EIC-ERC workshop on
Gene and Cell Therapy

29 June 2021

Genetic Engineering of Hematopoiesis to Treat Inherited Diseases and Cancer

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OSPEDALE SAN RAFFAELE

FONDAZIONE Telethon

A New Medicine for the 3rd Millenium

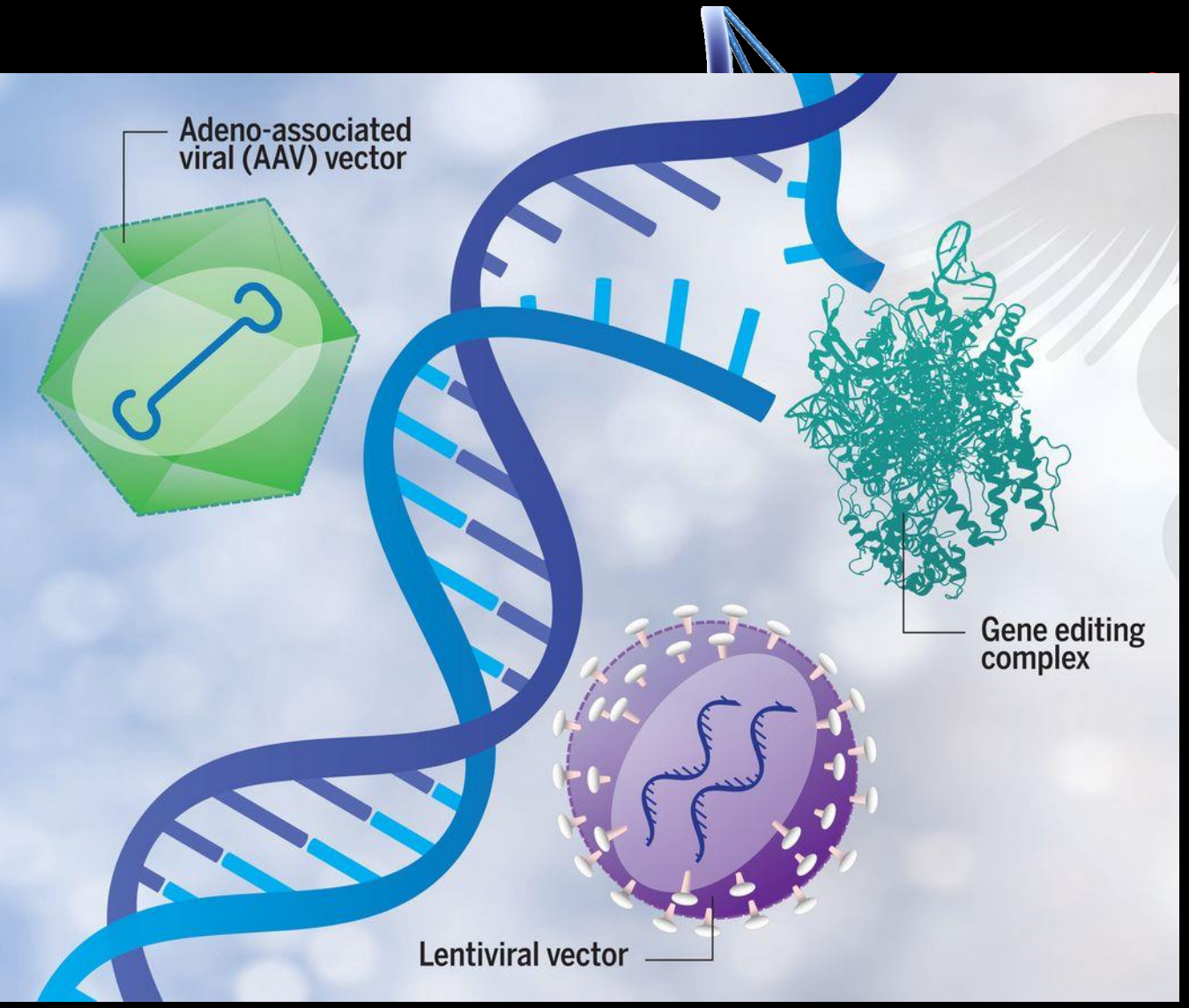
- *New technologies for transferring and editing genes (Gene Therapy)*
- *Effective strategies to isolate and transplant stem cells (Cell Therapy)*
- *Improved manipulation of biological weapons of immunity (Immunotherapy)*

- *Make possible to design new therapies*
  - for diseases until now without treatment

A New Medicine for the 3rd Millenium

- *New technologies for transferring and editing genes (Gene Therapy)*

The Gene Engineer's Toolbox



1995 00 2005 2010 2015 2020

Gene Replacement
- Lentiviral Vectors

Gene Editing
- ZFN
- CRISPR/Cas

Emerging Break-free Editors
- Base Editors
- Prime Editor

Epigenetic Editing

Salk Institute, 1995

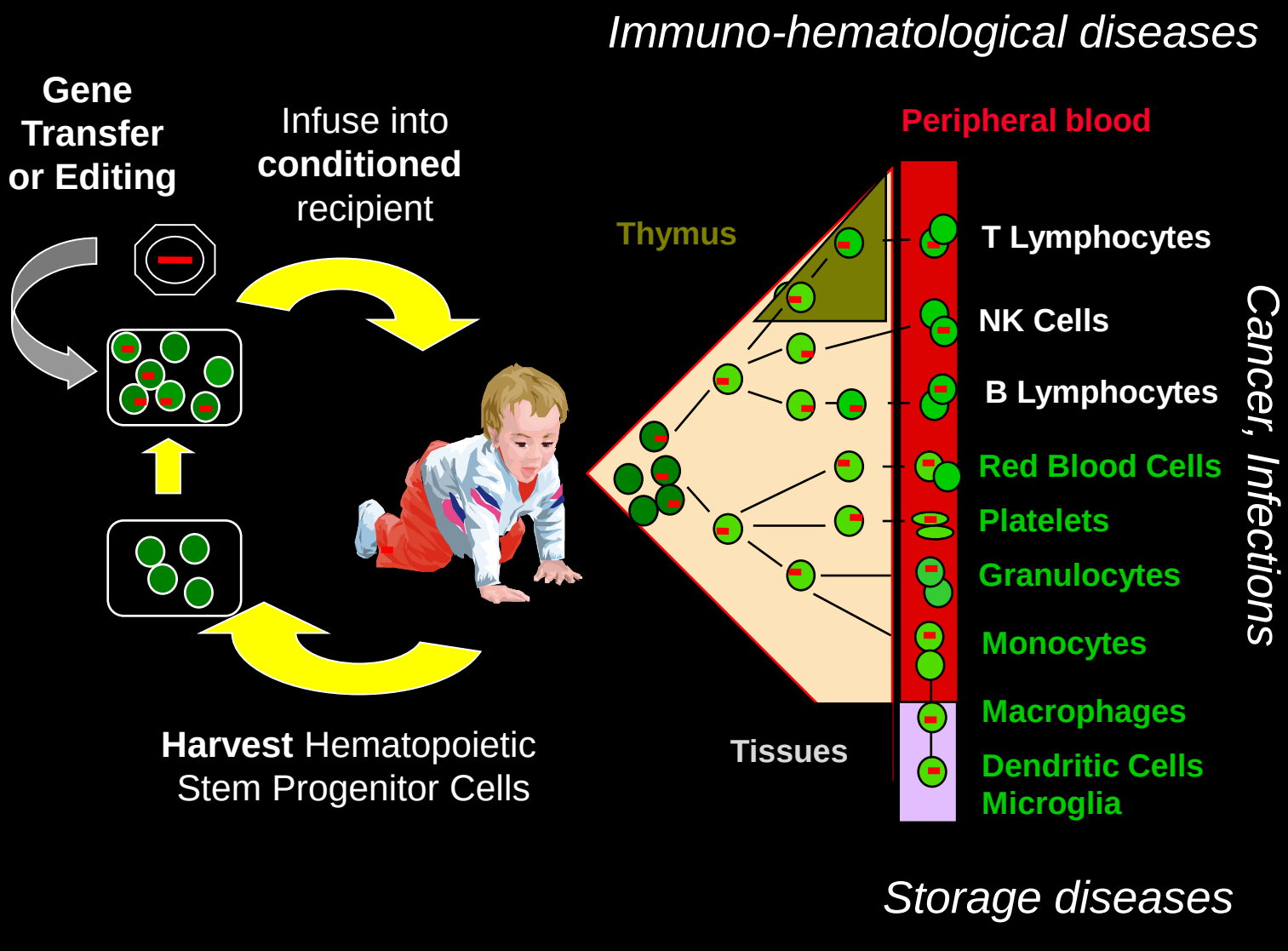
Challenges to Safe & Effective Gene Therapy

- *Achieve efficient & stable gene transfer*
  - in stem cells (*ex vivo*) or long-lived tissues (*in vivo*)
- *Regulate transgene expression*
  - Ectopic or constitutive expression may be toxic
- *Avoid immune response*
  - May neutralize therapy and clear transduced cells
- *Alleviate vector-related toxicity*
  - Inflammatory response to *in vivo* administration
  - Risk of insertional mutagenesis: integration may activate oncogenes, disrupt tumorsuppressor genes
  - Off-target DNA breaks, translocations in editing

A New Medicine for the 3rd Millenium

- *New technologies for transferring and editing genes (Gene Therapy)*
- *Effective strategies to isolate and transplant stem cells (Cell Therapy)*
  - exploiting their regenerative potential

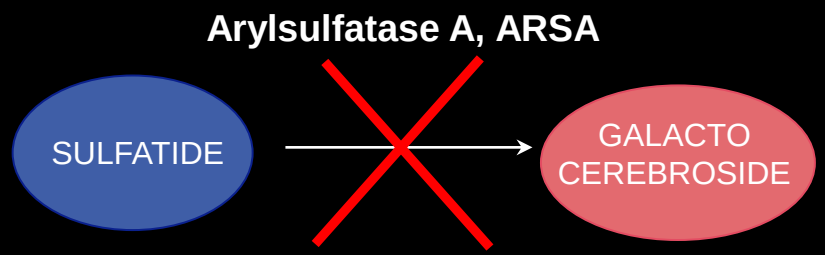
Hematopoietic Stem Cell (HSC) Gene Therapy



Clinical Testing of HSC Gene Therapy

- *Pioneering work with γ-RV in PID*
  - ADA-SCID 1st GT on EU market *(Strimvelis)*
- *Expanded applications with Lentiviral Vectors*
  - Up to 90% stable marking
  - No genotoxicity to date
  - Tiget trials: 120 pts, up to 10 yr follow up, persistent clear clinical benefit
  - *Metachromatic Leukodystrophy, Wiskott-Aldrich Syndrome, β-thalassemia, MPS-I*
  - 2 therapies already approved for EU market *(Zynteglo, Libmeldy)* with GSK/OTL
- *Similar findings in several other trials & sites*
  - ~350 pts; up to 19 (11 for LV) yr follow-up
  - *ALD, X-SCID, Sickle CD, CGD, MPS-IIIb, Fanconi, Fabry…*

Rationale for HSC GT of Metachromatic Leukodystrophy



Genetic deficiency of ARSA

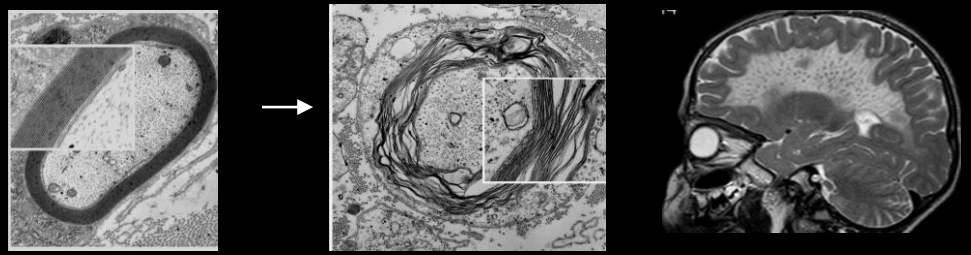
- Storage of myelin byproduct
- Degeneration of oligodendrocytes, microglia and neurons
- Severe dismyelination

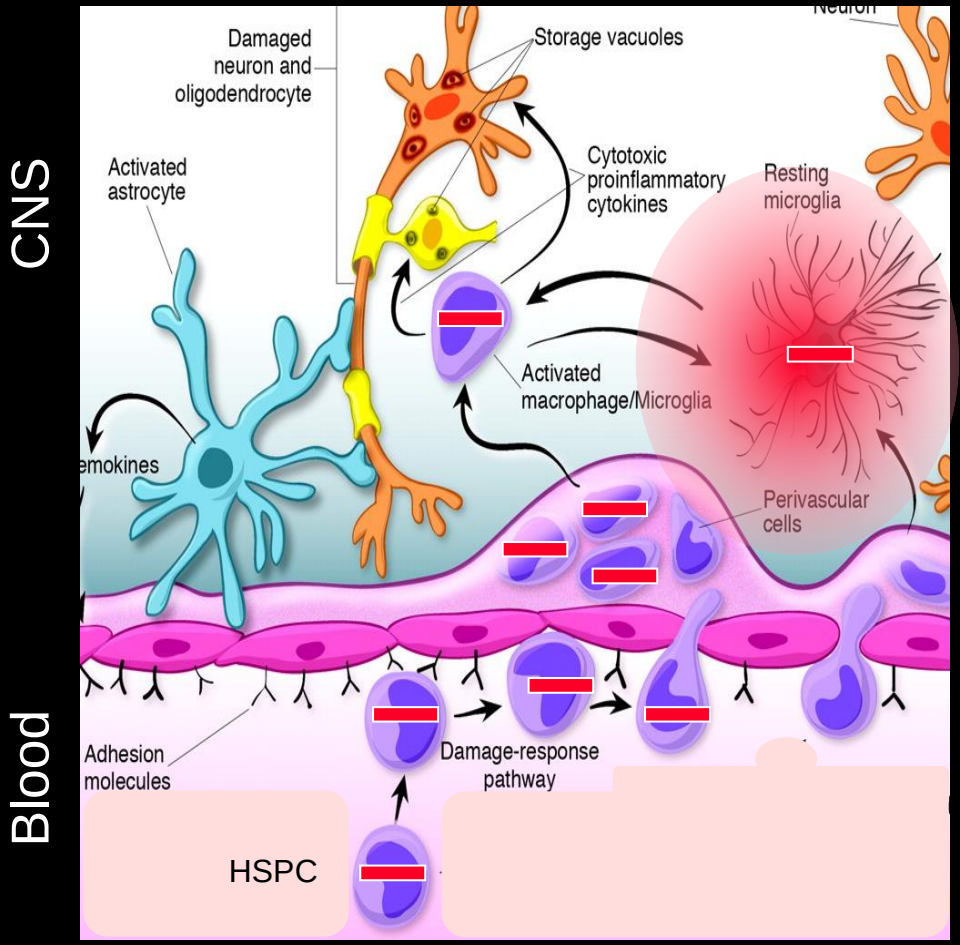
Prognosis

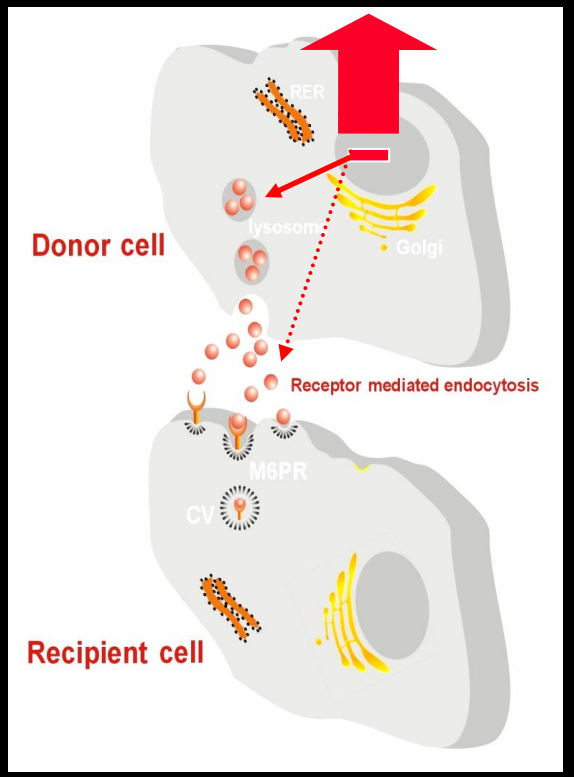
- fatal within 10 years from onset

Therapy

- HSC transplant poorly efficacious







After conditioning, some microglia reconstitution from infused progenitors

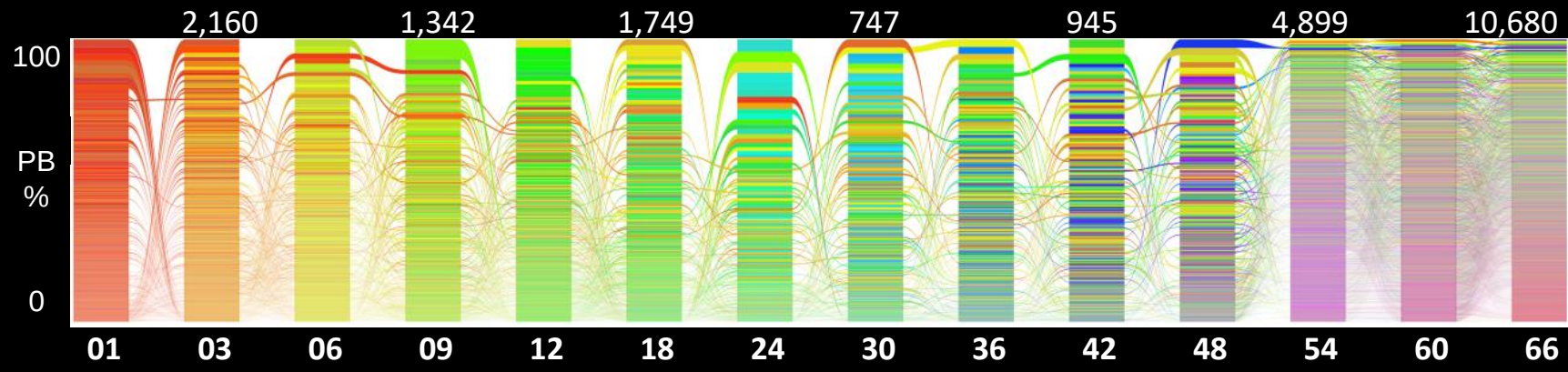
Biffi et al., J. Clin Inv. 2004 and J. Clin Inv. 2006; Capotondo et al., PNAS 2012

Clinical Benefit of HSC Gene Therapy in MLD

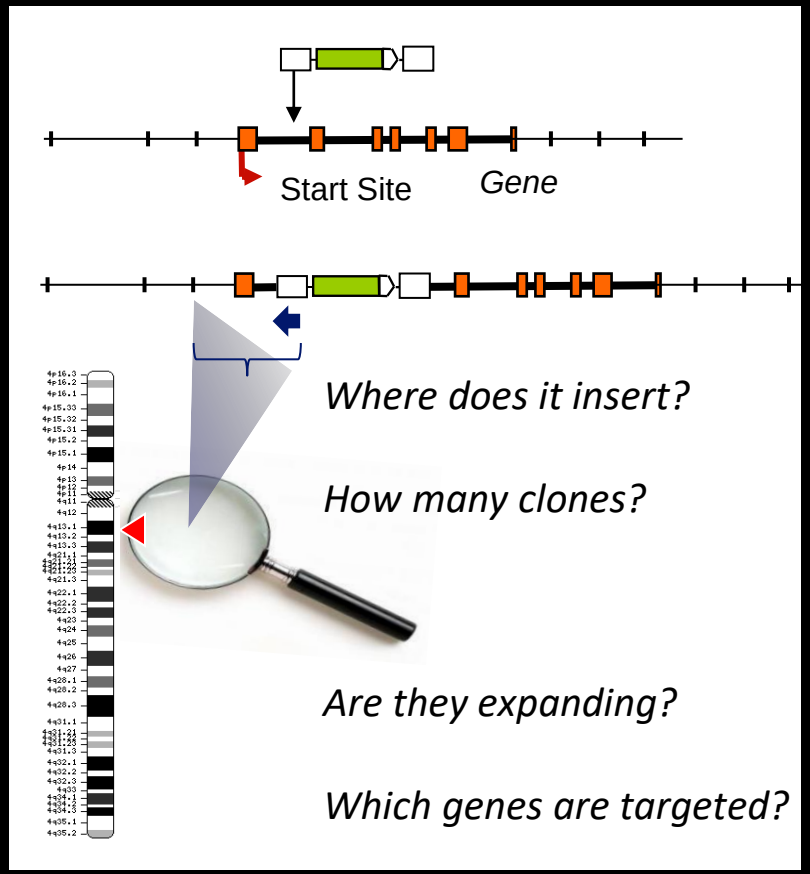
Biffi, Montini et al, Science 2013; Sessa, Lorioli et al., Lancet 2016
Fumagalli, Calbi et al. in revision

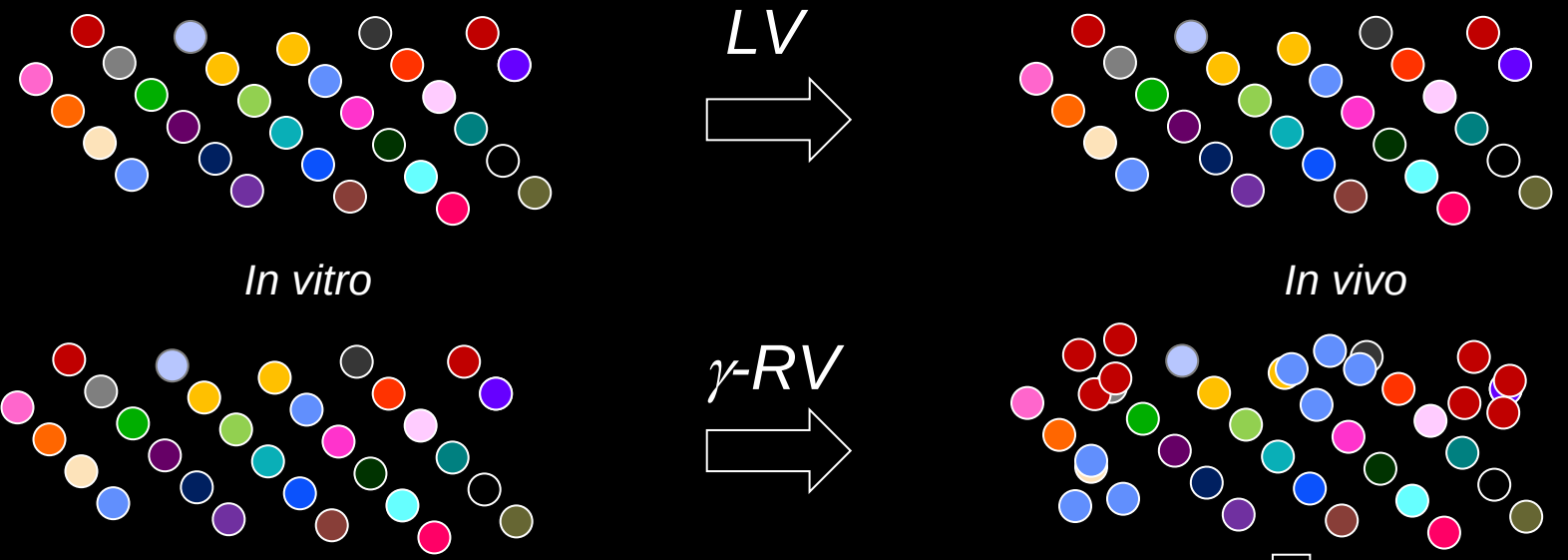
Genotoxicity

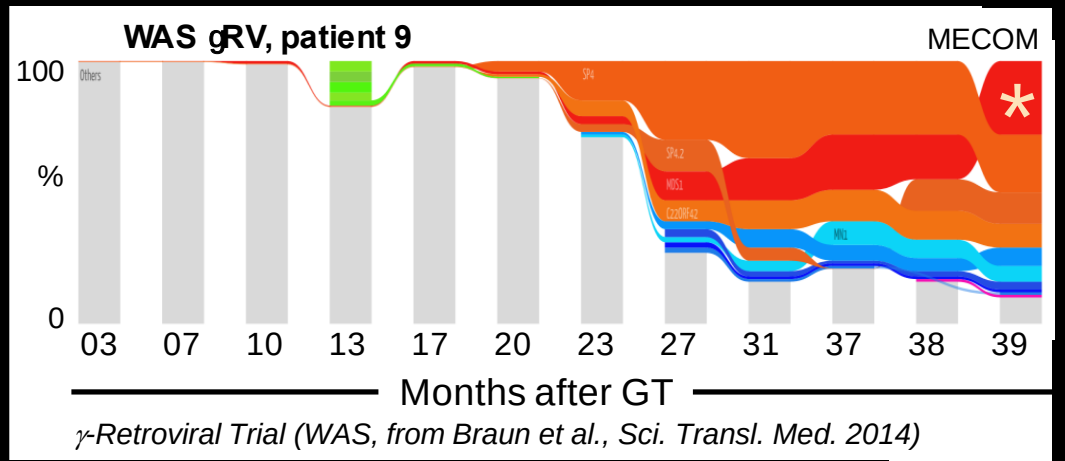
Lentiviral Trial (Milan)



Vector Integration Site Analysis



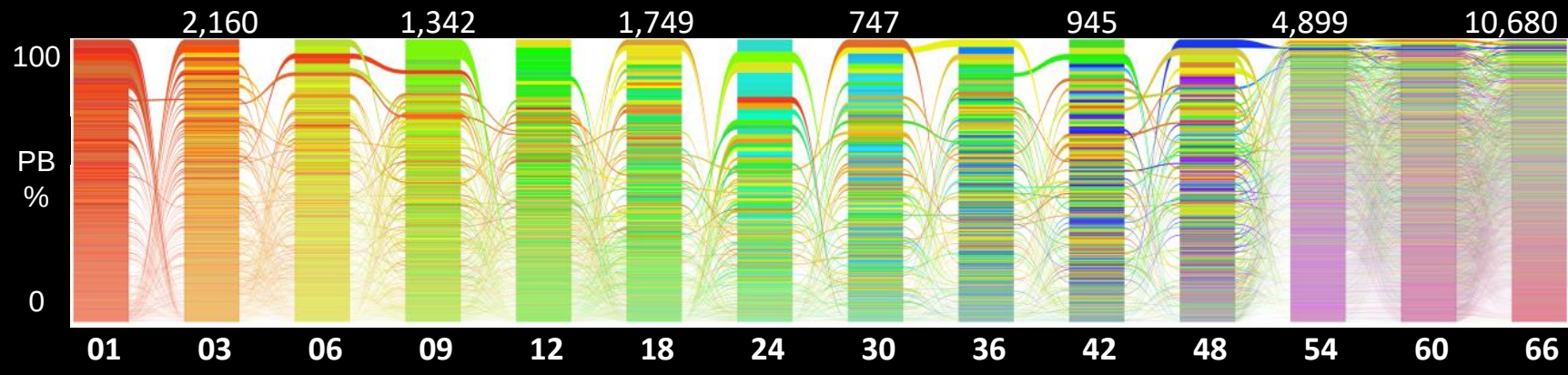




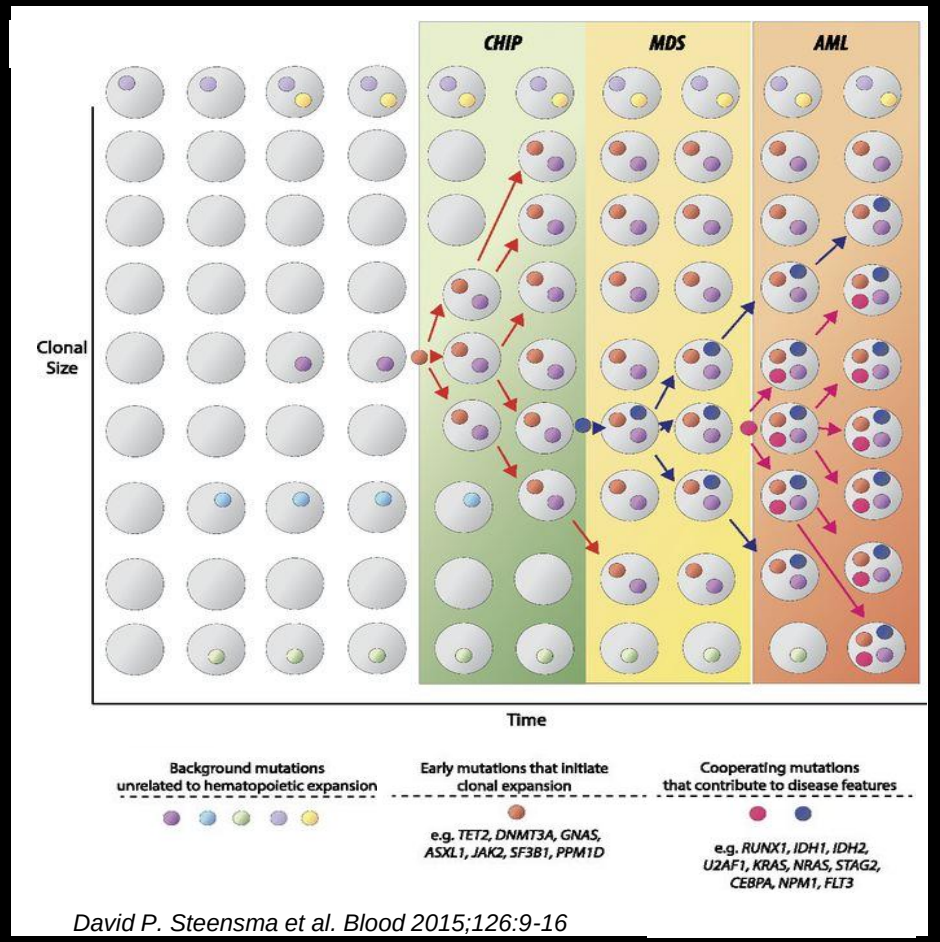
γ-Retroviral Trial (WAS, from Braun et al., Sci. Transl. Med. 2014)

Genotoxicity

Lentiviral Trial (Milan)



Age-related Clonal Hematopoiesis



David P. Steensma et al. Blood 2015;126:9-16

A Future Outlook for HSC Gene Therapy

- *Autologous HSC GT may become preferred to allogeneic HSC transplant in genetic diseases*
  - Available to every patients
  - Abrogates risk of graft vs. host disease & rejection
  - Mixed chimerism sufficient for full benefit
  - Enhanced benefit by increased gene dosage
- *Beyond gene replacement*
  - Target delivery of biotherapeutics at disease sites
    - CNS via microglia progenitors

- *Outstanding challenges*
  - gene transfer rate sometimes limiting
  - and variable among patients
  - genotoxic conditioning
  - delayed rate of engraftment
- *Concerns (long-term)*
  - residual genotoxic risk
  - long-term stability of transduced cells graft
- *Expected improvements*
  - increased HSC input (improved harvest, ex vivo HSC expansion)
  - milder conditioning regimens (non mutagenic)

A New Medicine for the 3rd Millenium

- *New technologies for transferring and editing genes (Gene Therapy)*
- *Effective strategies to isolate and transplant stem cells (Cell Therapy)*
- *Harness biological weapons of immunity (Immunotherapy)*
  - direct them against tumor cells or pathogen infected cells

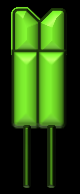
Cell & Gene Mediated Tumor ImmunoTherapy

Engineer T-cell specificity against tumor

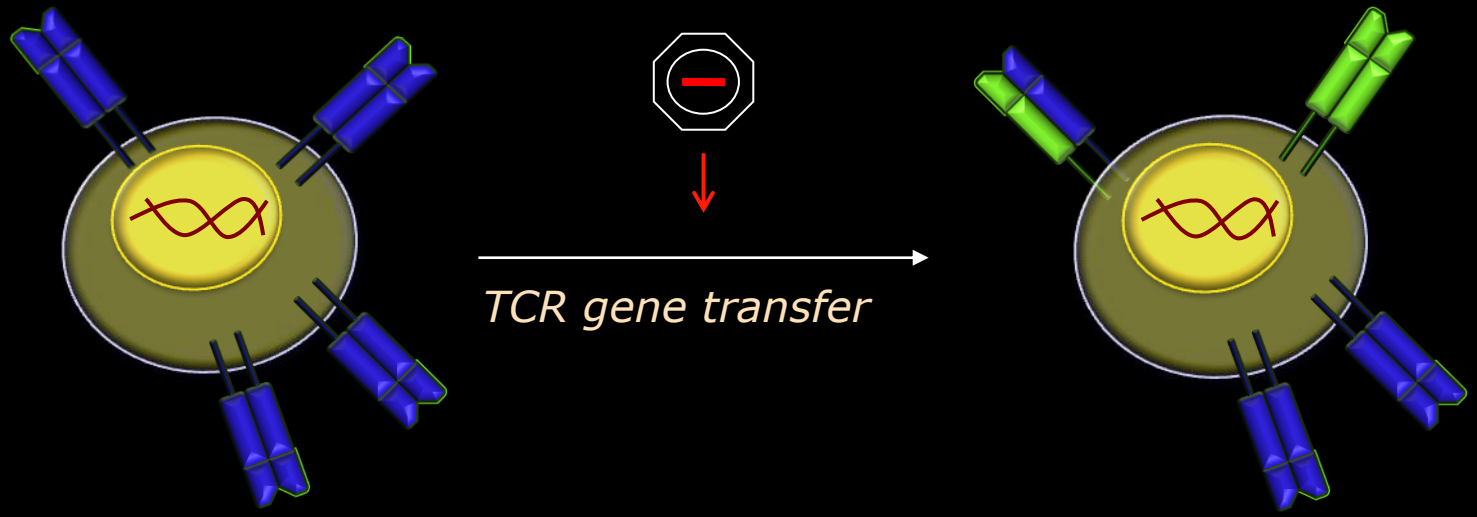
TCR/CAR gene transfer

Generate new T Cell Receptor against Tumor Antigen

- Isolated from rare tumor-infiltrating lymphocytes (TCR)
- Built as artificial Chimeric Antigen Receptor (CAR)



T cells from patient

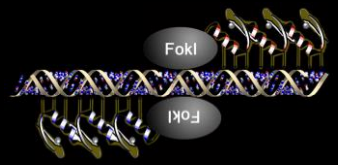


Hurdles: TCR dilution & misparing, competition with CAR

Poorly effective & potentially autoreactive T cells

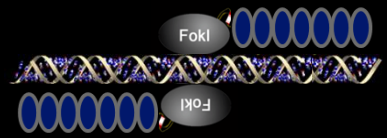
DNA “Nano-Surgery”

ZFNs



CRISPR/Cas9

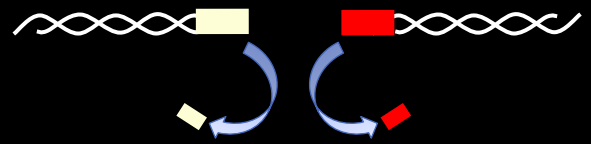
TALENs



DNA DSB
at target site

Gene Knock Out

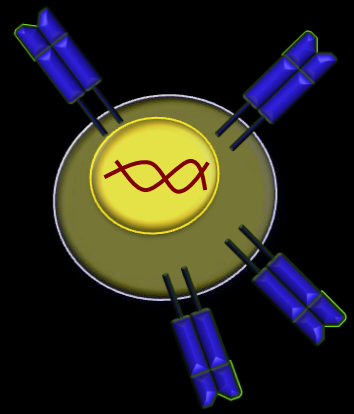
Repair by
Non Homologous
End Joining

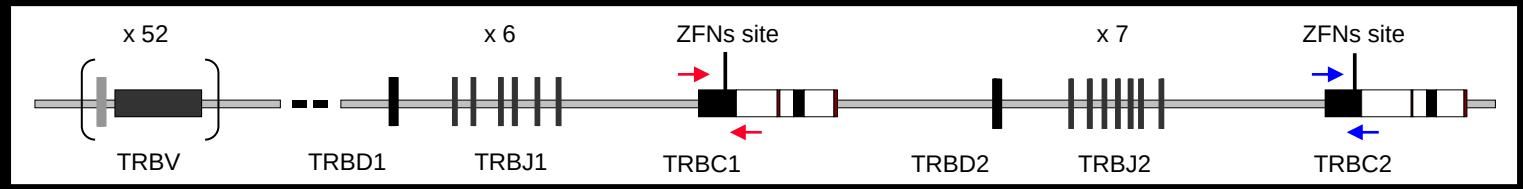


Loss of Function

Disrupting TCR Genes in Lymphocytes

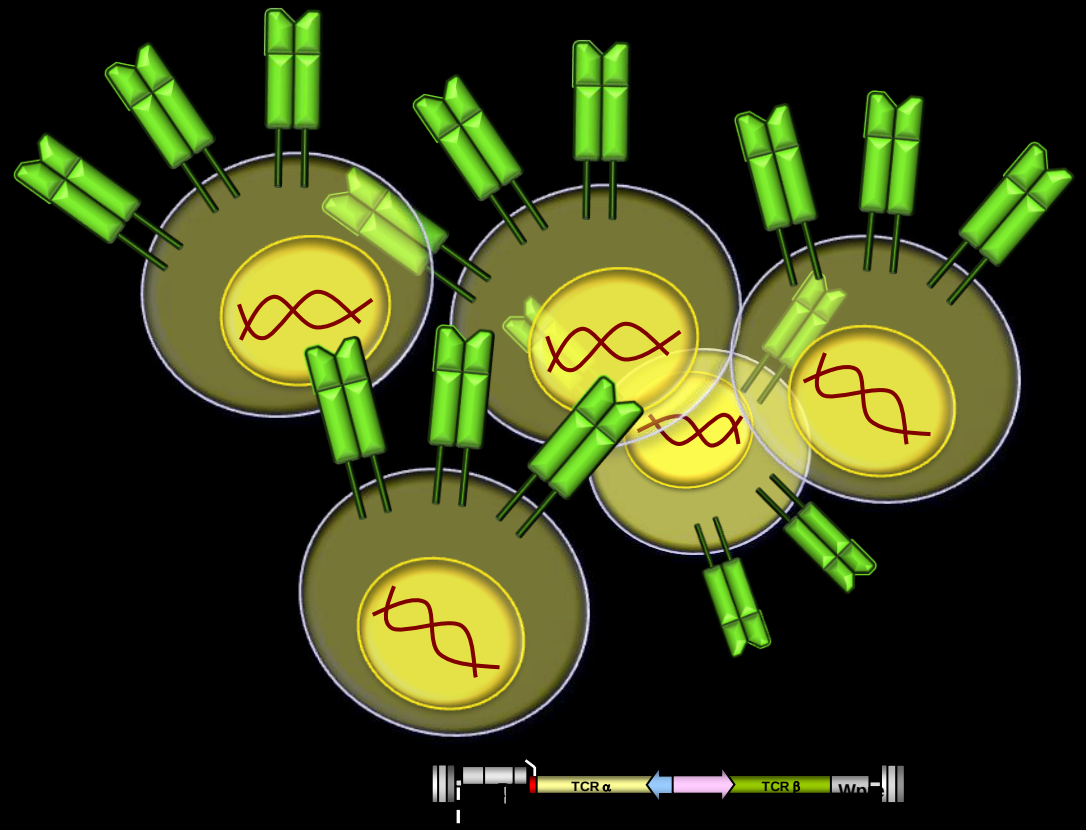
TRBC ZFNs





TCR β locus
(Chr. 7q34)

Genetic Editing of TCR Specificity

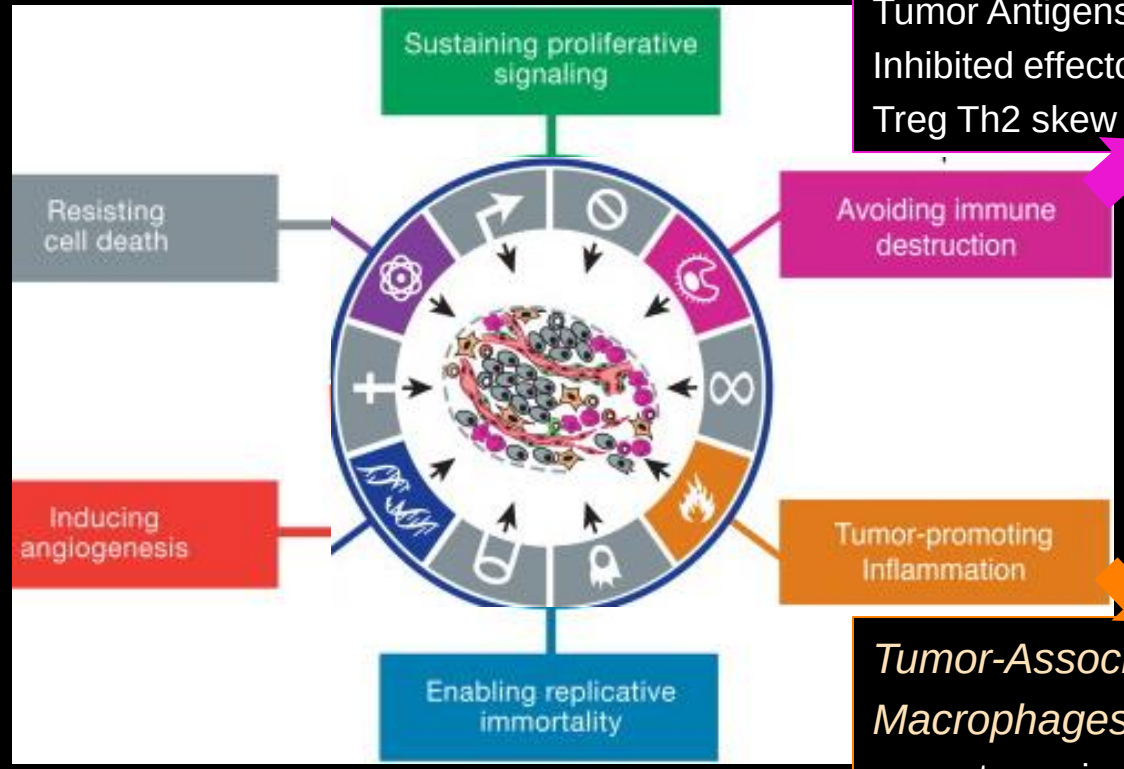


Provasi et al, Nat Medicine, 2012; Casucci et al, Blood 2013; Mastaglio et al, Blood 2017

Adoptive Cancer Immunotherapy

- *CART & TCR transfer*
  - Remarkable efficacy in some tumors (ALL, Lymphoma)
- *Ongoing improvements*
  - Enhanced activity
    - Editing out checkpoint controls
    - Physiological Expression
  - Improved specificity & better control of toxicity
    - Switches, conditional suicide, synthetic circuitry
    - Allogeneic source
      - *off-the-shelf product*

- *Poorly effective on solid tumors*
  - Immunosuppressive tumor micro environment limits recruitment & activity



T cells: limited reactivity to Tumor Antigens
Inhibited effector function, Treg Th2 skew

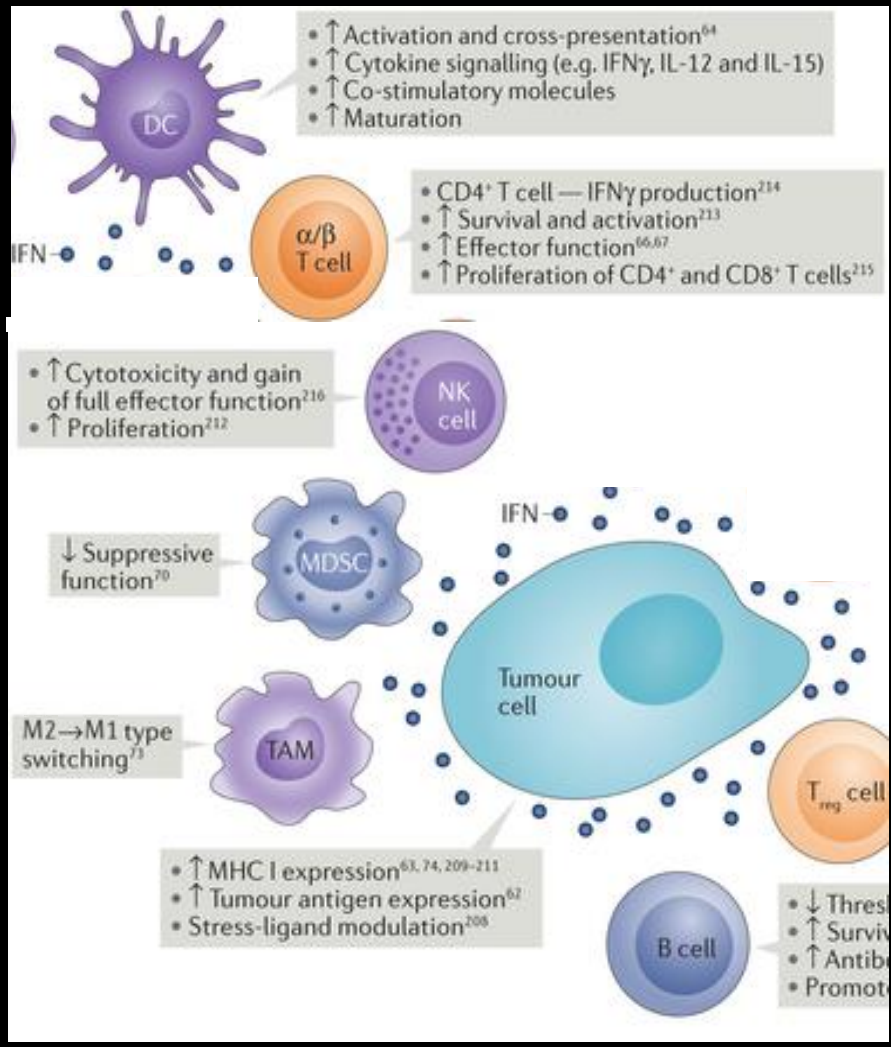
Tumor-Associated Macrophages (TAM) promote angiogenesis, tissue remodeling, invasion, immunosuppression

Tumor-targeted Gene Delivery of Immuno-activating Cytokines

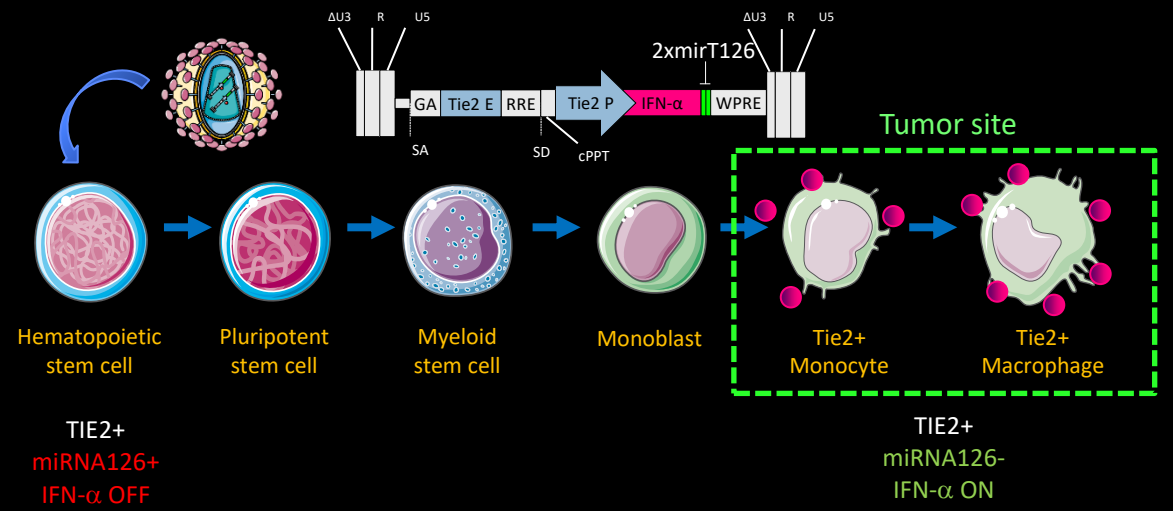
Stable expression targeted to disease sites

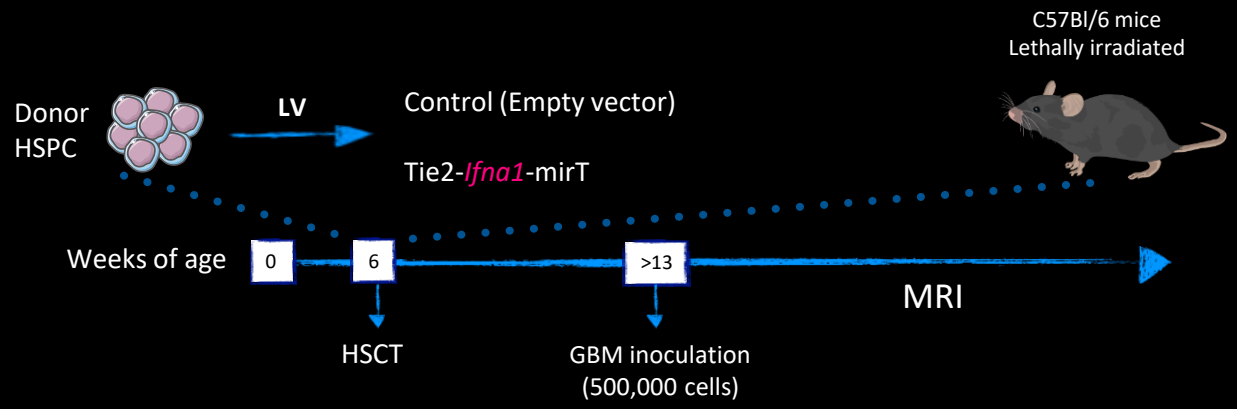
- Sustained local concentration within therapeutic window (no peak & troughs)
- Preventing off-target effects & reducing toxicity
- May prevent de-sensitization & counterregulatory effects

Pleiotropic Immunoactivation by IFN-α



Tumor-targeted Gene Delivery of IFN-α by TAM Inhibits GBM



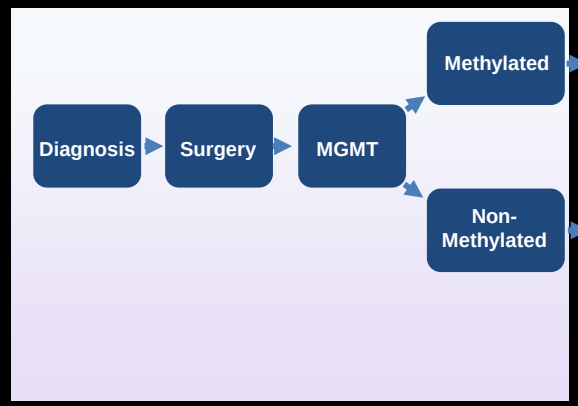


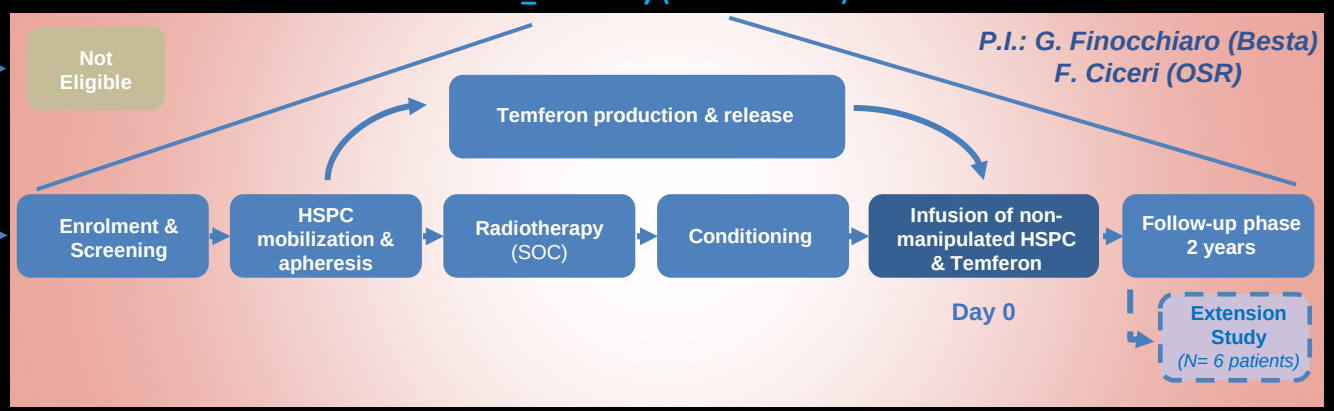
Escobar et al., Sci. Transl. Med 2014 & Nat. Comm 2018;

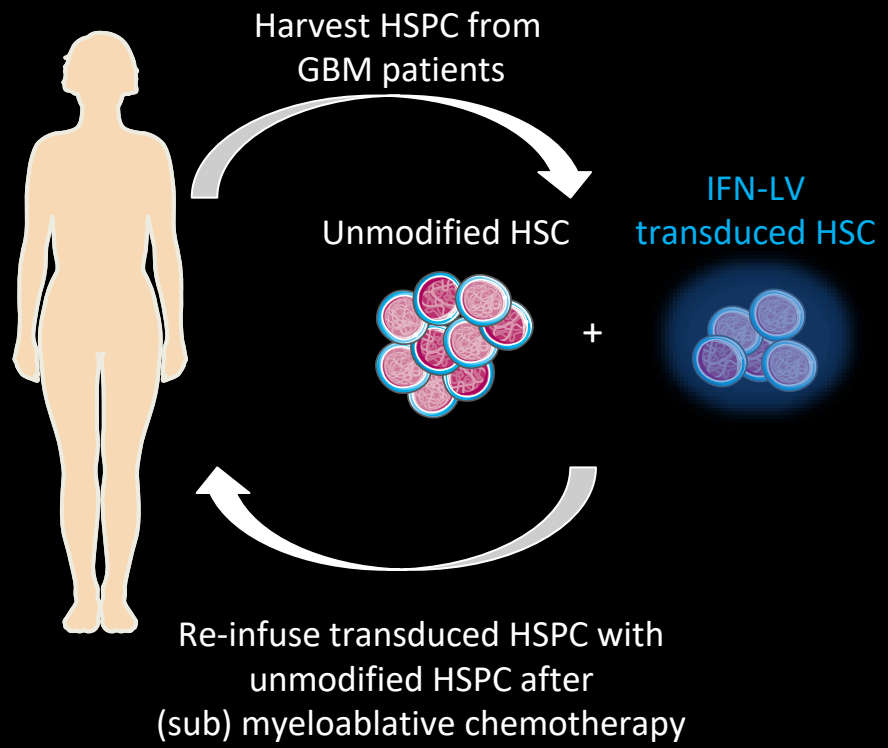
Translating IFNa Gene Therapy to the Clinic

Phase I/IIa
single-center, open-label dose escalation study

genenta science

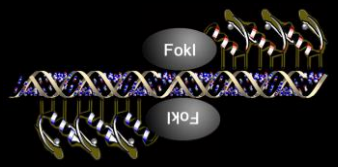


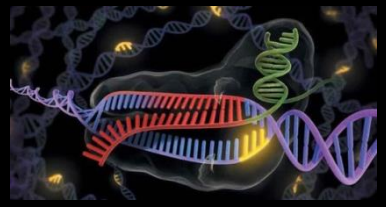


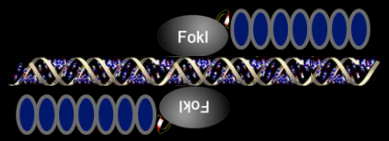


Gene Therapy 2.0: Gene Editing

ZFNs CRISPR/Cas9 TALENs





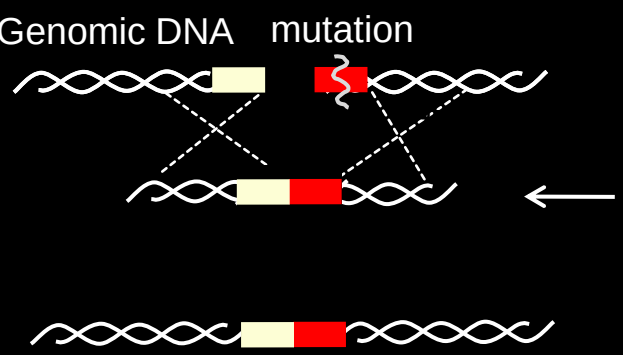


DNA DSB
at target site

Repair by
Homologous
Recombination

↓

Gene Correction

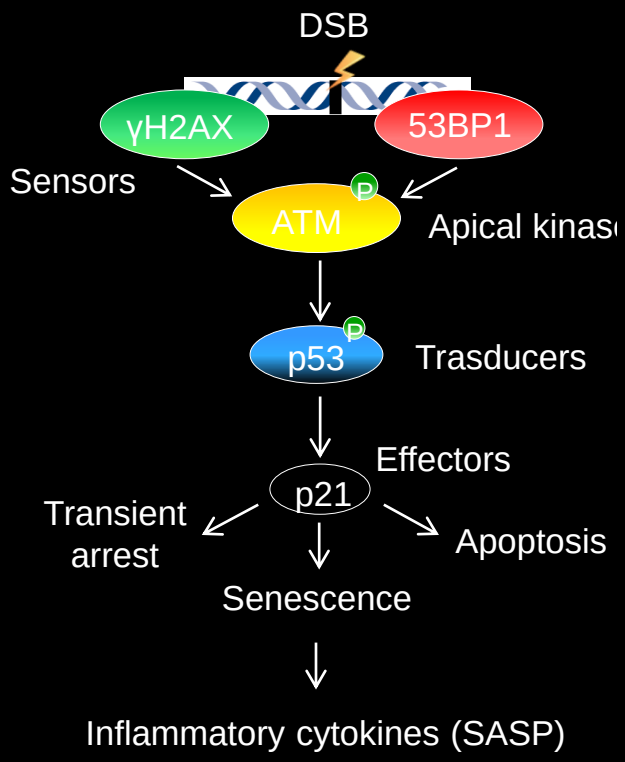


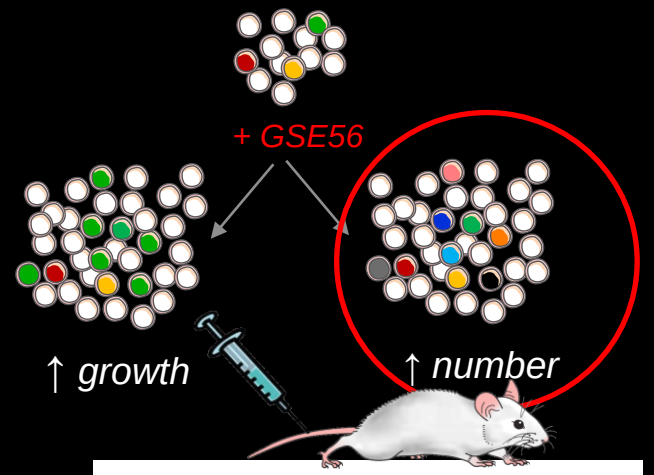
Gene correction
"Writing DNA"

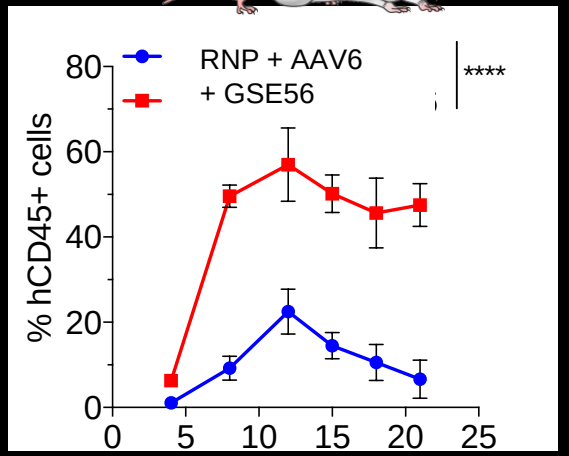
restores gene
- function and
- expression control

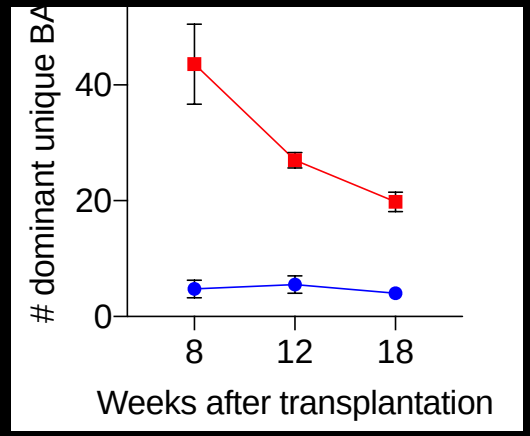
- *genotoxic risk*
  - circumscribed to off target activity
  - challenging to comprehensively define
  - potential for translocations, large deletions and bi-allelic hits
- *constrained in HSC by*
  - low efficiency of HDR
  - need for DNA template codelivery

Optimizing HSC Gene Editing









Schiroli, Conti et al, Cell Stem Cell 2019; Ferrari, Jacob et al., Nat Biotech, 2020

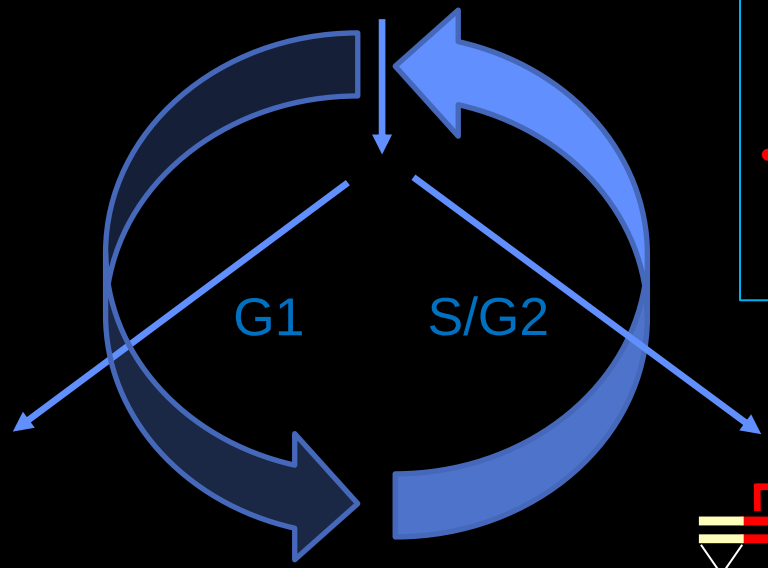
Towards Clinical Testing of HSC Gene Editing

Sickle Cell disease, β-thalassemia

- rescue of fetal hemoglobin by
- disruption of γ-globin repressor expression (erythroid enhancer)

Frangoul…Corbacioglu; N Engl J Med. 2021

DNA DSB at target site

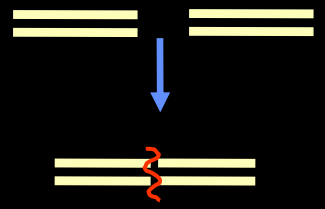


Primary immunodeficiencies
IL2RG, CD40L, RAG1/2

- unregulated gene expression pose risk of transformation or malfunction
- selective advantage of corrected progeny compensates for low editing efficiency
- Suitable risk-benefit ratio

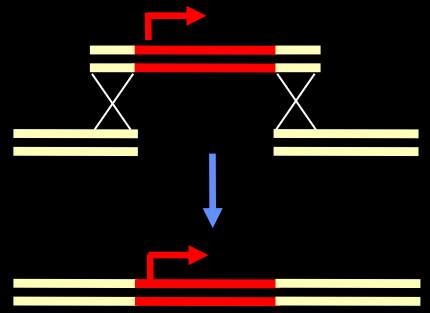
Schiroli et al, Sci Transl Med 2017
Vavassori, Mercuri et al, EMBO Mol Med 2020

NHEJ
Non Homologous End Joining



Gene Disruption

HDR
Homology Driven Repair



Exogenous donor template

Gene Correction / Transgene Insertion

Summary: Choosing the Right Tool

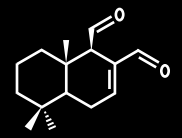
Lentiviral Vectors
for *Gene Replacement*
Genome-wide insertion
Variable expression level
Highly Efficient
Clinically tested
Compatible with long-term HSC
Residual insertional genotoxicity?

Nuclease-based Editing
for Gene Correction
Targeted insertion
In situ reconstitution
Constrained in HSC
Early clinical stage
Impact on long-term HSC TBD
Off-target activity,
large genomic rearrangement?

Base (and Prime) Editors
for Correcting Mutations
Single/few base edit
DNA Break-less/free
Efficient
R&D stage
Unknown
Sequence-independent
off-target activity?

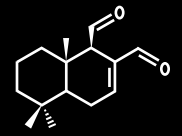
An Evolving Pharmacology

- *Small drugs*

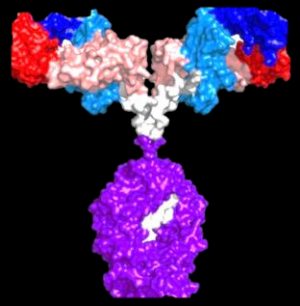


An Evolving Pharmacology

- *Small drugs*



- *Biologicals*



- *Genes*
  - Stable sustained expression
  - Targeted and regulated
- *Cells*
  - Regenerative potential of stem cells
  - Killer action of Lymphocytes

Cell & Gene Therapy: the Challenges Ahead

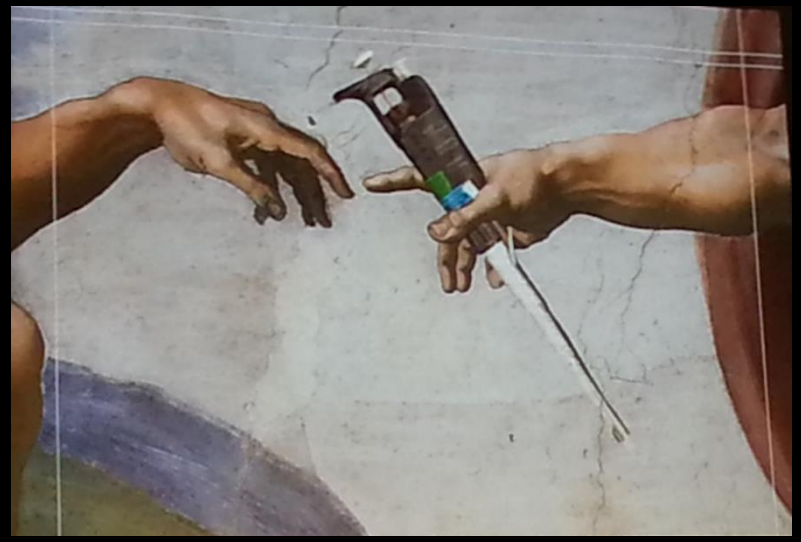
- *Biology*
  - Still limited understanding of stem cell and immune regulation

  Need to overcome:
  - *innate responses* to exogenous nucleic acids and DNA breaks
  - *biological constraints* to survival, engraftment or regeneration imposed by disease
  - *immune barriers* to gene transfer & cell transplant
  - *evasive resistance* to immune therapies in cancer progression
- *Safety*
  - “Live” drugs unprecedented complexity
  - long-term effects
- *Bedside delivery*
  - Need for multidisciplinary expertise
- *Society*
  - Personalized medicine manufacturing
  - marketing pipeline
  - costs and sustainability
  - Fair equitable access

Cell & Gene Therapy: the Ethical Concerns

Somatic gene therapy

- managed under existing ethical norms and regulatory regimes for advanced therapies
- Limited to treatment of severe disease or disability
- evaluated in the context of risks and benefits as other medical treatments
- enhancement of human capacities discouraged or unacceptable at this time



Emerging potential for Germline gene editing

- Although strongly discouraged for technical, scientific and ethical reasons, it has already been attempted

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