Advanced Genetic Engineering of Hematopoiesis for the Treatment of Inherited Diseases

ASGCT 22nd ANNUAL MEETING
Gene Editing Workshop
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SAN RAFFAELE TELETHON INSTITUTE FOR GENE THERAPY
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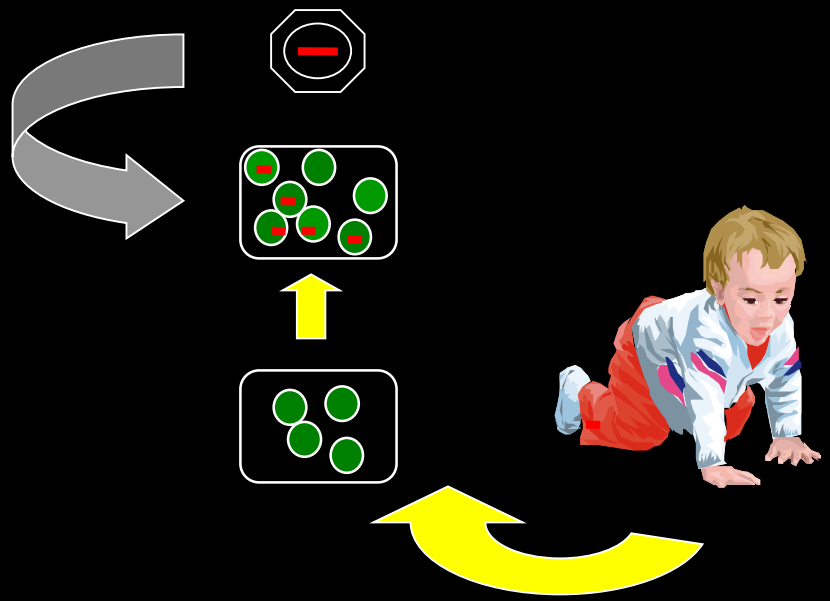
Therapeutic Potential of HSC Gene Therapy

Immuno-he

Gene Transfer



Harvest Hematopoietic Stem Progenitor Cells

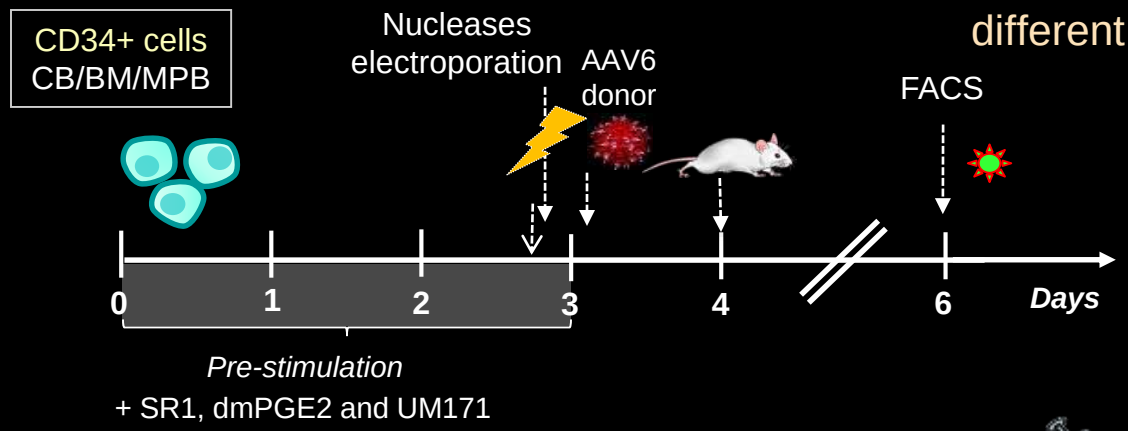
Tissues

Storage diseases

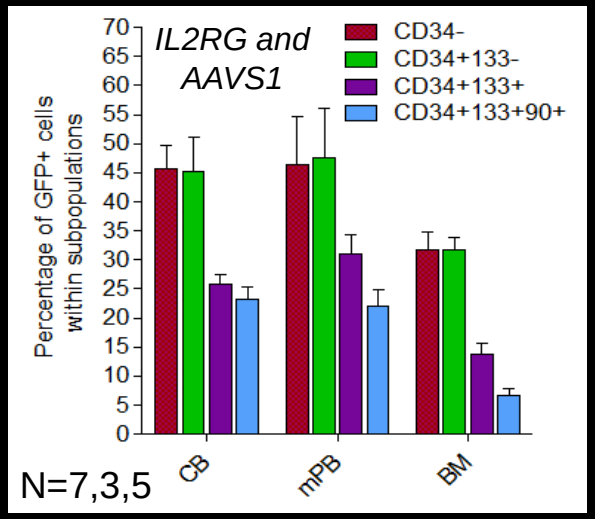
Therapeutic Potential of Targeted Gene Editing in HSC Gene Therapy

- *Targeted insertion in a safe locus*
- *Correct in situ mutations*
  - restores gene *function* and *expression control*
- *genotoxic risk limited to off target activity*
  - circumscribed to small fraction of genome
- *often constrained by*
  - unavailable or low efficiency of HDR
  - need for DNA template co-delivery

Optimized HSPC Gene Editing Protocol



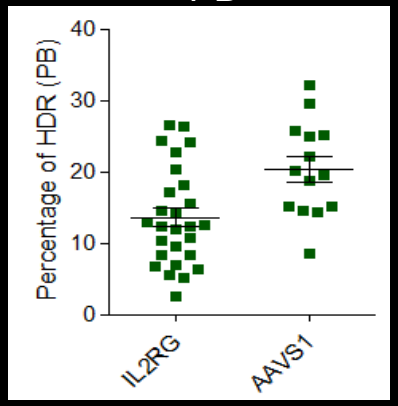
HSPC from different sources

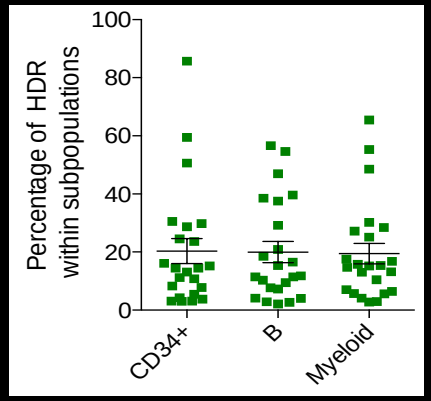


- ~50-70% HDR editing in bulk CD34+ cells
- Independent from the nuclease platform
- Lower but substantial efficiency in primitive cells (20-40%)
- Long-term multi-lineage engraftment of edited HSPC
- Reproducible on HSPC from different cell sources

IL2RG and AAVS1 site BM analysis

PB





12-20 wks after transplant

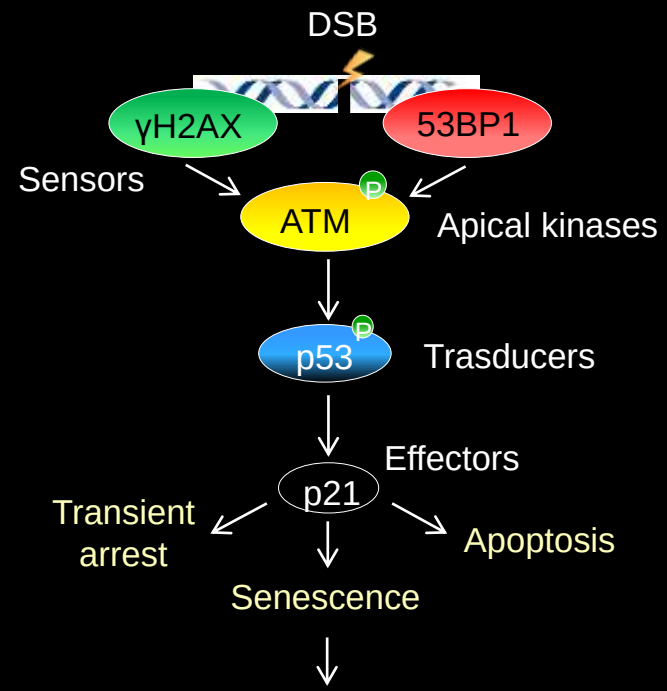
Genovese et al., Nature 2014

Schiroli, ..., Genovese, Naldini* Science Translational Medicine 2017*

Petrillo et al., Cell Stem Cell 2018

Impact of DNA Damage Response on HSPC

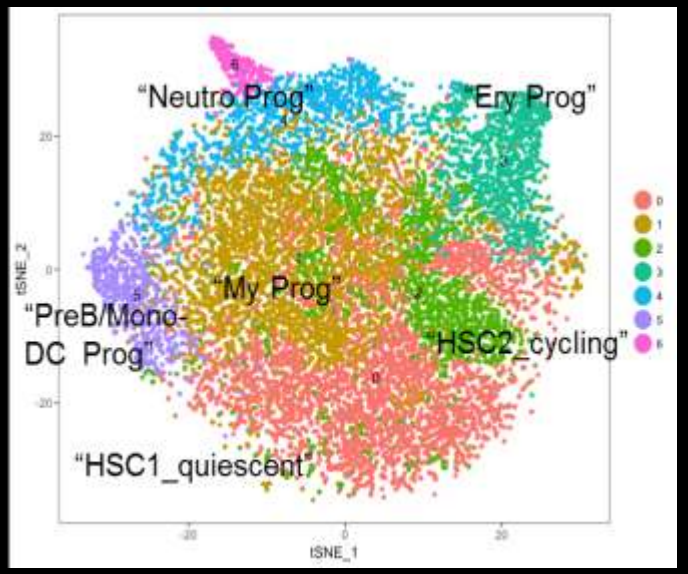
- Although improved, the yield of HDR-edited HSPC remain limiting
  - Does it reflect biological response(s) to DNA DSBs impacting long-term repopulation activity?



G. Schiroli A. Conti

Collab. R. Di Micco

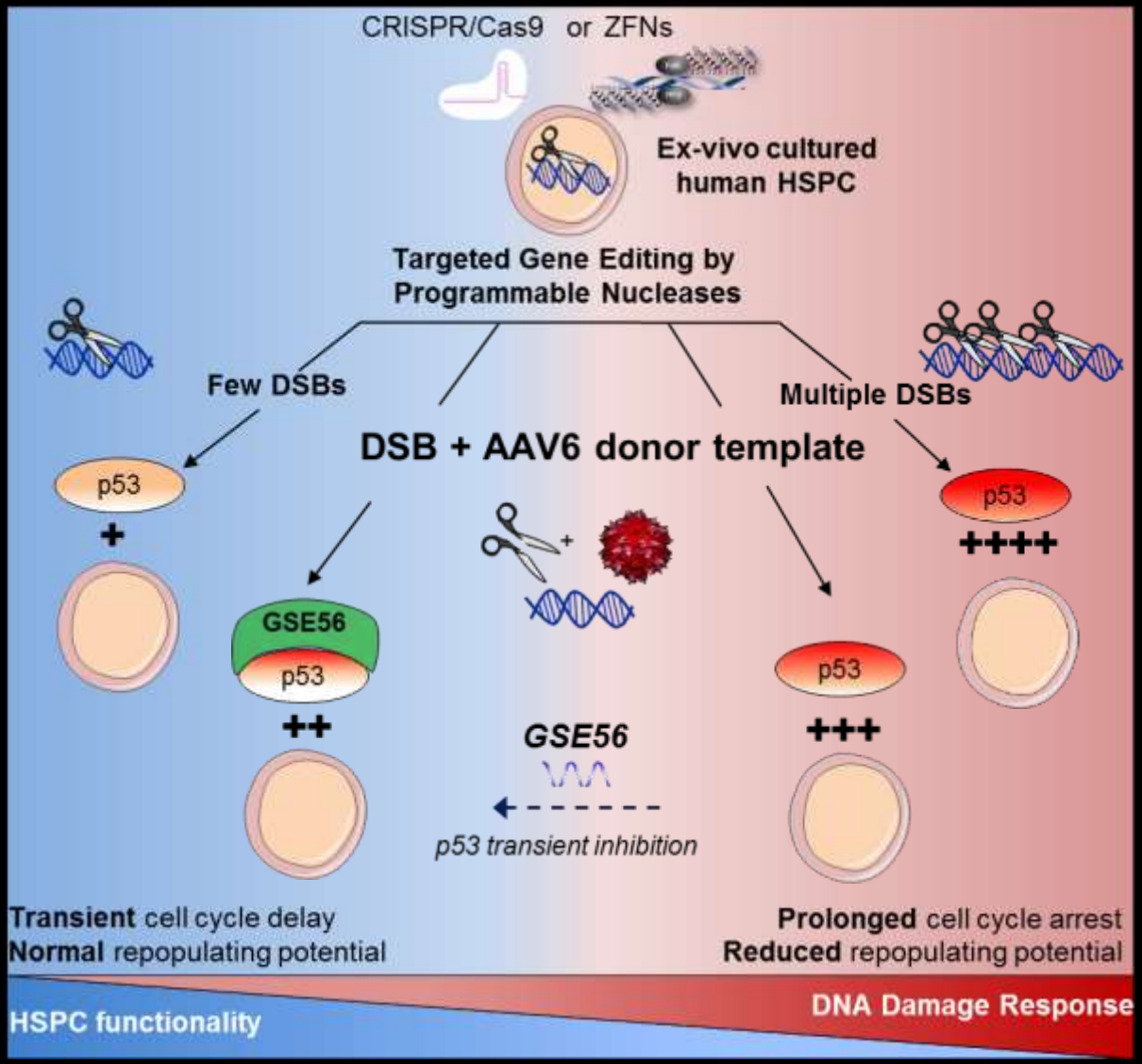
Single cell transcriptomic



Non-linear dimensional reduction with supervised approach (gene list from *Fares et al., Blood 2017*)

Milyavsky.. Dick, Cell Stem Cell 2010
Mohrin.. Passegué, Cell Stem Cell 2010

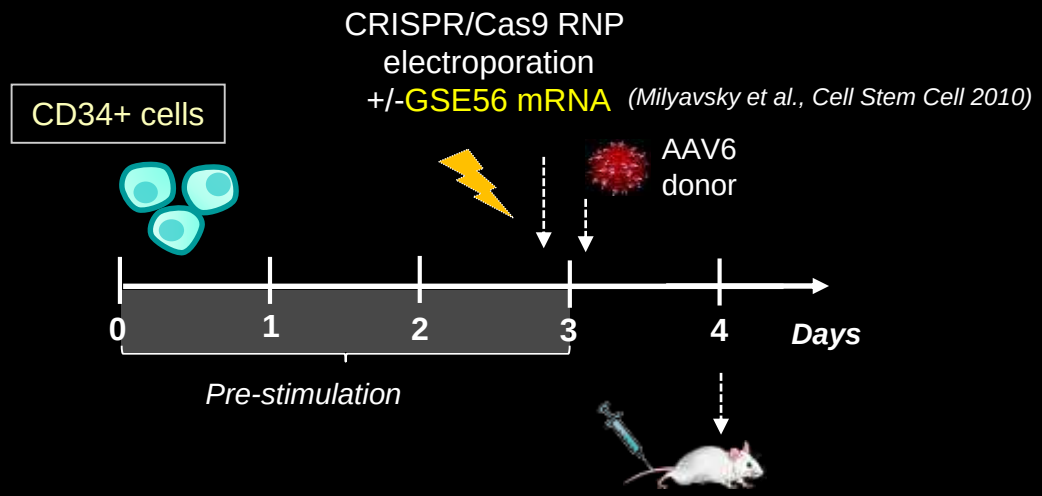
HSPC Response at Single-Cell Resolution



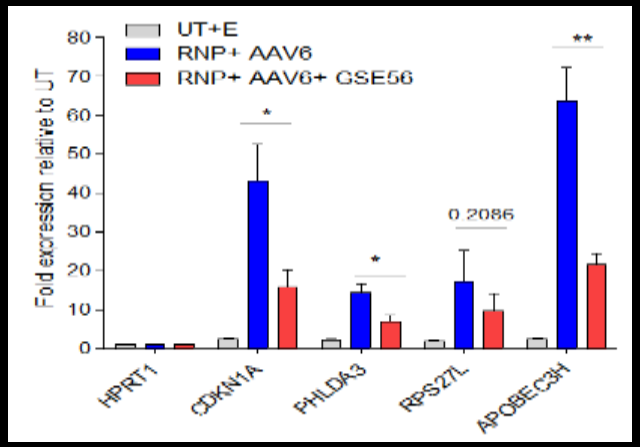
Schiroli, … Genovese, Naldini*, Di Micco*; Cell Stem Cell 2019*

OR 961: Schiroli, May 2nd – Excellent Award

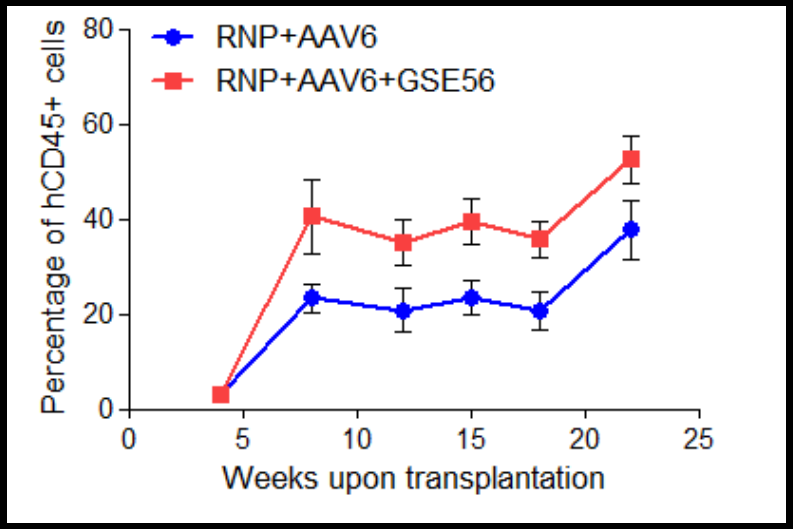
Inhibiting p53 response improves edited HSC fitness



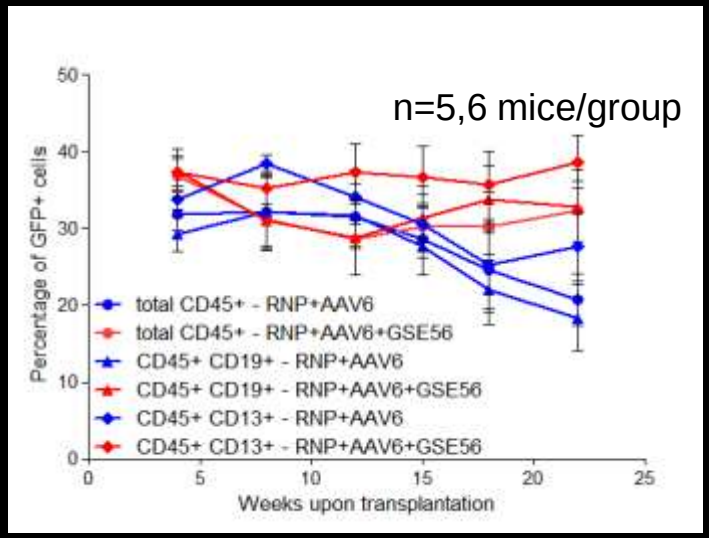
p53 Transcr Signature



Engraftment of human cells (PB)



% GFP in Human PB cells



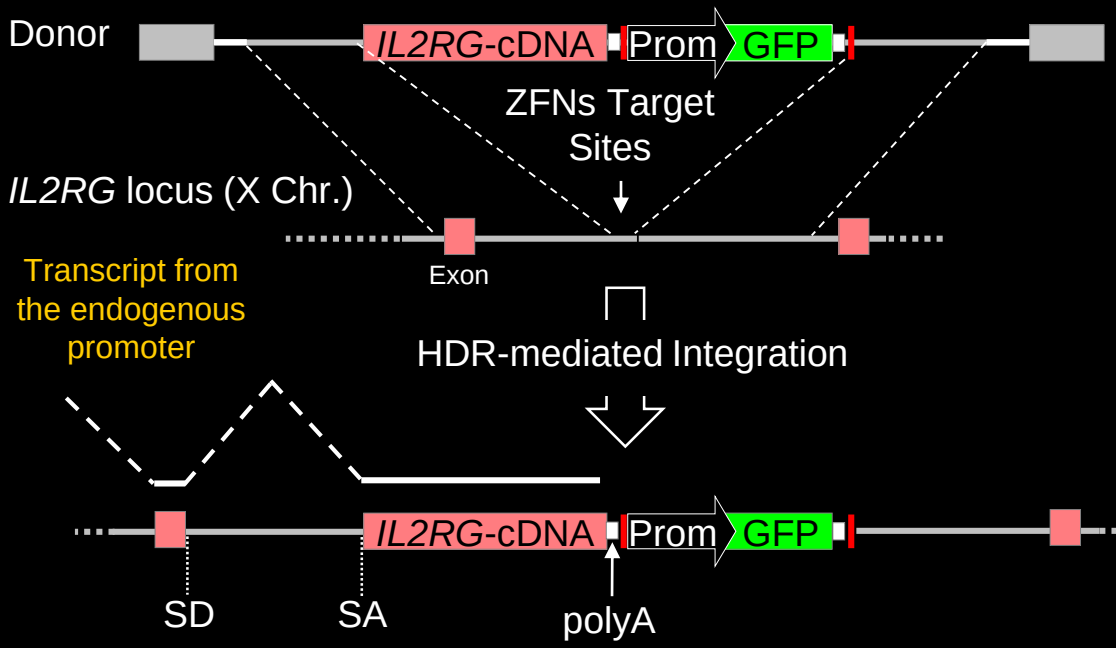
Schiroli, … Genovese, Naldini*, Di Micco*; Cell Stem Cell 2019*

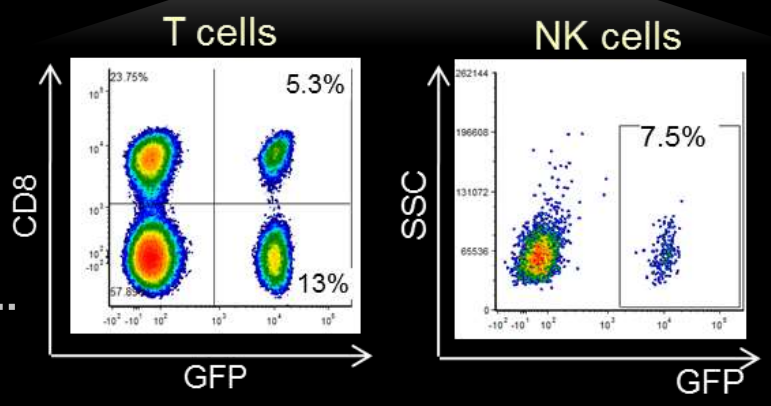
ASGCT OR 961: Schiroli, May 2nd – Excellent Award

Towards Clinical Testing of Targeted Gene Editing in HSC Gene Therapy

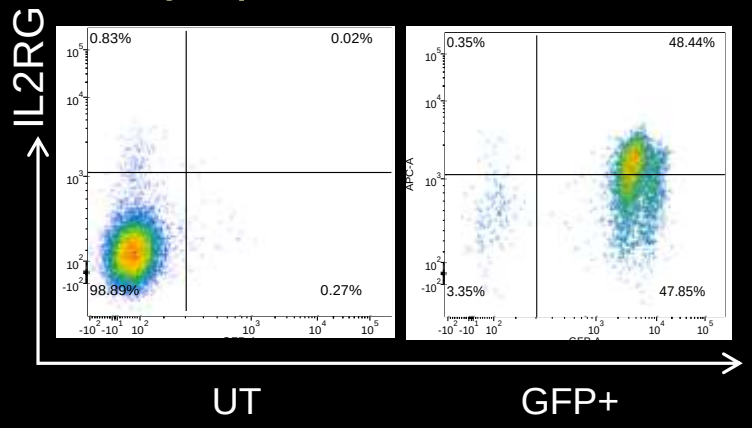
- *Primary immunodeficiencies such as: IL2RG, RAG1/2, CD40L,*
  - provide best risk-benefit ratio for first clinical testing
  - unregulated gene expression may pose risk of transformation or malfunction
  - selective advantage of gene corrected progeny may compensate for low editing efficiency
  - lymphoid progenitors may provide therapeutic benefit in the absence of long-term engrafted HSC

“One Size Fits all” Gene-correction Strategy for SCID-X1

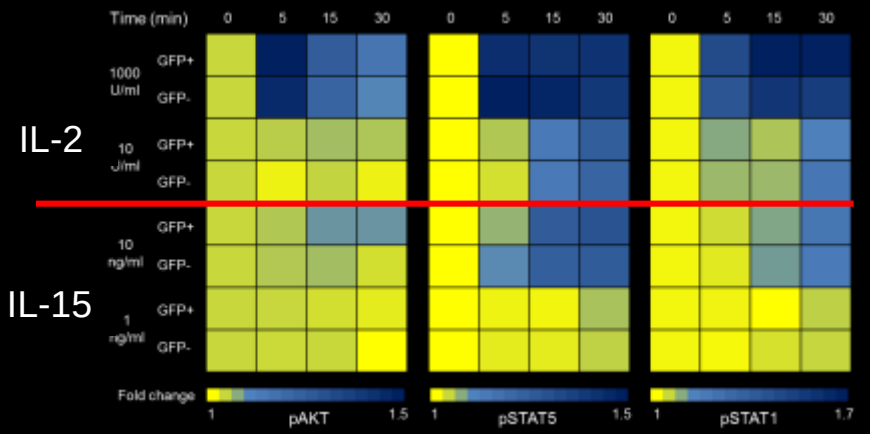




♂ Lymphoid cells IL2RG -/-



IL2RG signalling pathway analysis



Modeling SCID-X1 Gene Correction in Mice

Tranplant
Wild-type + SCID HSPC
at decreasing ratio

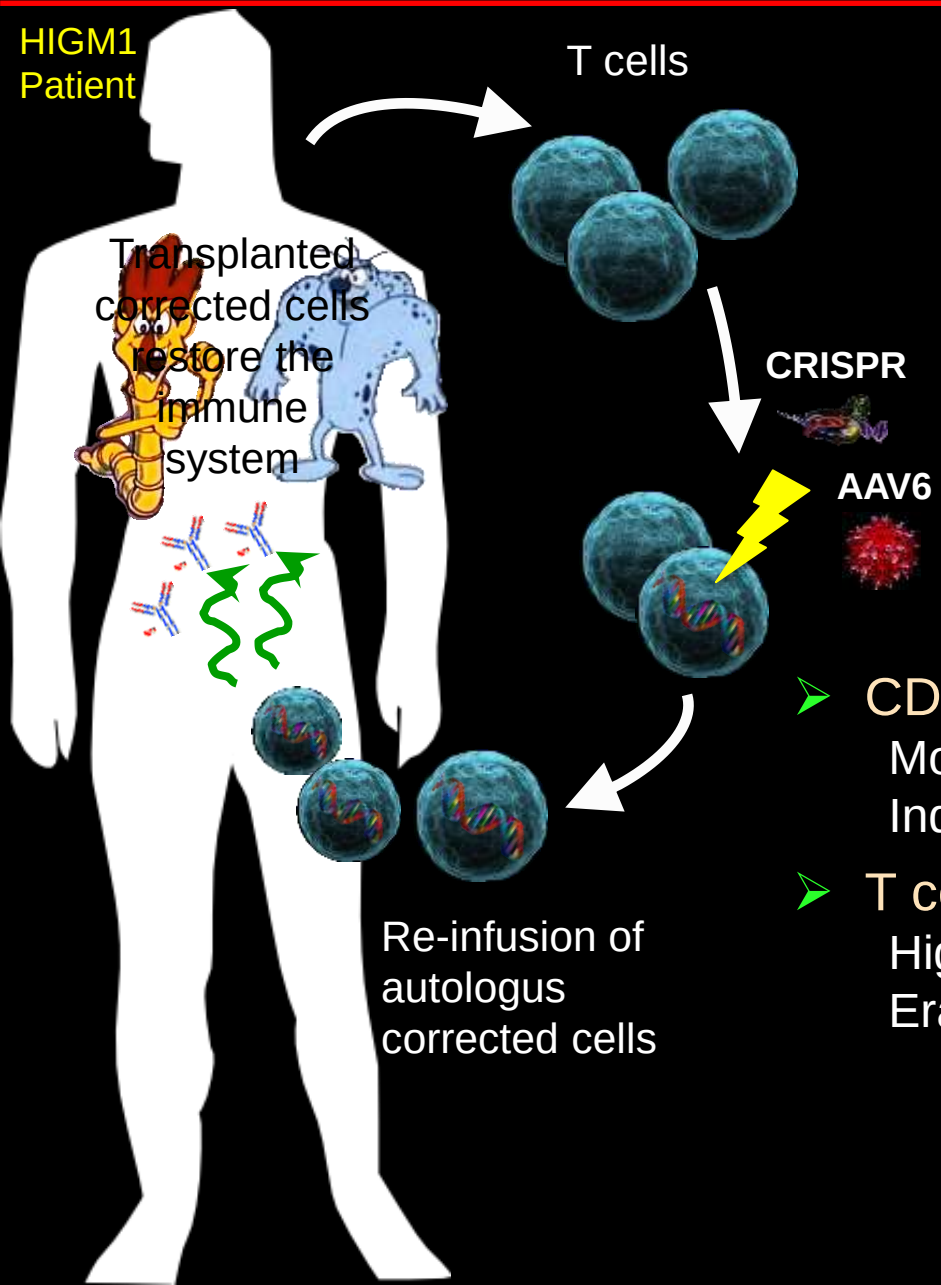
SCID-X1 mice

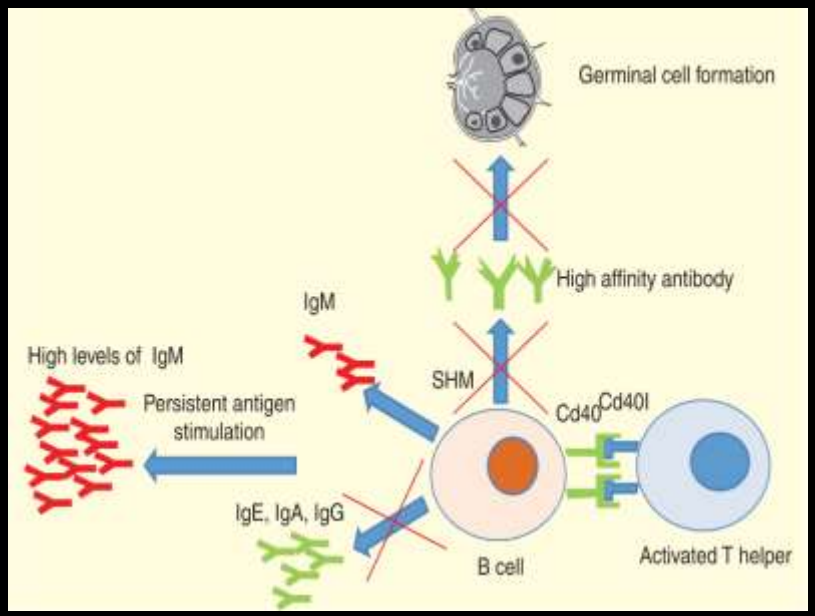
- *Required threshold of functional HSPC to rescue SCID-X1 disease in mouse model*
  - 10% of HSPC transplant input
  - within reach of optimized protocols & reagents
- *Establishes rationale for clinical translation*
- *Road map for the development of HSPC gene editing strategies*

RAG1: In coll. with A. Villa and L. Notarangelo

Schiroli, ..., Genovese, Naldini* Science Translational Medicine 2017*

Gene Correction of *CD40LG* on T cells and HSC





- CD40LG deficiency cause HIGM1 syndrome
  - Mostly expressed on activated CD4+ T cells
  - Induces B cell activation and Ig class-switching
- T cells well amenable to HDR gene editing
  - High safety of the clinical cell product
  - Eradicate life-threatening infections

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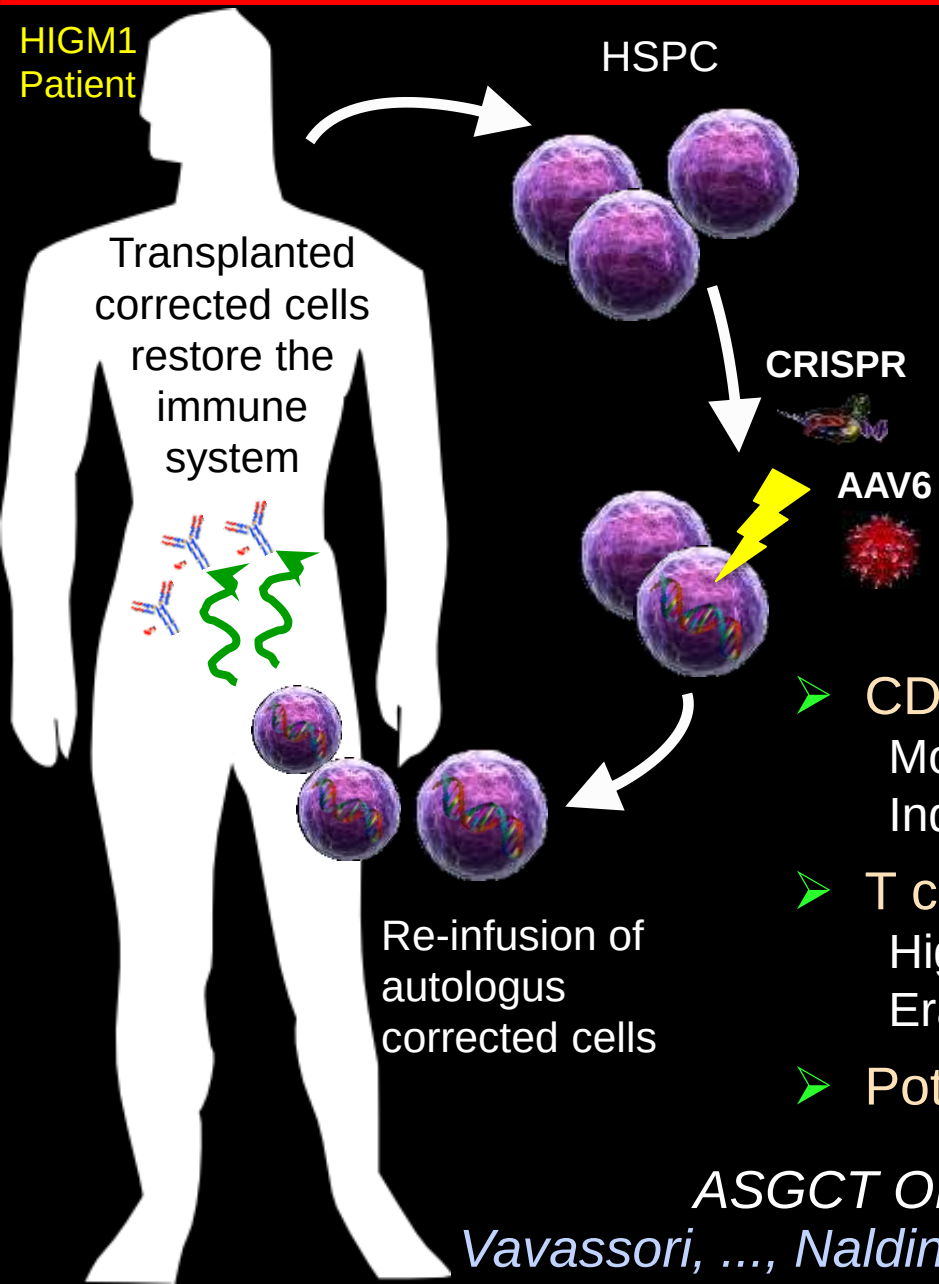

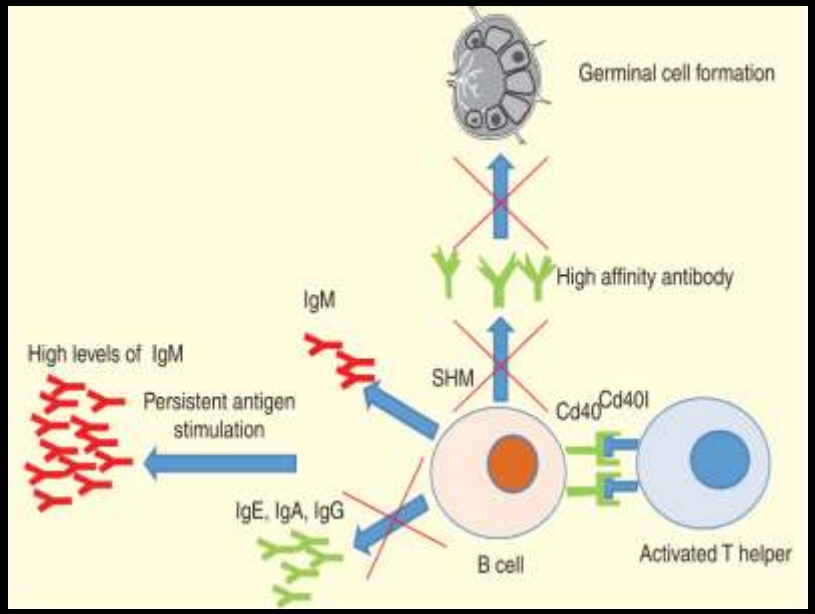

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- T cells well amenable to HDR gene editing
  - High safety of the clinical cell product
  - Eradicate life-threatening infections
- Potentially bridge to HSC Transplant

ASGCT OR 656: Vavassori, May 1st – Travel Award

Vavassori, ..., Naldini, Genovese*. Manuscript in preparation*

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- Martina Fiumara

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- Michael C. Holmes
- Anthony Conway

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- Carrie Margulies
- Jennifer Gori
- Vic Myer

We are looking for brilliant post-docs or PhD students