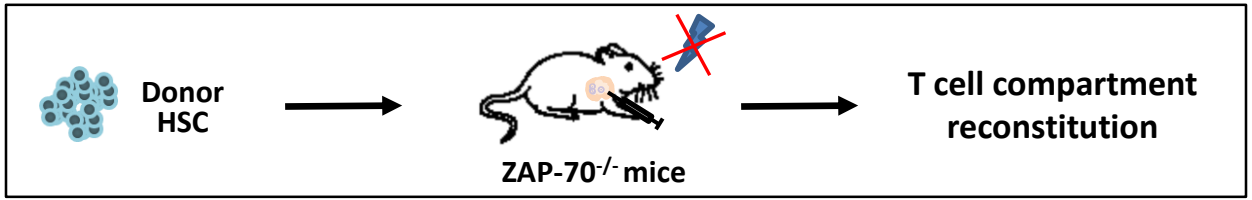


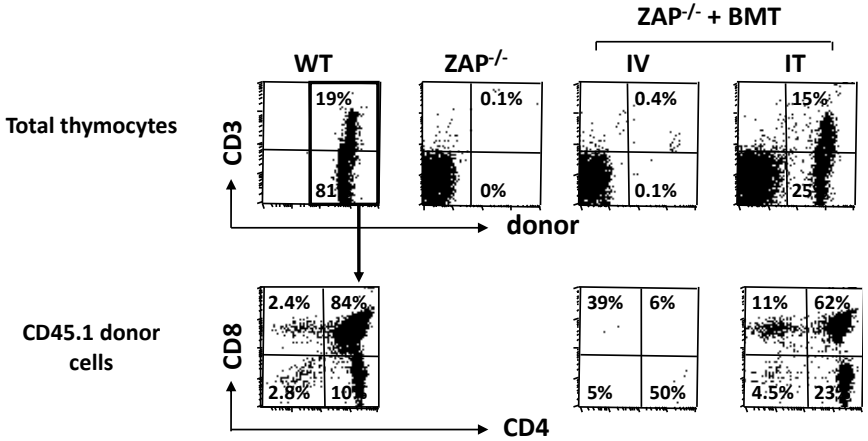
IT administration of progenitor cells results in:

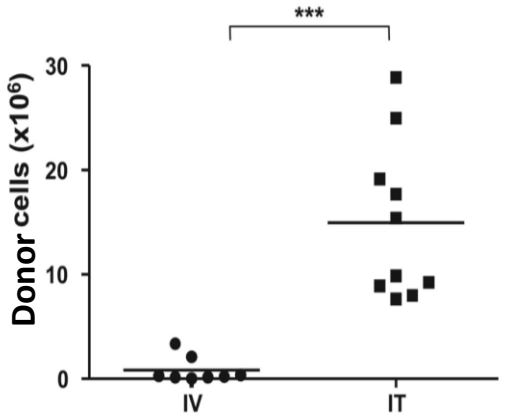
- faster/enhanced T cell reconstitution
- long term thymopoiesis

Adjali et al., PNAS, 2005 Vicente *et al.* , Blood, 2010 De Barros et al., Blood, 2013

Targeting the thymus: enhanced T cell reconstitution







Thymus 25 weeks

IT injection of HSCs results in enhanced T cell reconstitution in the absence of conditioning

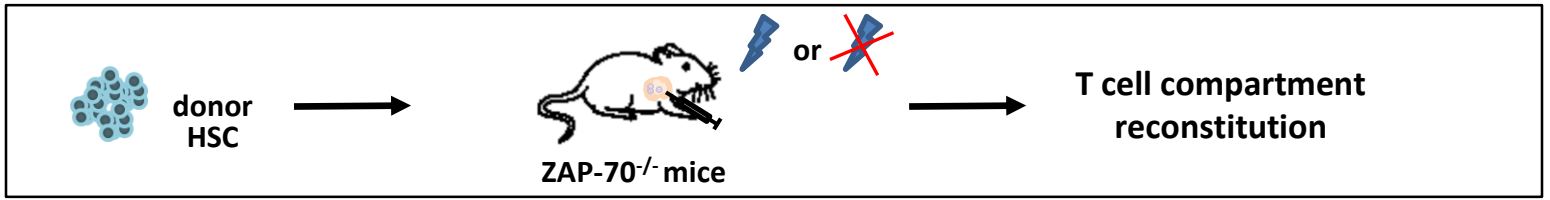
Vicente et al., *Blood* 2009

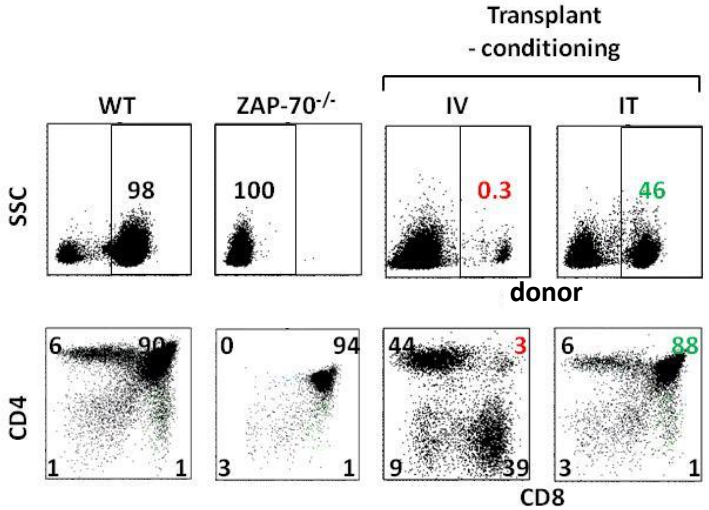
Can long-term thymopoiesis be achieved by the IT administration of HSCs?

- **Role of conditioning**

- Fate of HSCs in a semi-allogeneic setting
  <20% of patients have a histocompatible donor

Role of conditioning in the outcome of IV and IT HSC transplantation





Thymus 26 weeks

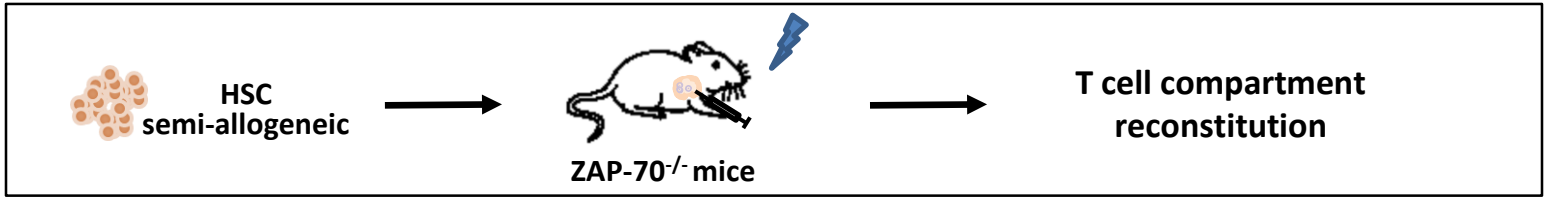
- **long-term donor-derived thymopoiesis:**
  - **dependent on conditioning following IV HSC administration**
  - **independent of conditioning after IT HSC administration**

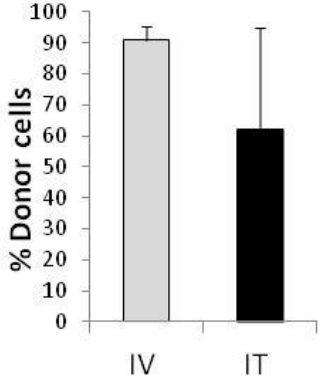
Can long-term thymopoiesis be achieved by the IT administration of HSCs?

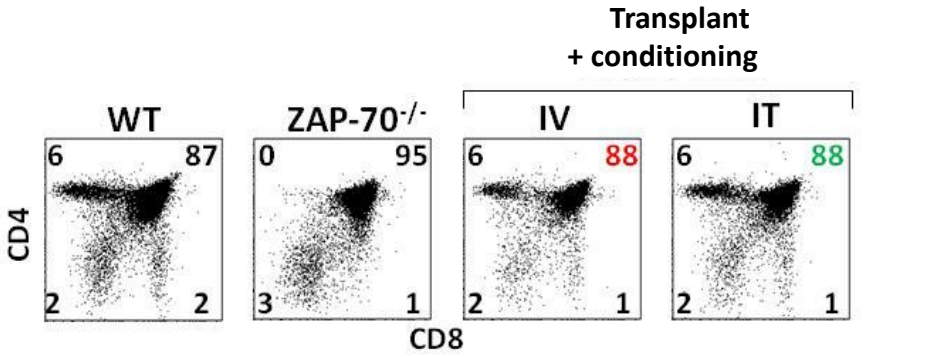
- Role of conditioning

- **Fate of HSCs in a semi-allogeneic setting**
  <20% of patients have a histocompatible donor

Both IV and IT injection of semi-allogeneic HSCs leads to long-term thymopoiesis in conditioned mice

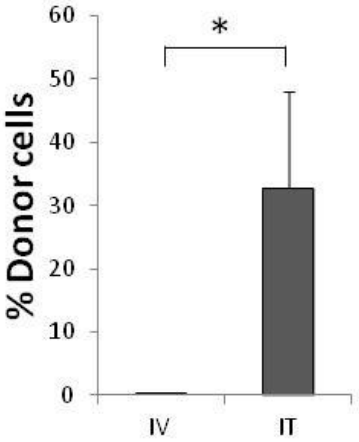


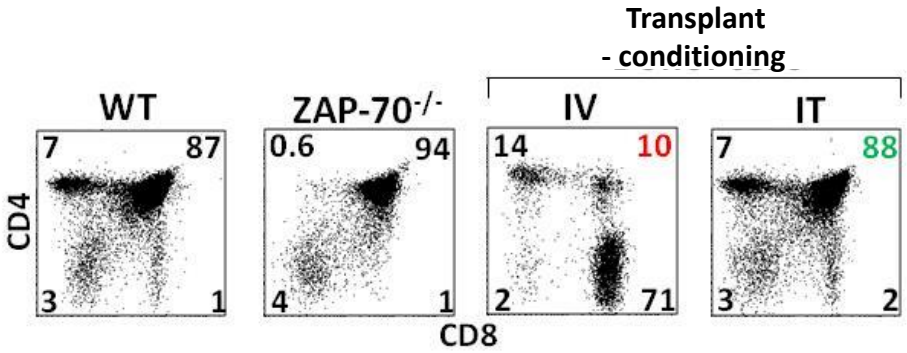




Thymus 26 weeks

In the absence of conditioning, only IT injection of semi-allogeneic HSCs leads to long-term thymopoiesis





Thymus 26 weeks

Conclusions

- **IT administration of HSCs:**
  - **Long-term thymopoiesis in the presence and even in the absence of conditioning**
    - **Irrespective of donor origin (histocompatible/ semi-allogeneic)**

- **Long-term thymopoiesis following IT transplantation:**
  - **Associated with the persistence of donor-ETPs**
    - **Extended myeloid potential as compared to WT ETPs**

Future Directions…

Effect of IT HSC transplantation on thymic architecture restoration
- **--> medulla generation**
- **--> T reg differentiation**

Elucidate the mechanisms resulting in thymus autonomous long term thymopoïesis

Feasibility of the thymic injection in SCID models with remnant thymus
- **--> RAG-/- mice**

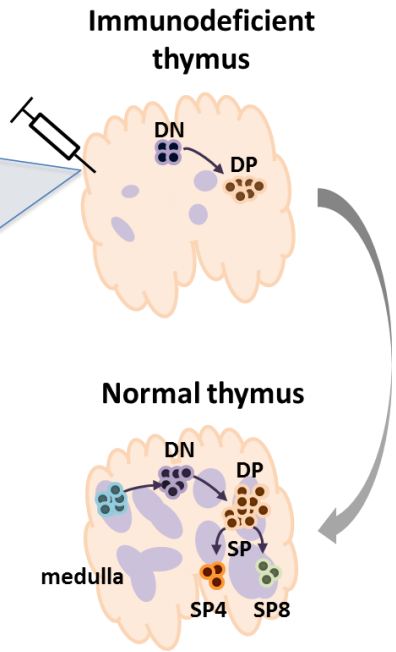
Feasibility of the thymic injection in humanized mouse models
- **--> NSG mice + CD34+ transplantation**

Concomitant injection of progenitors by IT and IV routes

Conclusions:

Targeting the thymus:

- Gene transfer
  → AAV
- Allogeneic progenitors
  → Donor HSCs



AAV gene transfer:

- Medulla formation
- Reconstitution of a functional T cell compartment >40 weeks

HSC transfer:

- Rapid and efficient engraftment
- Thymopoiesis by 2 weeks
- Long term thymopoiesis independent of donor origin and conditioning

Potential translation to the clinic:

Development of innovative therapeutic strategies for enhancing T cell development

Thank you !!

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Cédric ORFEO

MRI : Cytometry Platform
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RHEM

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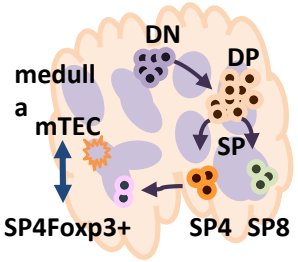
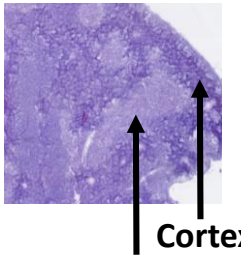
Carmen YONG

Former members :
Rita VICENTE
Stéphanie de BARROS

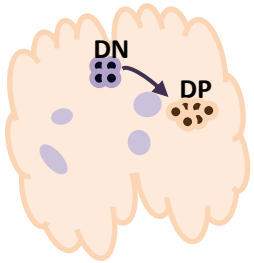
Treg generation requires the presence of a functional medulla

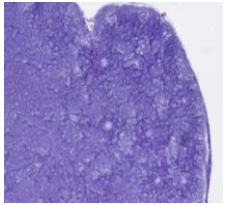
WT thymus

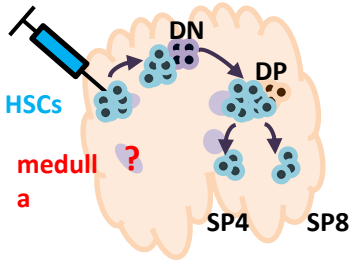




Immunodeficient thymus

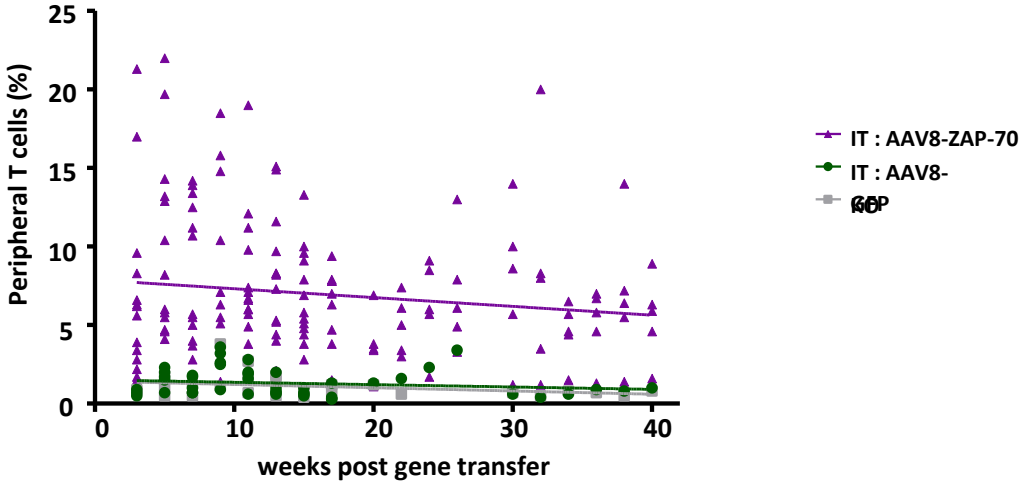






? What is the diversity of the cells that make up the thymic medulla

IT AAV8-ZAP-70 results in long-term maintenance of peripheral T cells

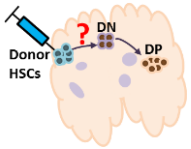


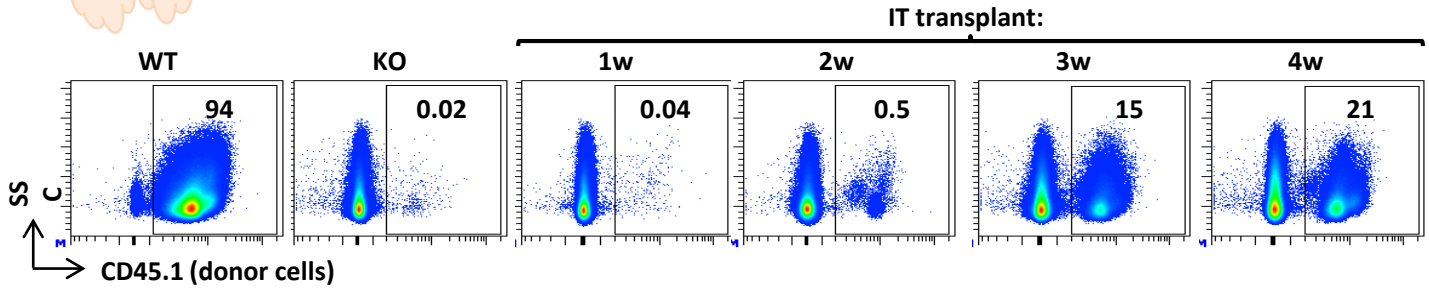
AAV8-ZAP-70 IT transfer:

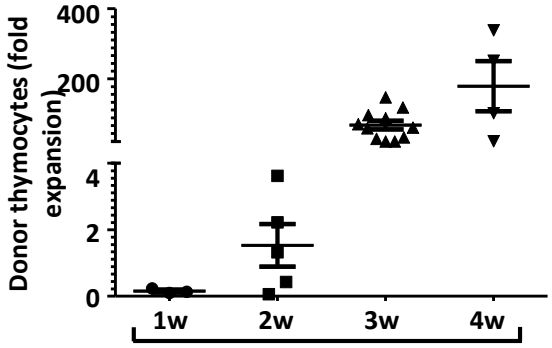
→ Rapid and efficient T cell reconstitution

→ T cells maintained for > 10 months

Rapid engraftment and expansion of donor HSCs following IT administration in ZAP-70$^{-/-}$ mice





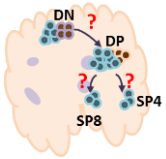


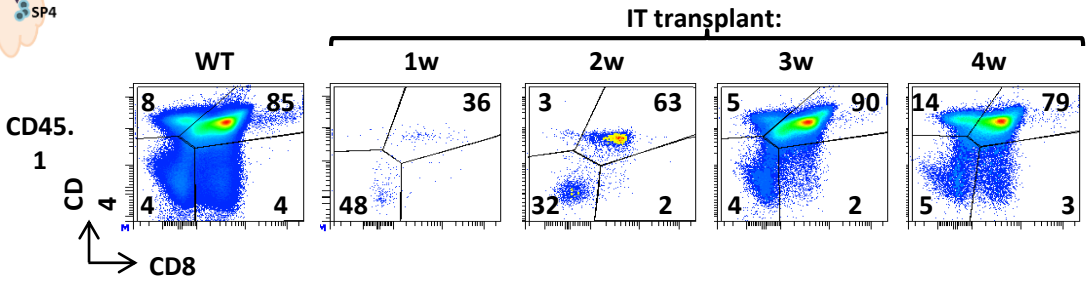
→ 100-fold donor thymocyte expansion by 3 weeks

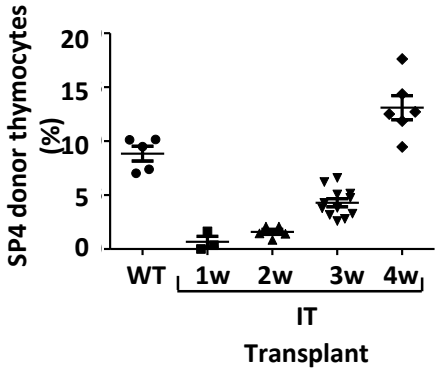
Selective advantage of donor progenitors

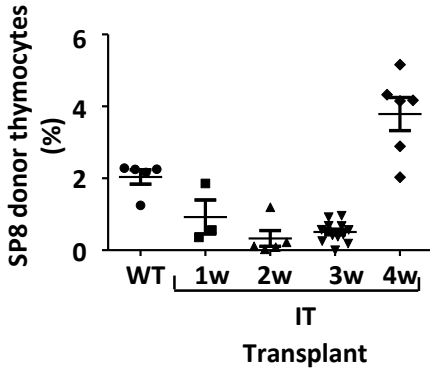
Thymus

IT HSCT results in a rapid kinetic of thymocyte maturation





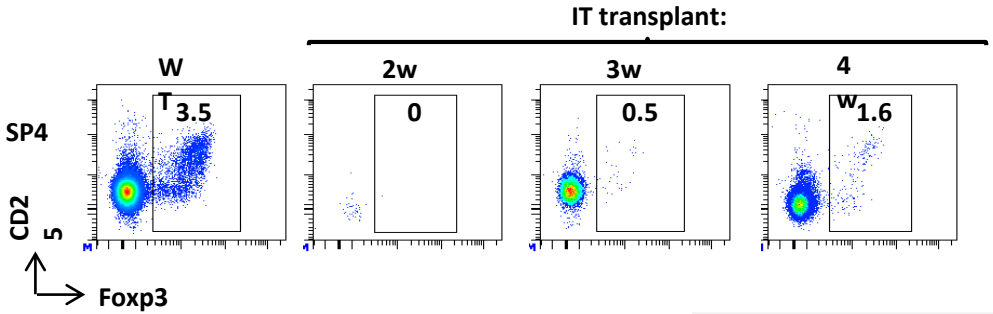


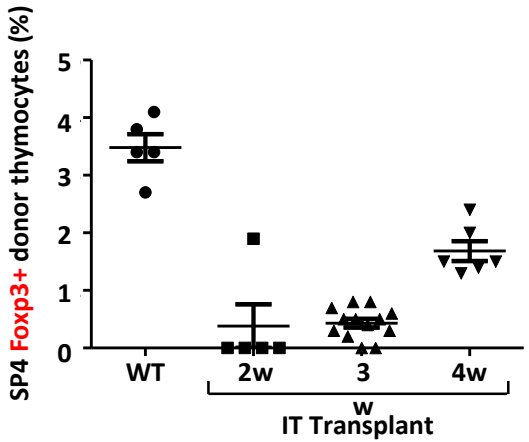


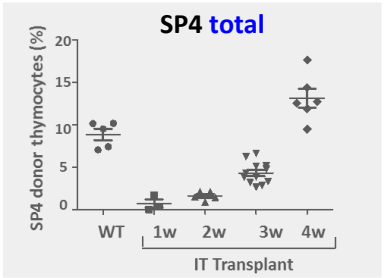
→ SP4 thymocyte differentiation by 3 weeks

→ SP8 thymocyte differentiation by 4 weeks

Delayed differentiation of SP4 Foxp3+ thymocytes following intrathymic HSC transplantation

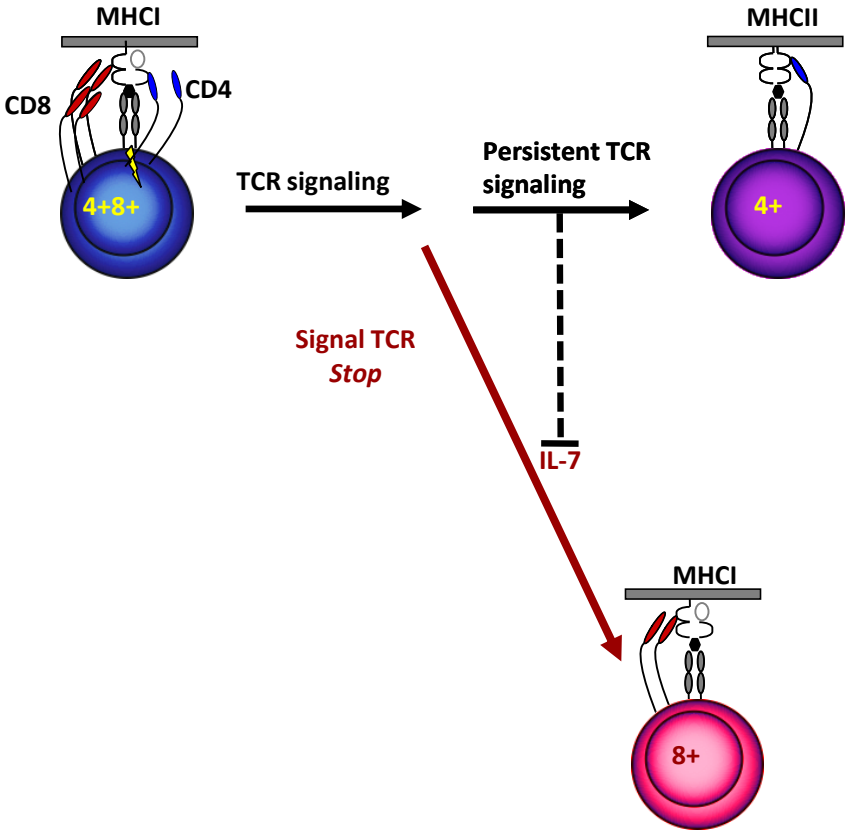






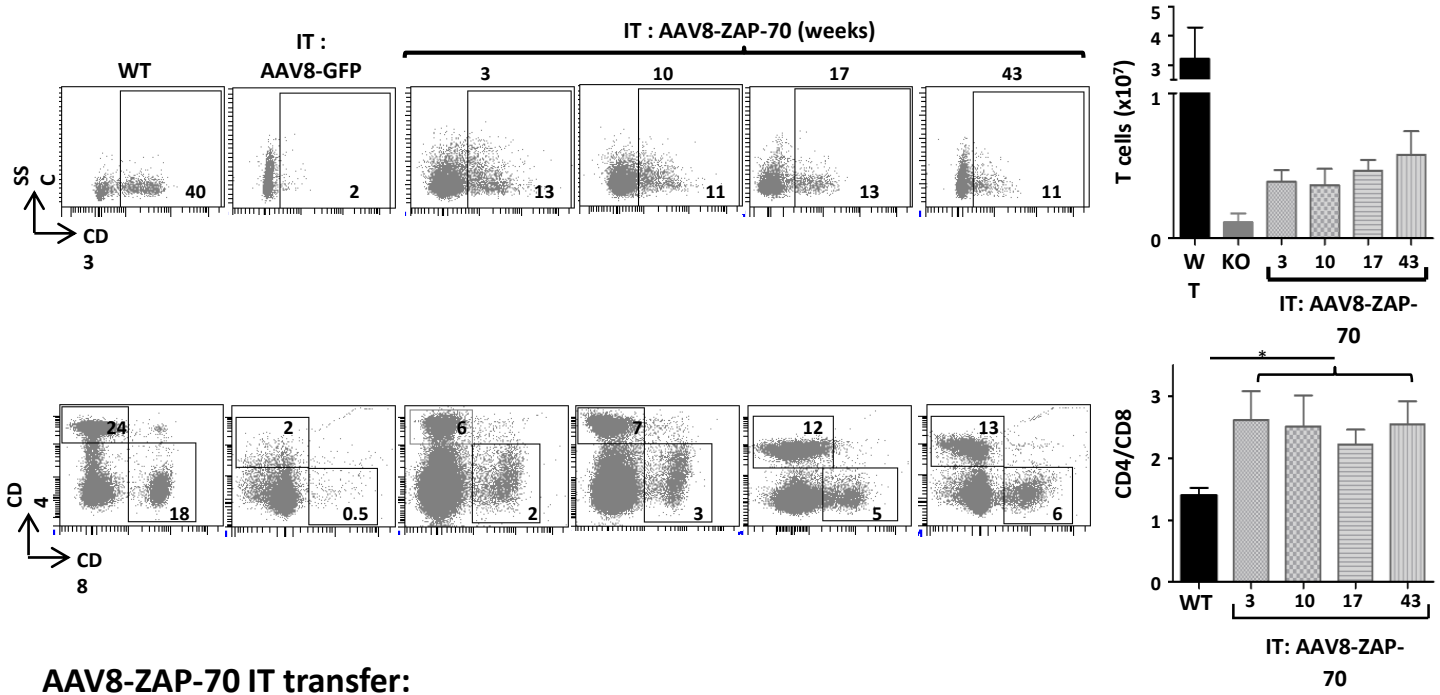
Thymus

The kinetic signaling model



Singer et al., *NRI*, 2008

IT AAV8 gene transfer induces long term peripheral T cell reconstitution

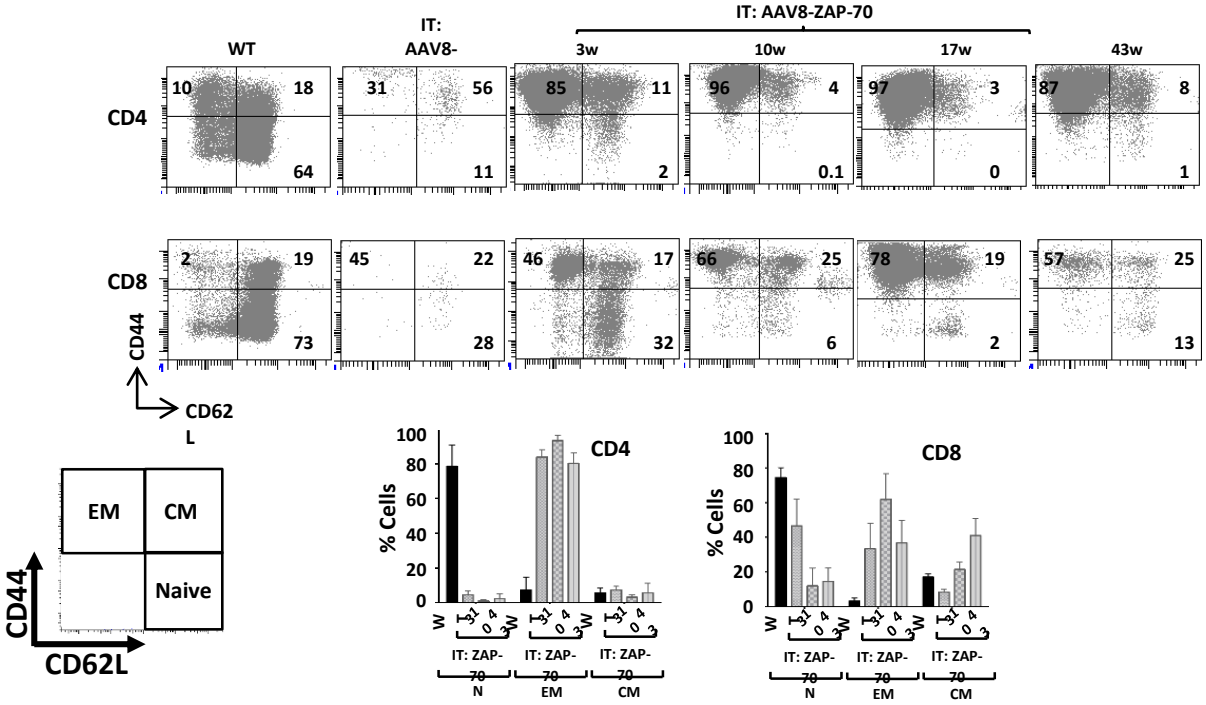


AAV8-ZAP-70 IT transfer:

→ CD4/CD8 ratio significantly skewed to a CD4 lineage fate

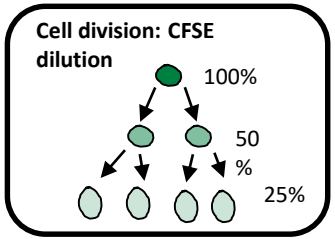
Lymph Nodes

AAV8-ZAP-70-transduced lymphocytes differentiate into effector cells

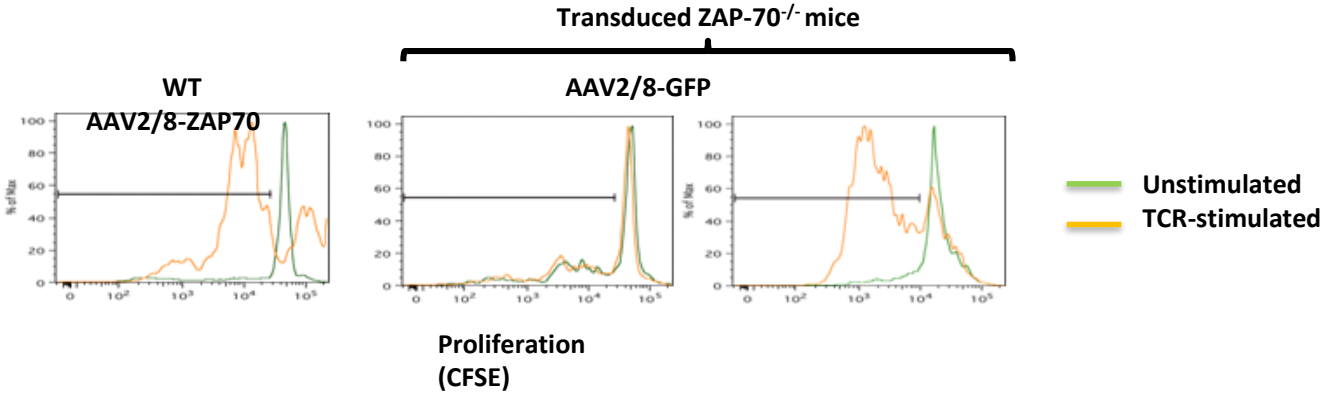


→ Significant increase in CM and EM T cells as compared to the majority of N T cells in WT mice

Proliferative capacity of ZAP-70 transduced T cells

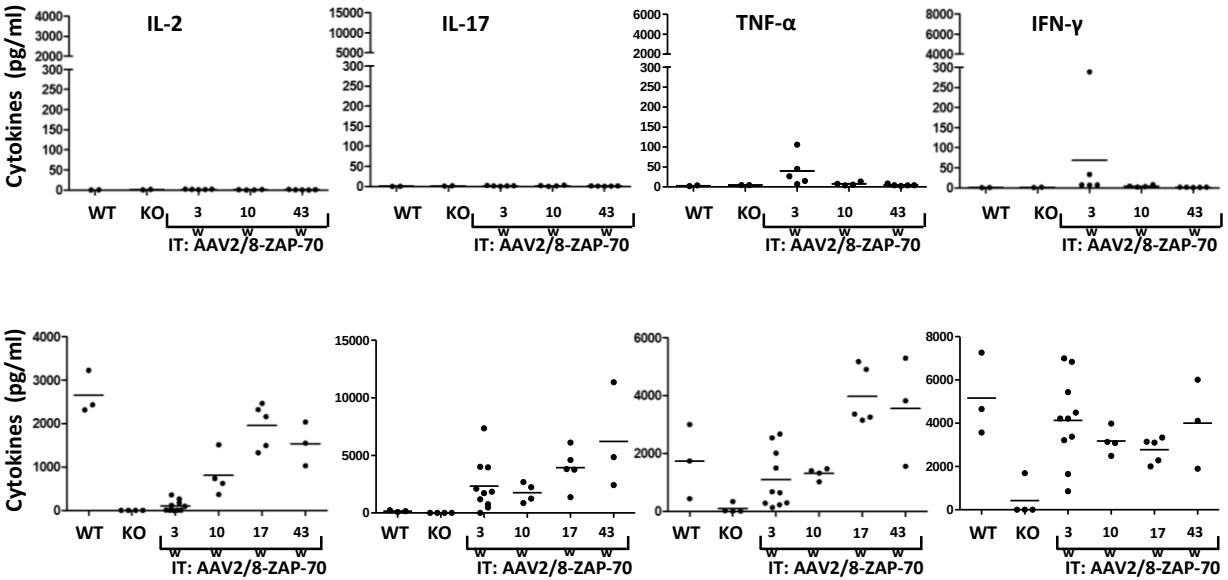


LN cells + CFSE → TCR stimulation (α-CD3 + α-CD28) → Proliferation → Flow cytometry



→ High proliferation in ZAP-70 transduced T cells following TCR stimulation

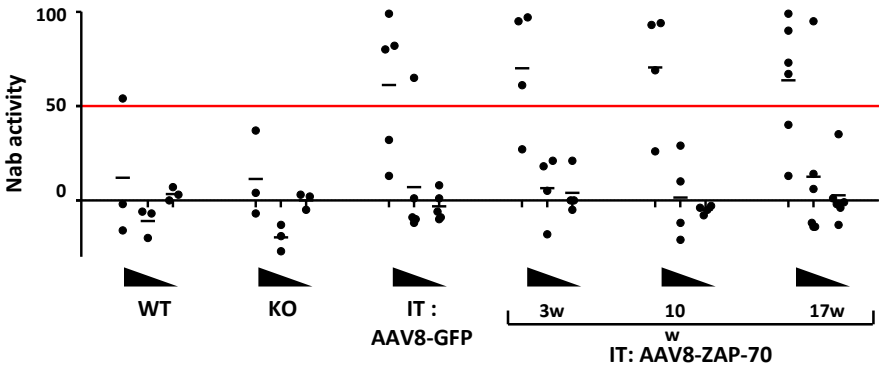
IT AAV8-ZAP-70 gene transfer generates functional T cells able to secrete cytokines upon TCR engagement



→ Increase TNF-α and IFN-γ levels in sera at 3w

→ Secretion of IL-17, TNF-α and IFN-γ by TCR stimulated-T cells

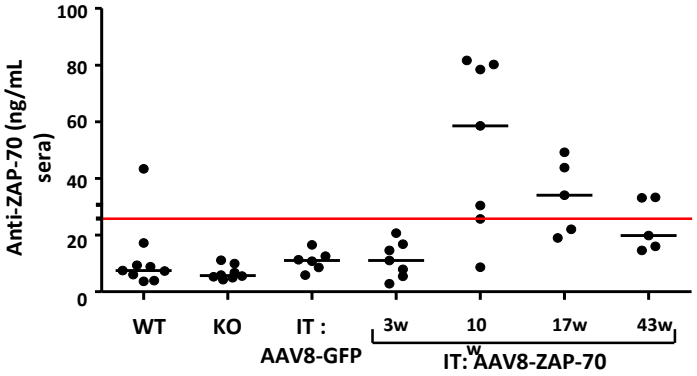
Induction of a T cell-independent humoral response to AAV8 capsid epitopes following IT vector administration



→ 10 of 16 AAV8-ZAP-70-treated ZAP-70$^{-/-}$ mice positive

→ Similar level with AAV8-GFP

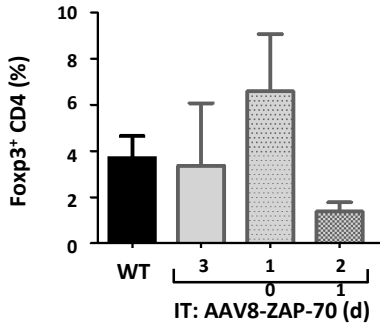
Production of anti-transgene following IT vector administration



→ Dilution anti-ZAP-70 antibodies at early time points (10w)

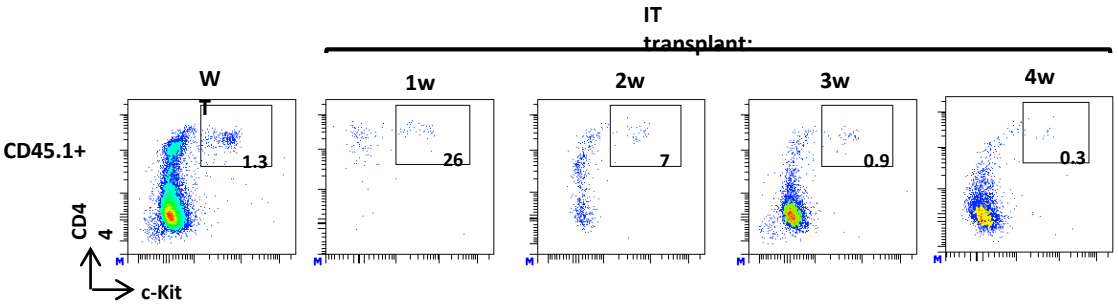
→ Decrease during time and low so no elimination of gene-transduced T lymphocytes

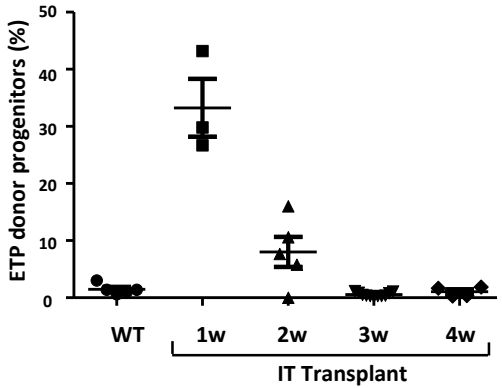
AAV8-ZAP-70-transduced thymocytes differentiate into regulatory T cells



→ Early development in the thymus

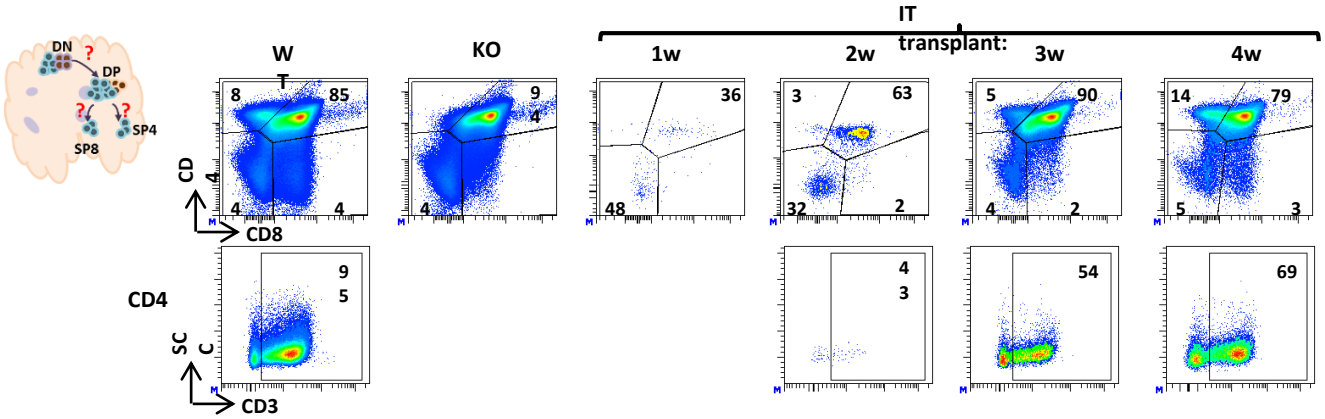
IT HSCT results in a dramatic increase in early thymic progenitors (ETP)



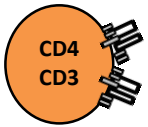


→ High % of donor ETP by 1w

IT HSCT results in a rapid kinetics of thymocyte maturation



Single Positive



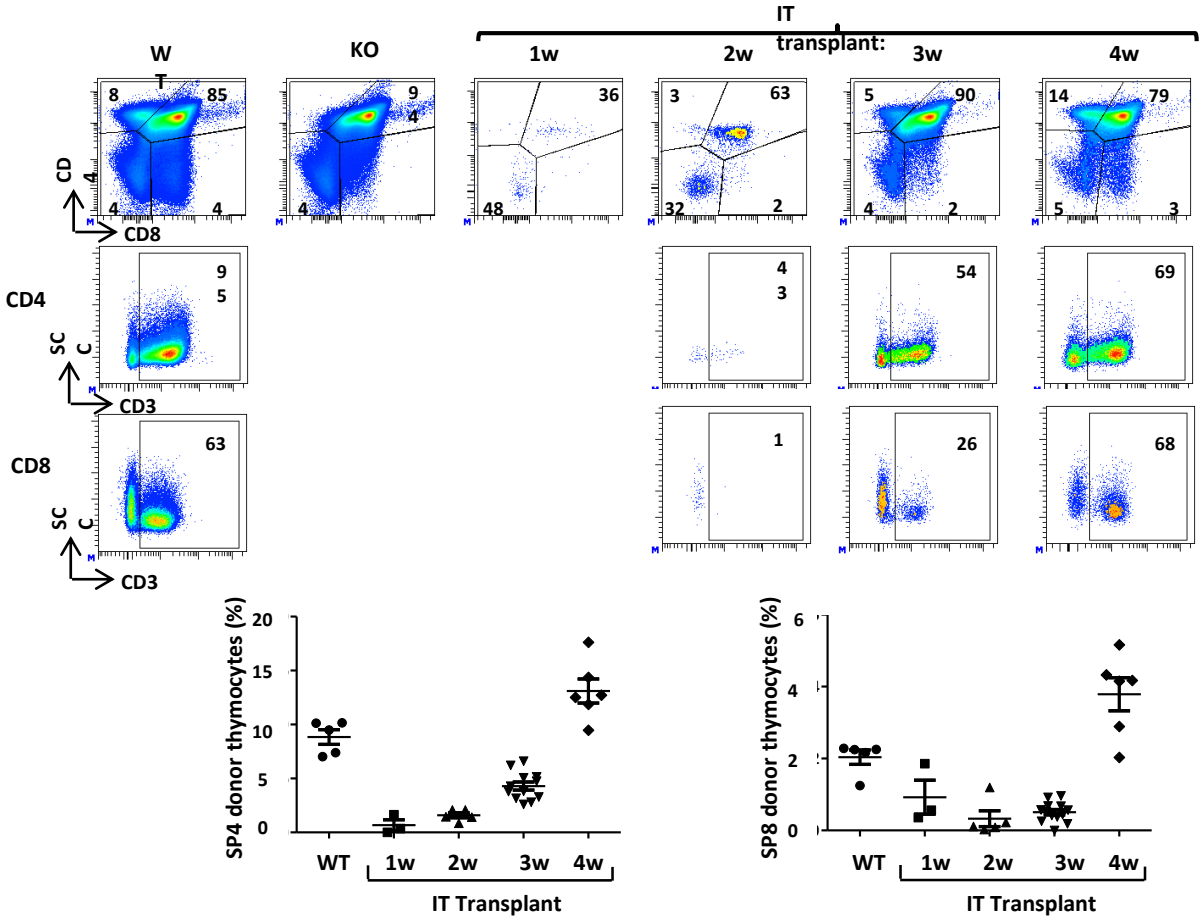
SP4

Lymphoid Tissue inducer

LTi: CD4+CD8- CD3-

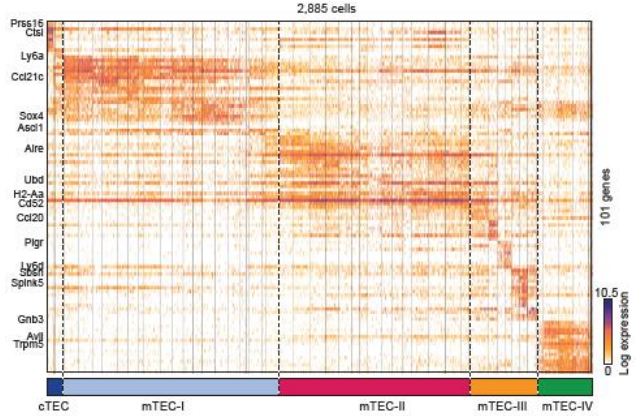
→ SP thymocyte differentiation by 3w

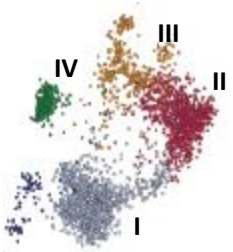
IT HSCT results in a rapid kinetics of thymocyte maturation



→ DP thymocyte differentiation by 2w

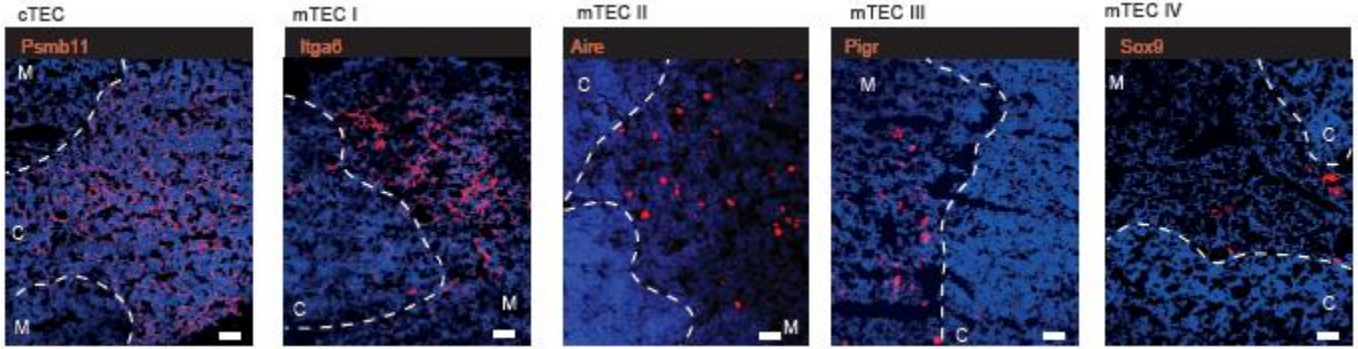
Heterogeneity of TEC compartment: new mTEC subtypes

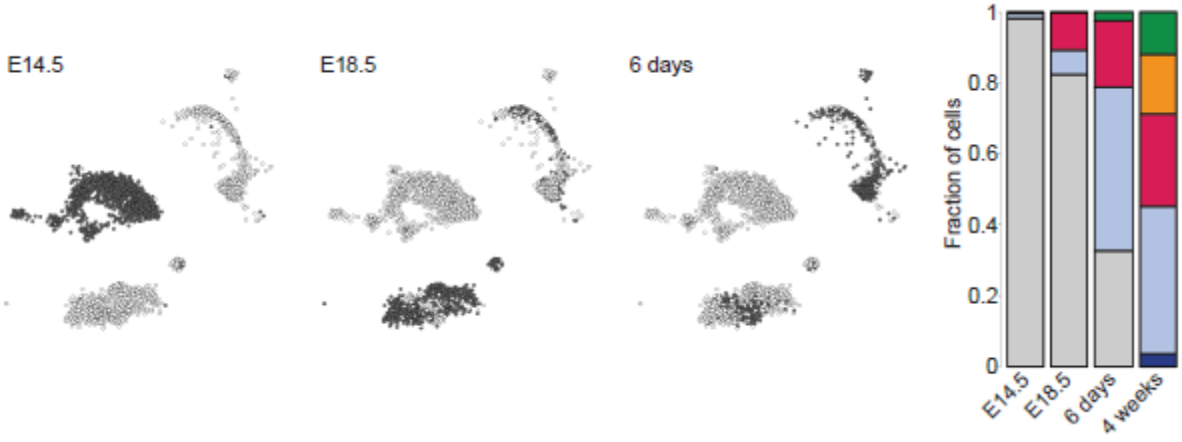




De *novo* map of thymic stromal populations

Identification of 4 mTEC subsets




E14.5
E18.5
6 days
Fraction of cells
1
0.8
0.6
0.4
0.2
0
E14.5
E18.5
6 days
4 weeks

Thymus targeting

- **Alternative strategy applicable for both cell and gene therapy**
- **Potential treatment for immunodeficiencies and other clinical conditions requiring rapid T cell reconstitution**
- **Clinical application (non-traumatic, rapid and feasible)**