GENE THERAPY-BASED ORPHAN DRUGS

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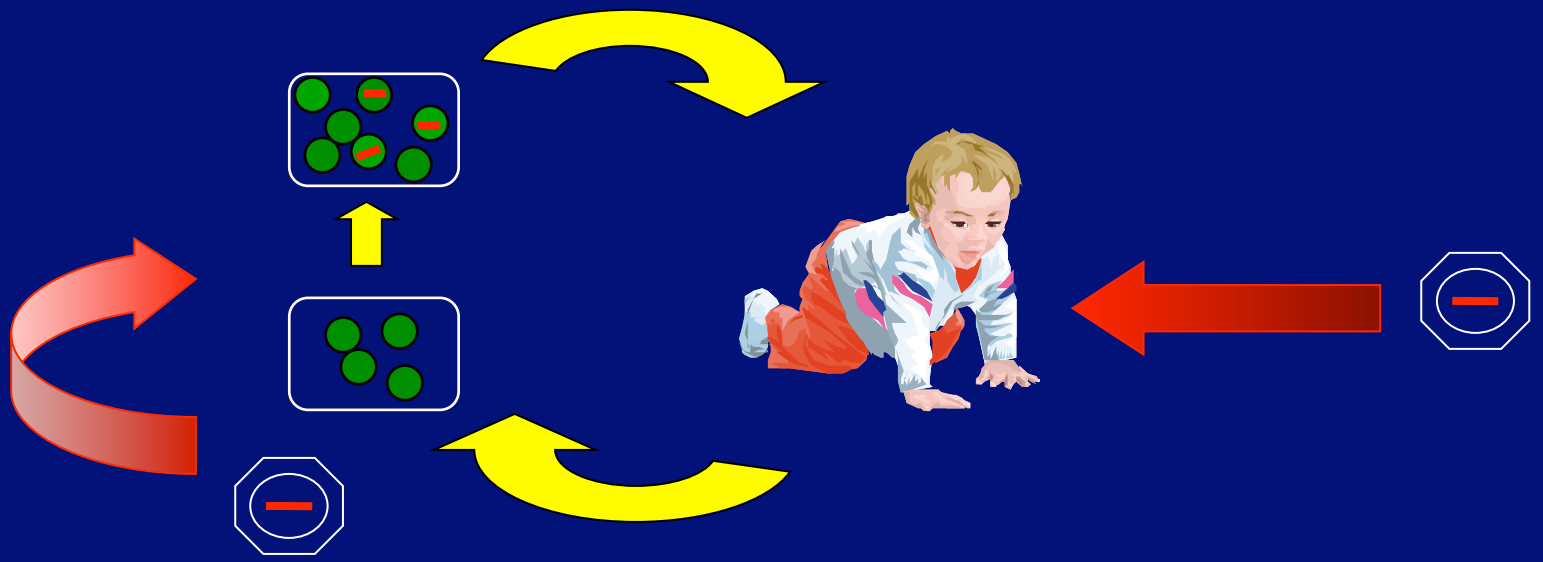
Gene therapy strategies

Ex vivo approaches

***In vivo* approaches**



Retrovirus
Adenovirus
Lentivirus

Adenovirus
AAV
DNA/Liposomes
Lentivirus

Gene therapy-based EU designated Orphan Drugs

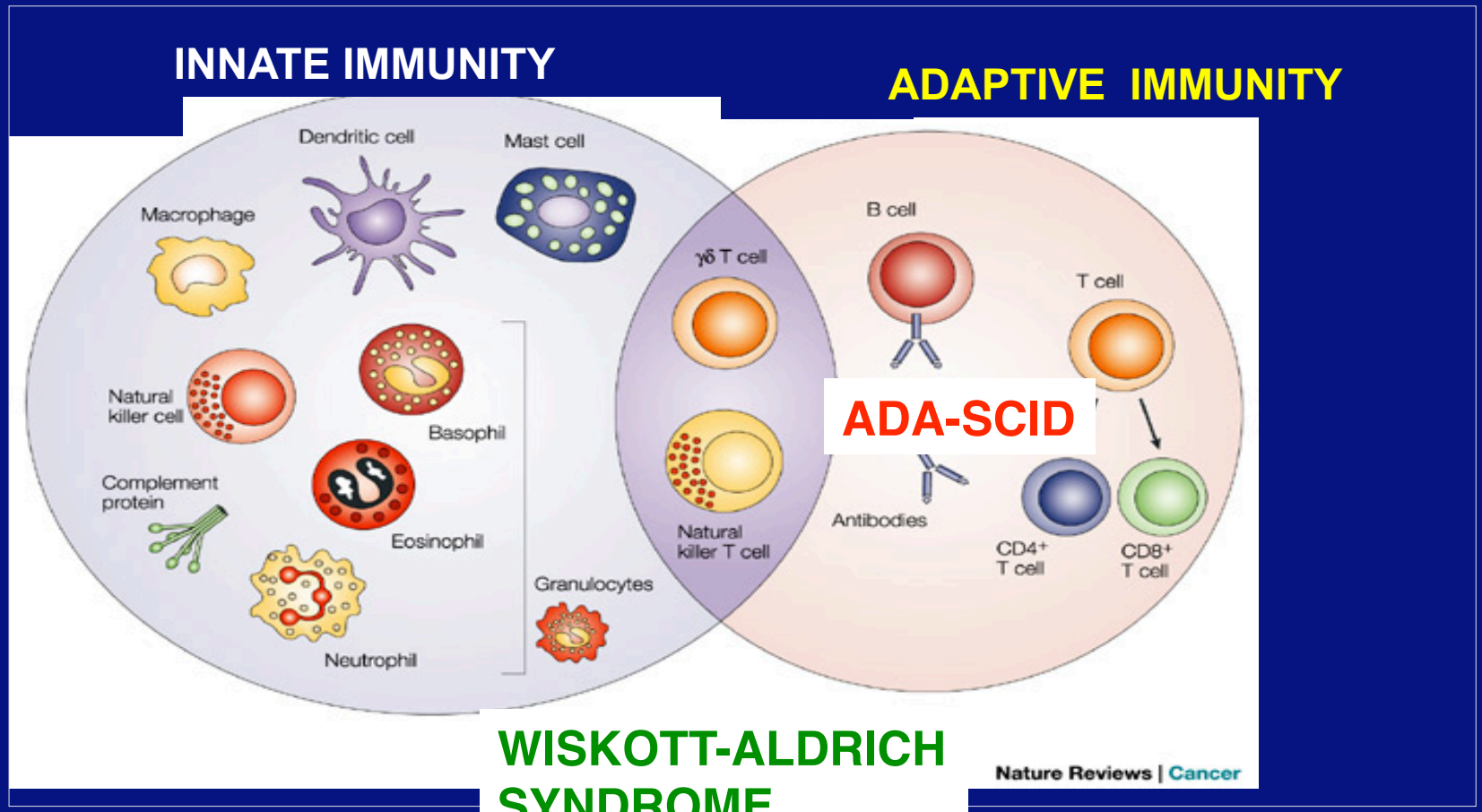
Ex vivo

Hematopoietic System	SCID-X1 ADA-SCID Wiskott-Aldrich Syndrome Beta-thalassemia
CNS & PNS	Metachromatic Leukodystrophy

In vivo

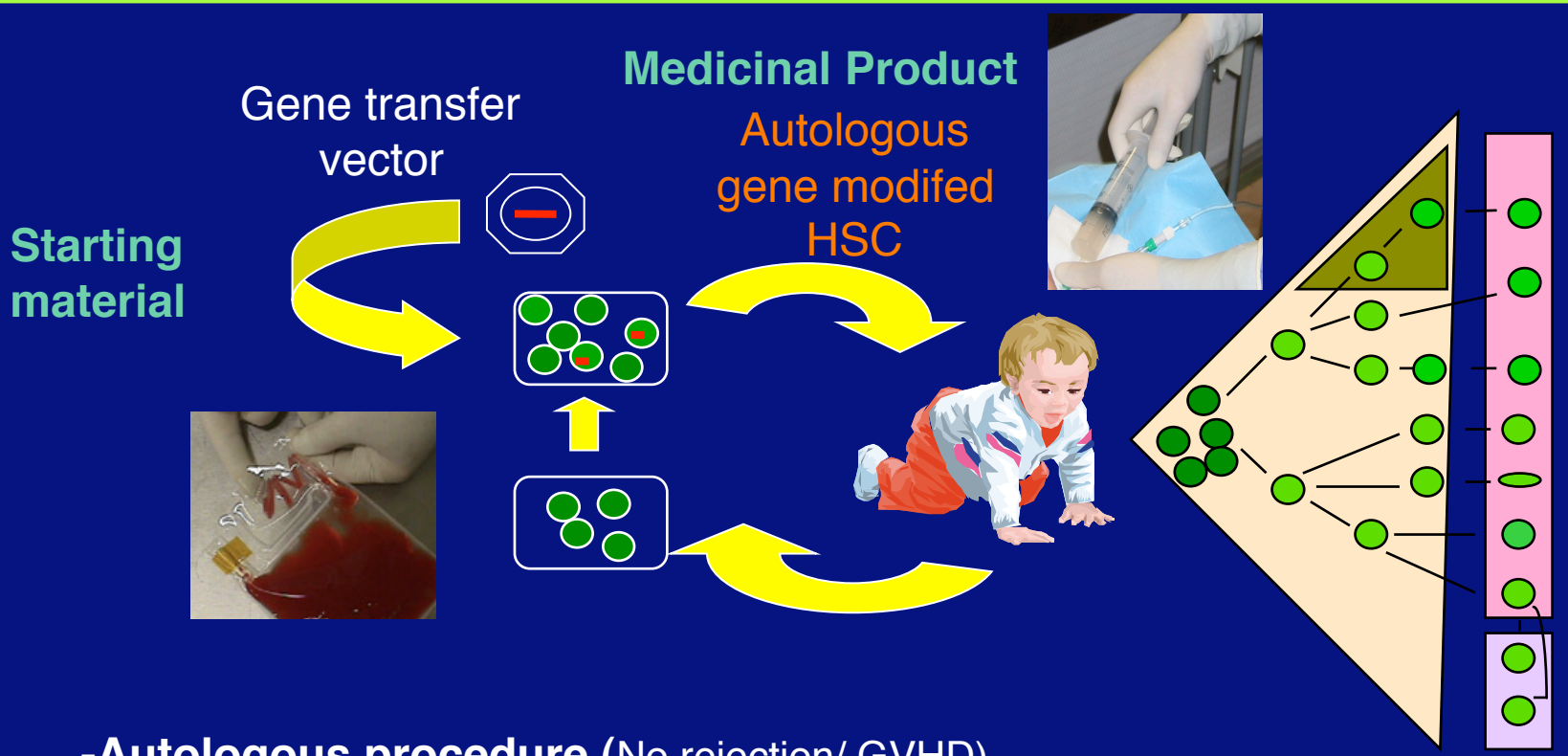
Muscle	Duchenne Muscular Distrophy Alpha-Sarcoglycanopathy Gamma-Sarcoglycanopathy
Multiple organs	Glycogen storage dis. type II (Pompe's disease)
Eye	Retinitis pigmentosa Leber's amaurosis Stargadt's disease
Skin	Epidermolysis bullosa

Primary immunodeficiencies



- Alterations in development and/or functions of adaptive/innate immunity
- Higher susceptibility to infections
- Failure to thrive
- Increase risk of autoimmunity and cancer

HSC gene therapy for primary immunodeficiencies



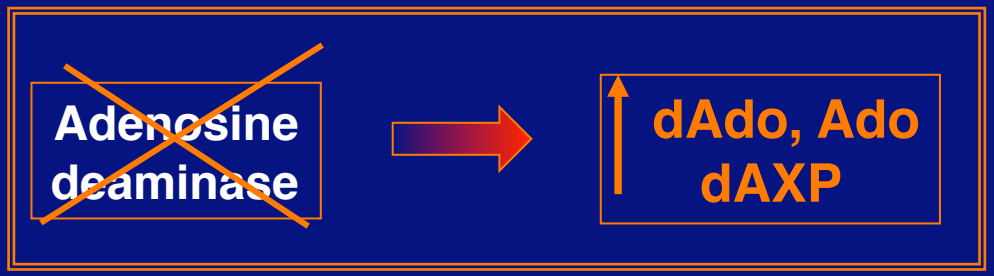
-Autologous procedure (No rejection/ GVHD)

-Reduced toxicity

-Selective advantage for gene corrected cells

-Data on safety and efficacy from preclincial studies and pilot studies

Adenosine Deaminase-deficient SCID



Autosomal recessive
1:375,000-1,660,000

Immunodeficiency

- T, B, NK, lymphopenia
- Severe recurrent infections
- Autoimmunity

Metabolic Disease

- Bone and growth abnormalities
- Organ toxicity (lung, liver)
- Neurological and behavioral alterations

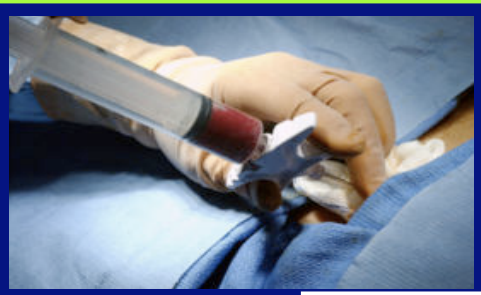
TREATMENT OPTIONS

- ⇨ Bone Marrow Transplantation
- ⇨ Enzyme Replacement Therapy (PEG-ADA)
- ⇨ HSC Gene Therapy

RATIONALE FOR GENE THERAPY

- **Scientific rationale**
  - The ADA gene is constitutively and ubiquitously expressed
  - Gene-corrected lymphocytes have an advantage over ADA-deficient cells.
  - 10% of normal ADA expression may be sufficient
- **Unmet medical need**
  - 90% of children lack an histocompatible donor in the family
  - High risk of bone marrow transplant from alternative donors (30-65% survival)
  - Treatment with bovine enzyme (PEG-ADA) (80% survival) not a definitive cure, not always effective, very expensive

Gene transfer protocol into autologous bone marrow CD34+ cells



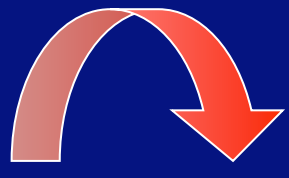
BM Harvest

No PEG-ADA

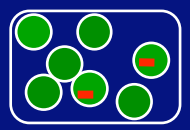
Busulfan
2 mg/Kg/day x 2
(days -3, -2)

Day -4: Purification of BM CD34+ cells

Day 0: Infusion



MLV LTR

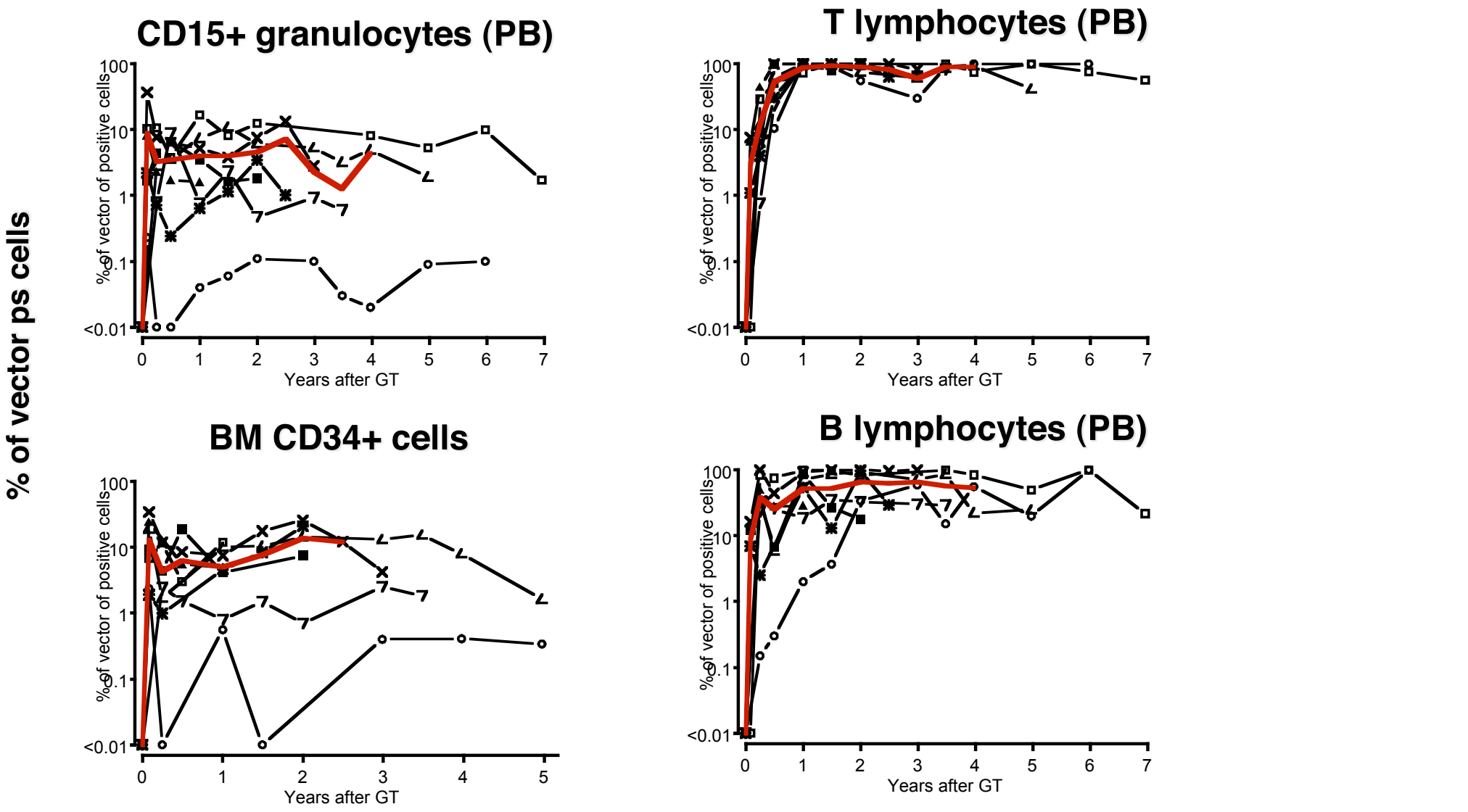


Days -3 to -1:
3 cycles of transduction
on retronectin + cytokines

Day-4: Prestimulation
(TPO, FLT3-ligand,
SCF, IL-3)

A. Aiuti, MG Roncarolo, C. Bordignon, 2002

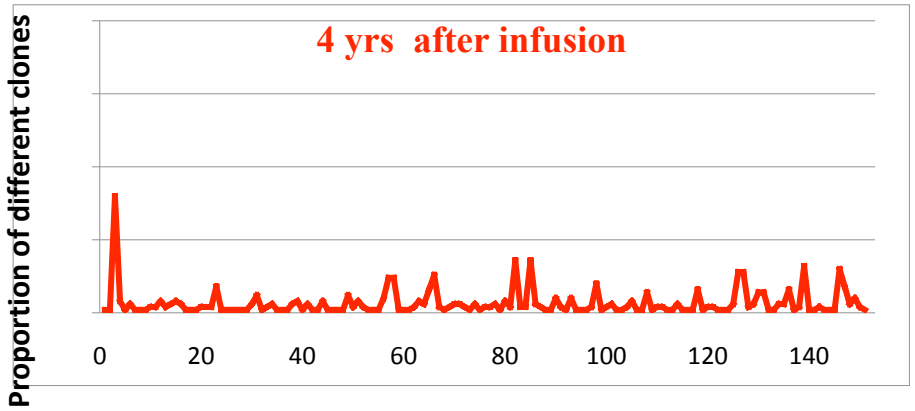
Long-term engraftment of gene corrected cells



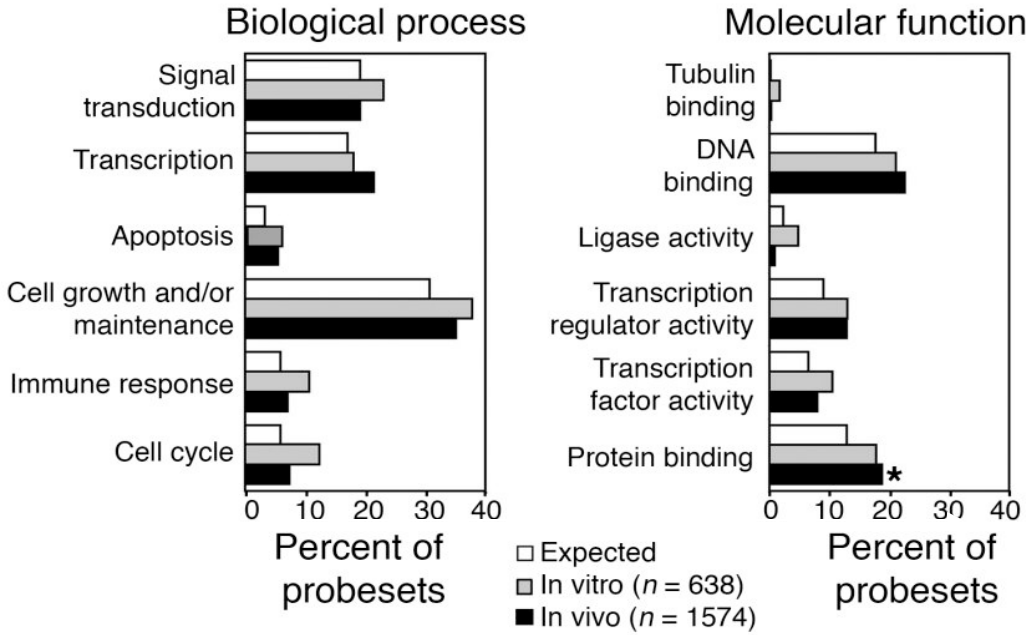
A. Aiuti et al. NEJM, 2009

POLYCLONAL VECTOR INTEGRATIONS and REPERTOIRE

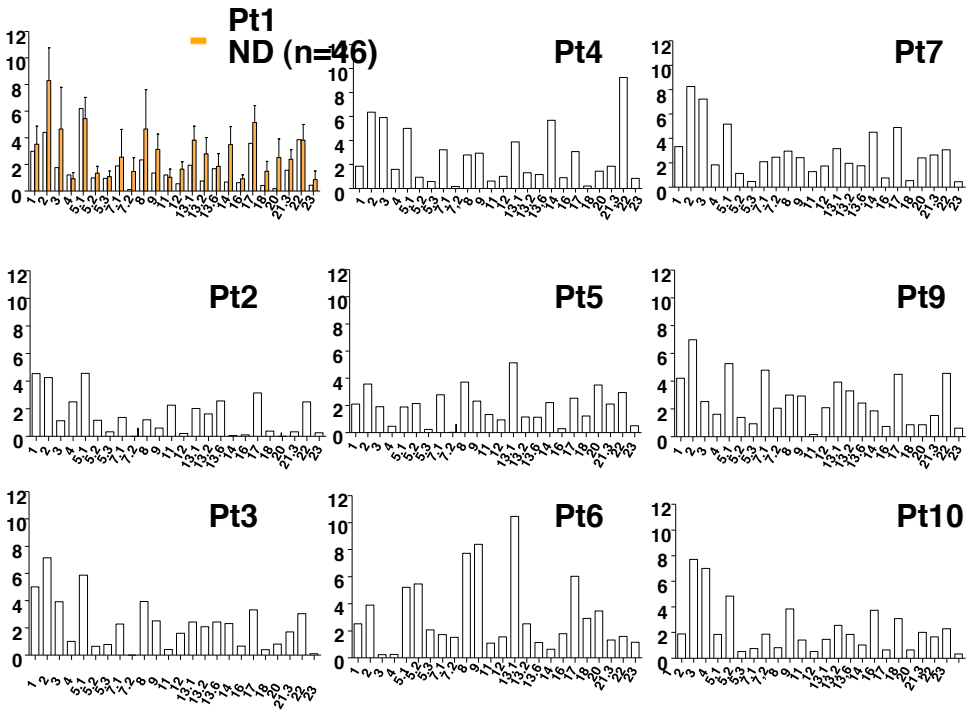
Diversity of integrations in T lymphocytes



No in vivo skewing

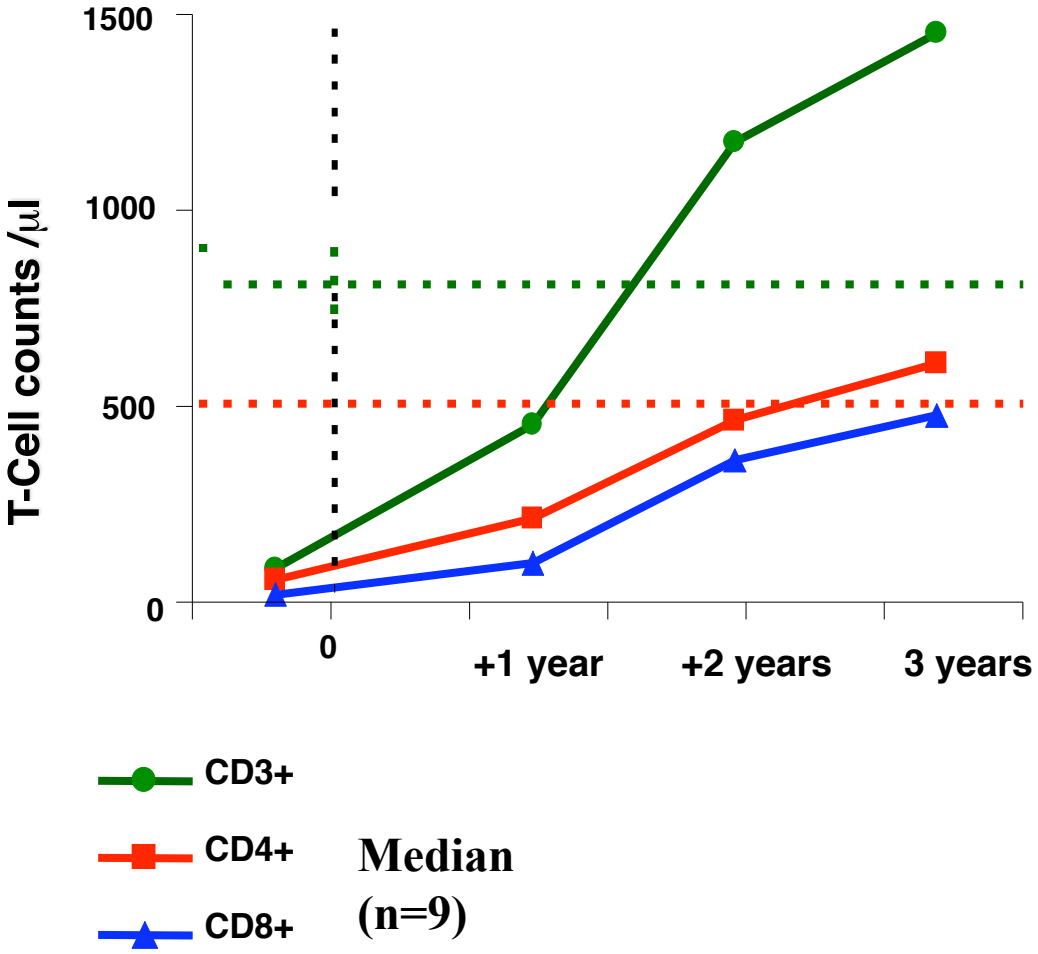


TCR Vbeta Repertoire

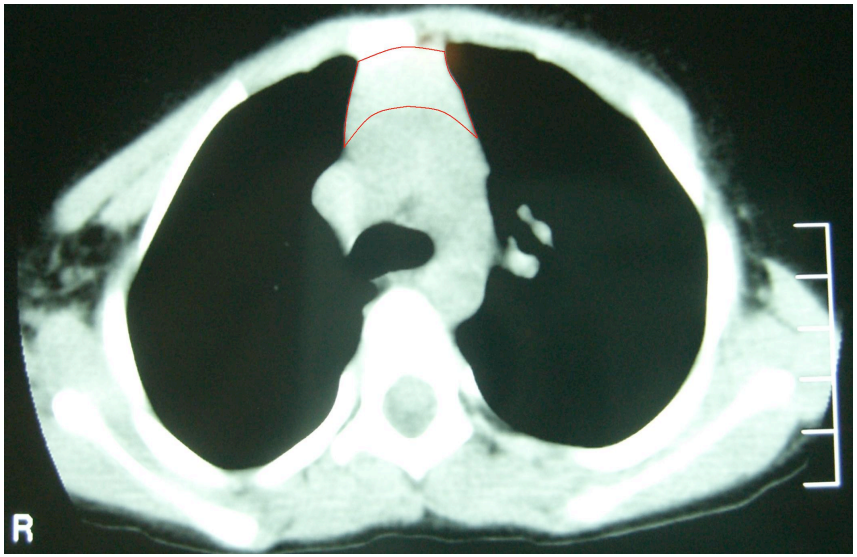


Aiuti et al., JCI 2007 and unpublished results

Immune reconstitution after GT

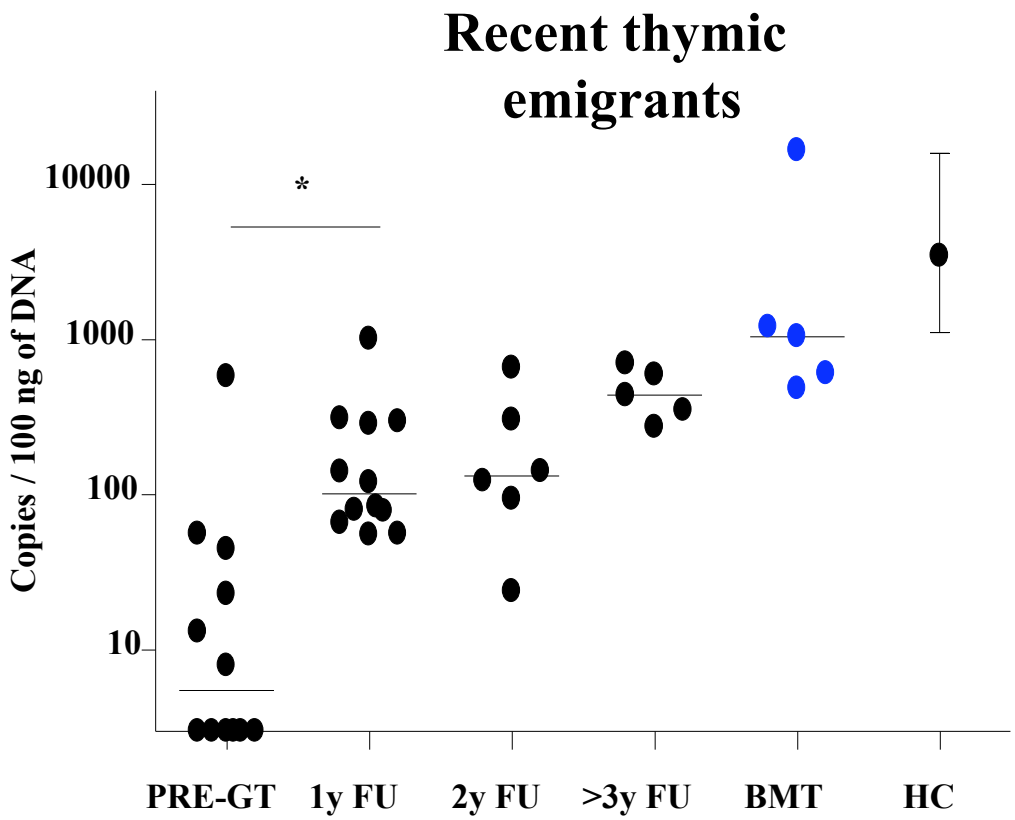


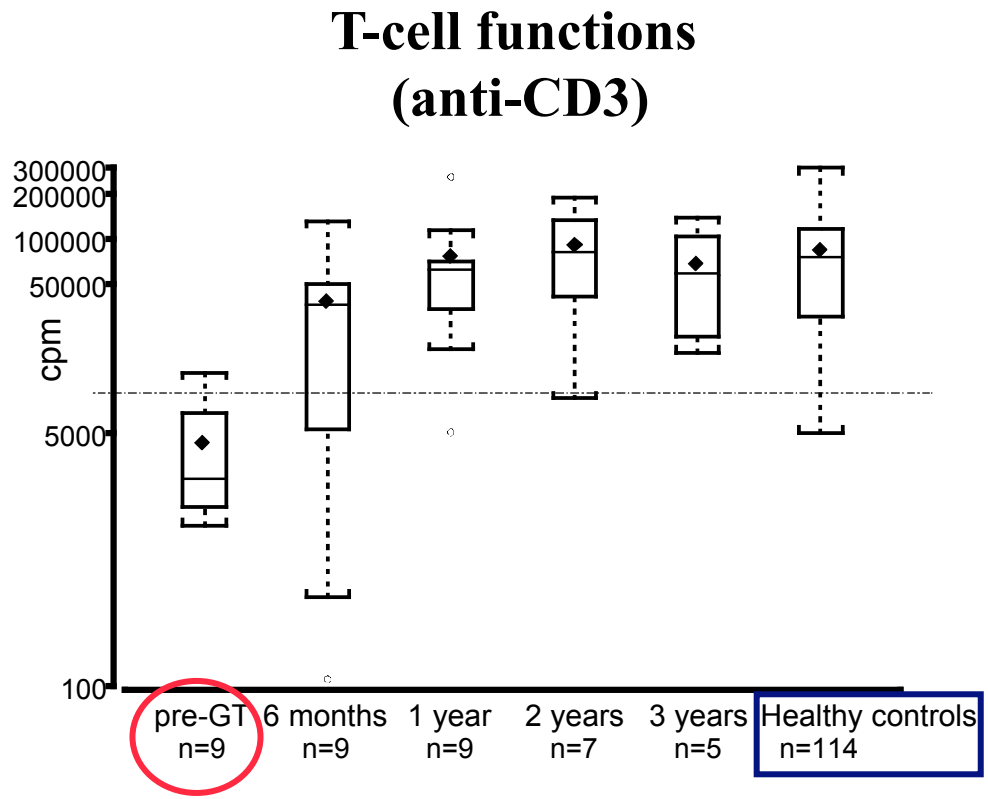
Thymus 3 yrs post-GT



5/5 patients showed increased thymic volume (CT scan)

Recovery of thymic functions





Aiuti et al., NEJM, 2009

Immune response and protection from infections

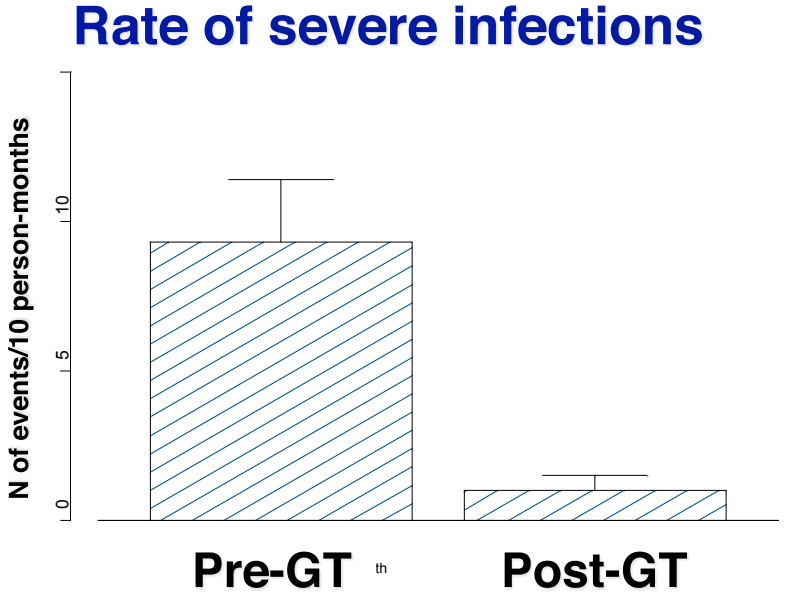
- IVIg discontinued in 6 pts with proven antibody response

(TT, DT, Pertussis, Haemophilus, Pneumo)

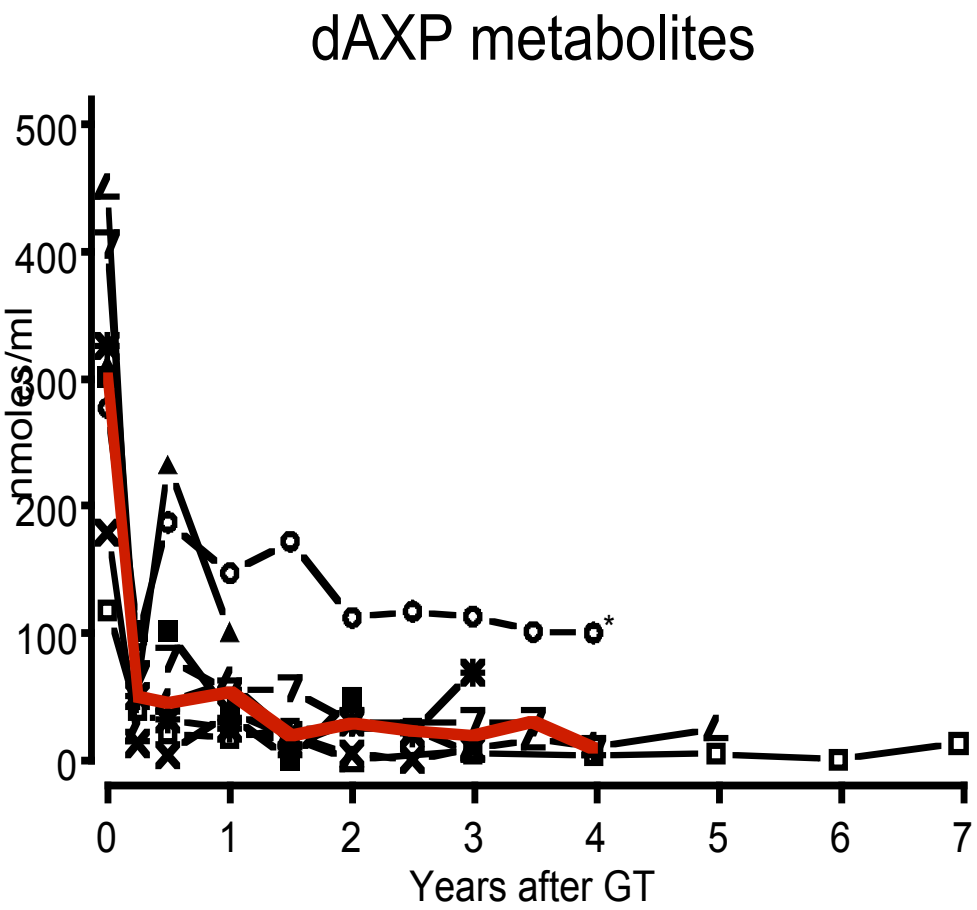
2 Pts ongoing IVIg discontinuation

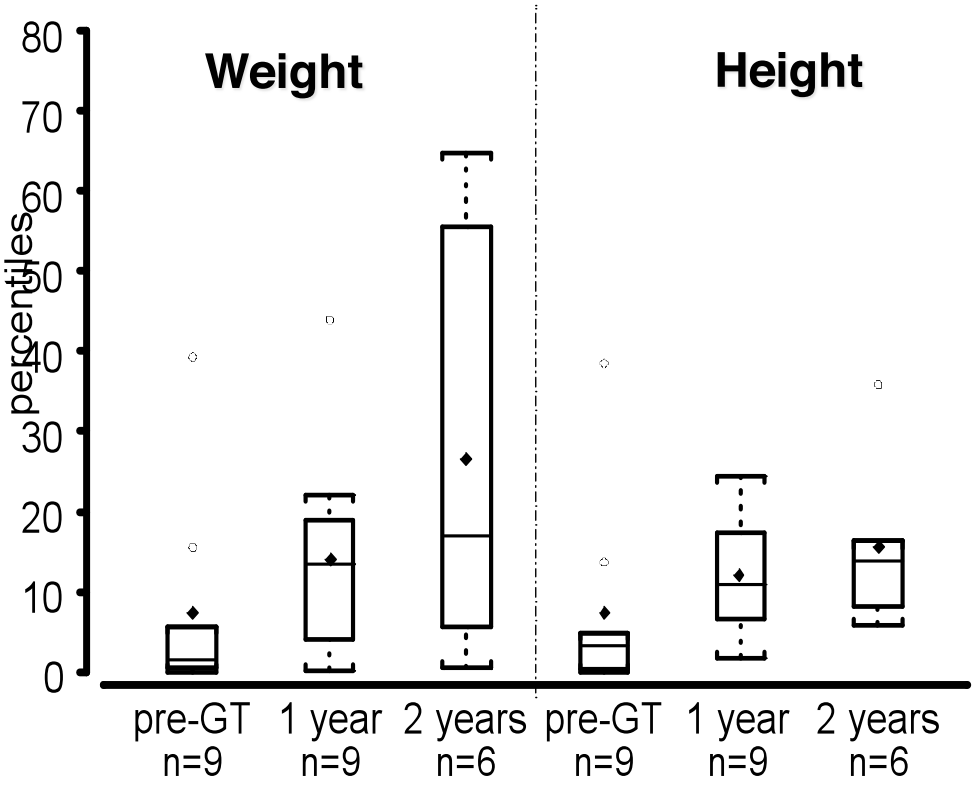
- MMR vaccine in 1 pt led to protective antibodies
- Four patients experienced varicella

without complications



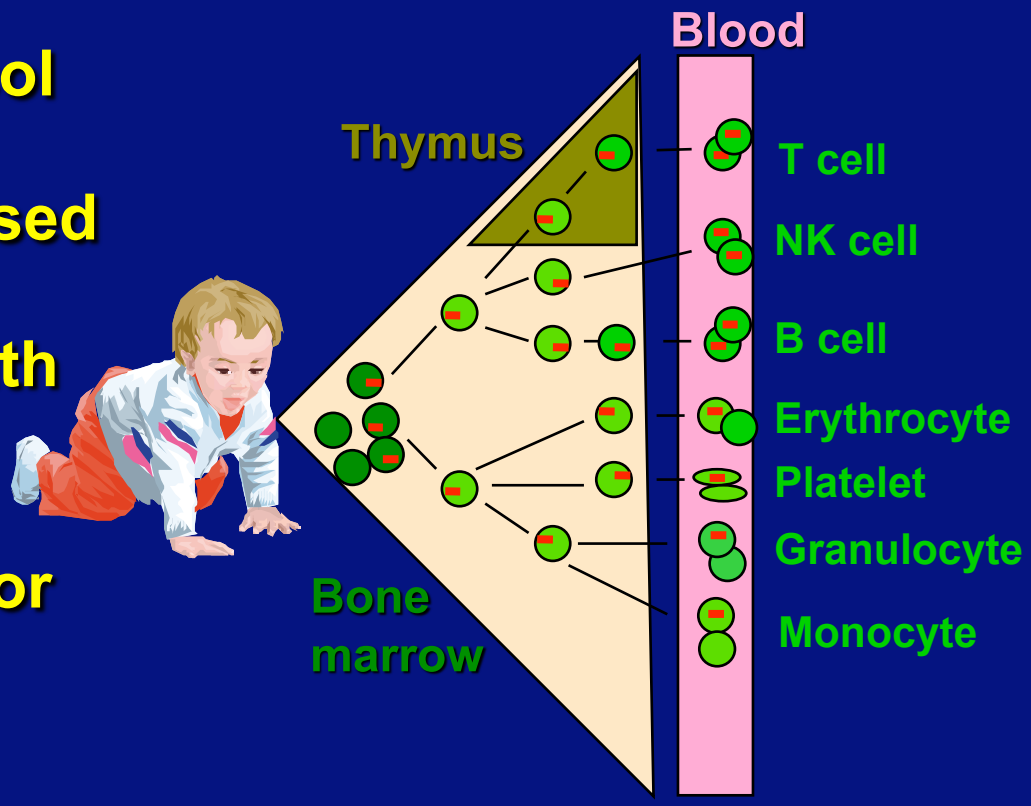
Systemic detoxification and growth improvement



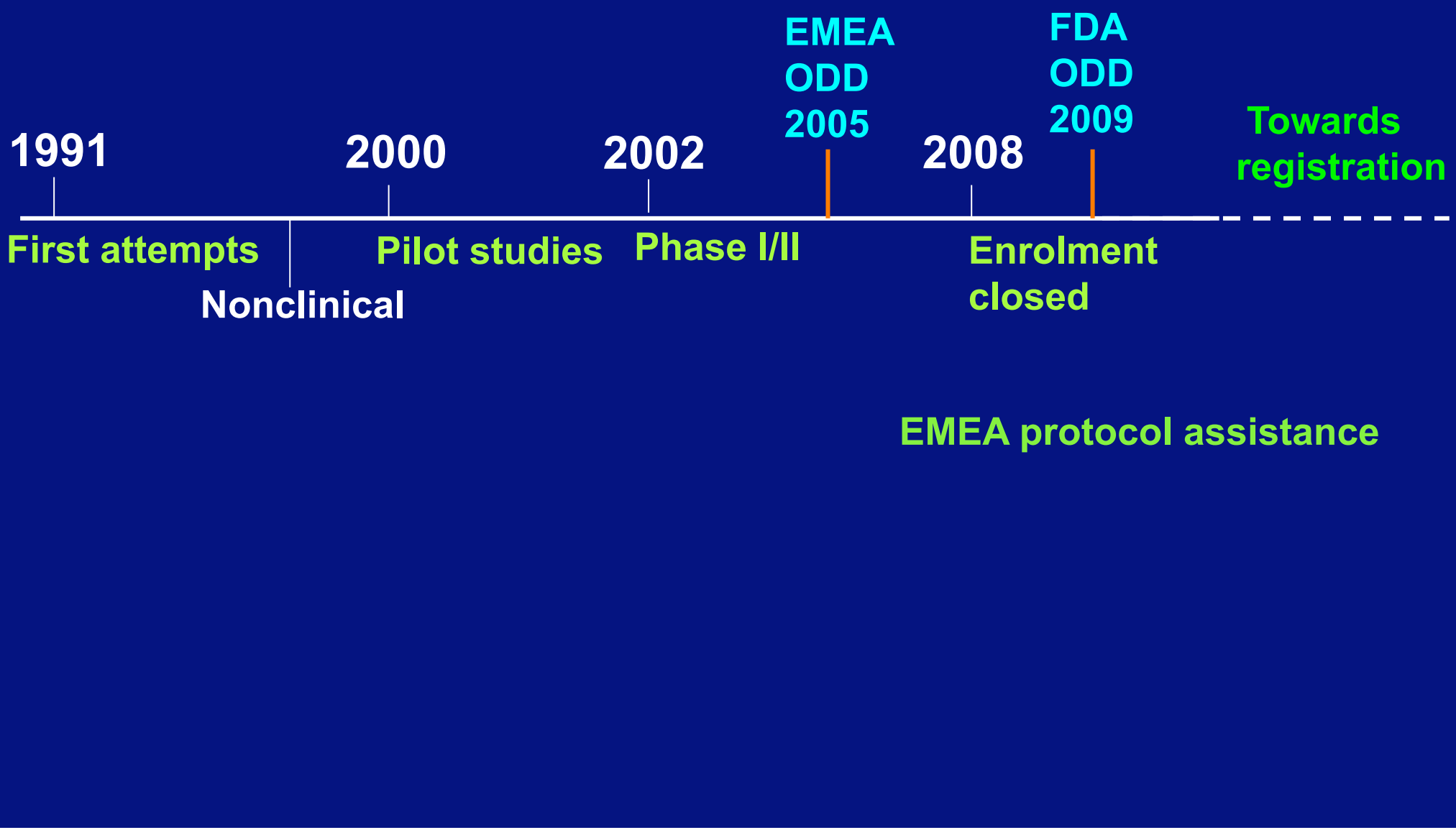


Outcome of ADA-SCID GT

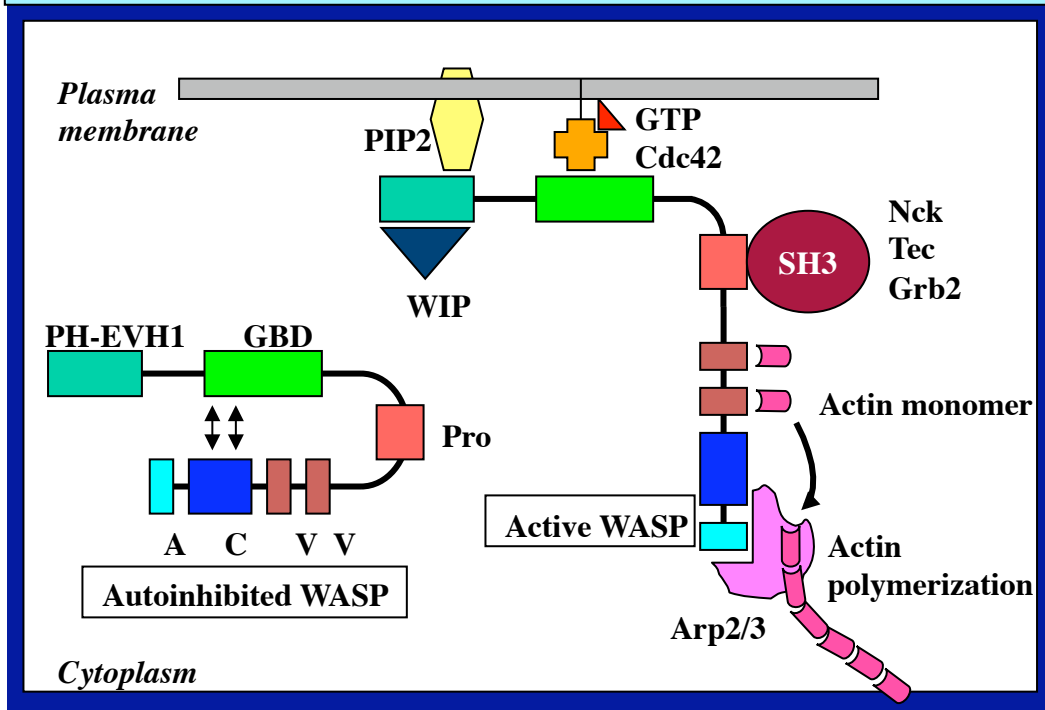
- Efficient transduction protocol
- Adequate cell number reinfused
- Competitive edge for HSC with low intensity myeloablation
- In vivo selective advantage for lymphoid cells
- Safety and clinical benefit



Clinical translation in ADA-SCID



Wiskott-Aldrich Syndrome (WAS)



CELLULAR DEFECTS

HSC	migration
T cells	migration, immune syn.
B cells	migration, Ig production
Platelets	reduced size / number
Dendritic cells	migration
Macrophages	adhesion, antigen uptake
NK cells	cytotoxic activity

X-linked, 1,250.000 newborn male

- Eczema
- Bleeding
- Infections
- Autoimmunity
- Tumors

Life expectancy:
15 years in severe forms
(WAS-negative)

Current Treatment

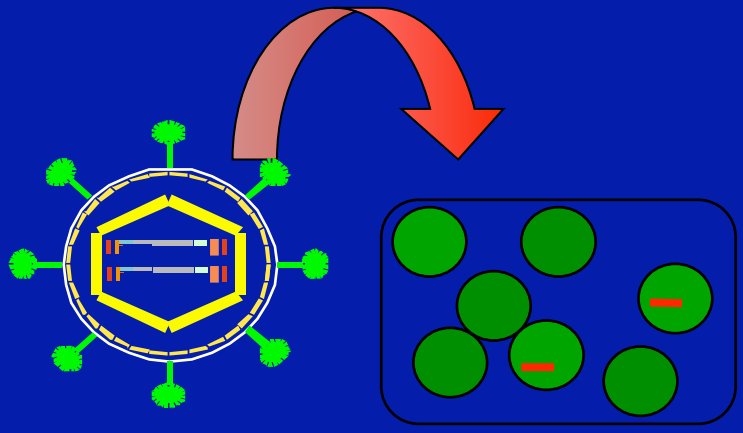
- Non curative: IVIg, steroid, splenectomy

>Allogeneic Transplant	(Survival)
- HLA-identical sibling	80-90%
- Matched Unrelated Donor	70-80%
- Haploidentical	40-55%

> If patients >5years poor outcome of BMT (<30% survival)

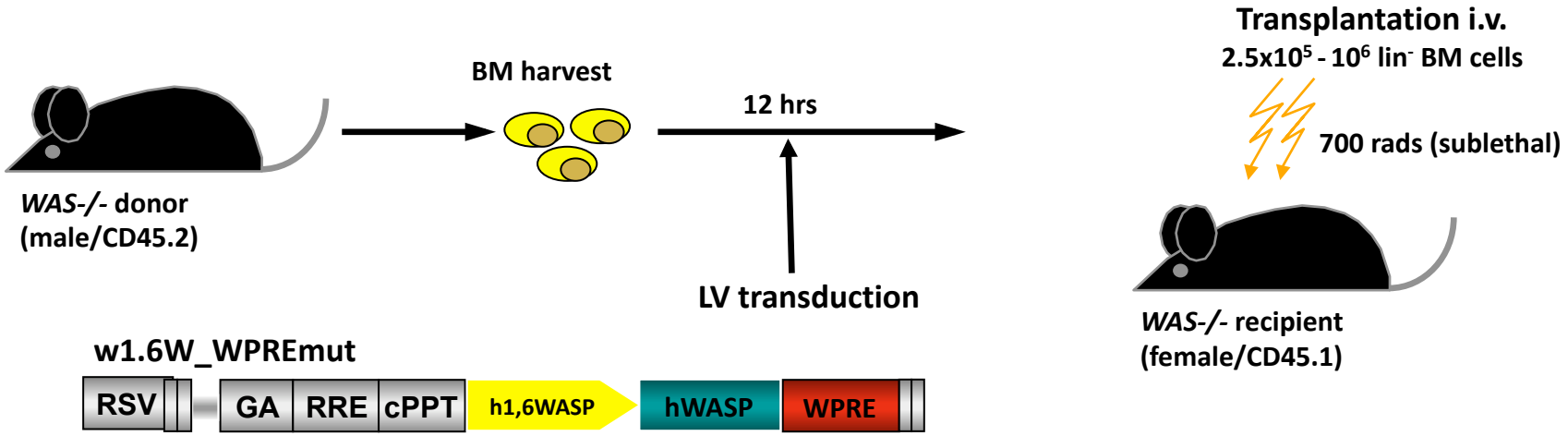
Filipovich, Blood 2001; Kobayashi, 2006; Ozsahin, Blood 2008

LENTIVIRAL VECTORS

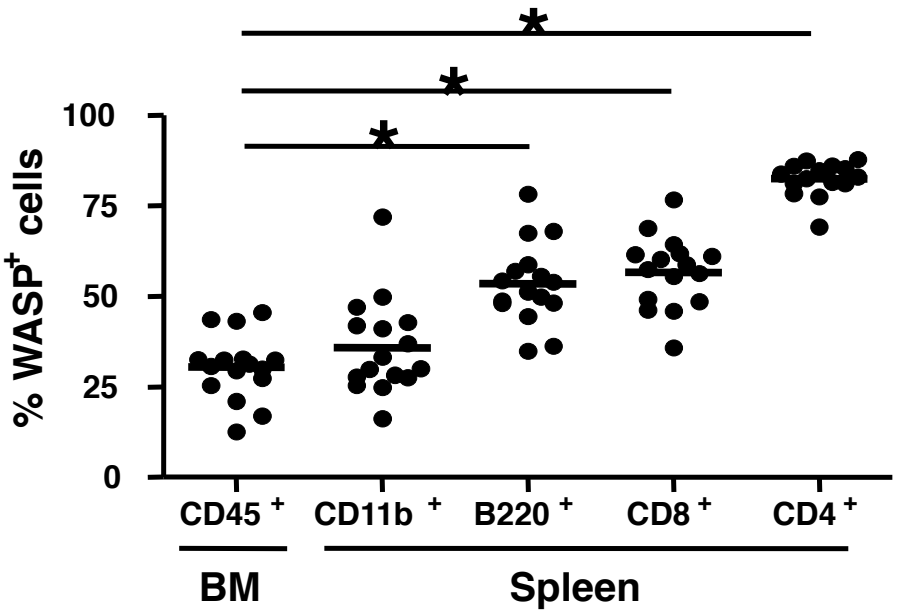


- HIV derived, self-inactivating system
- Safer integration profile
- Physiological promoter
- Improved GT efficiency into HSC

SAFETY AND EFFICACY OF WAS GT IN WAS KO MICE



Engraftment and selective advantage



98 mice followed for 4-16 months

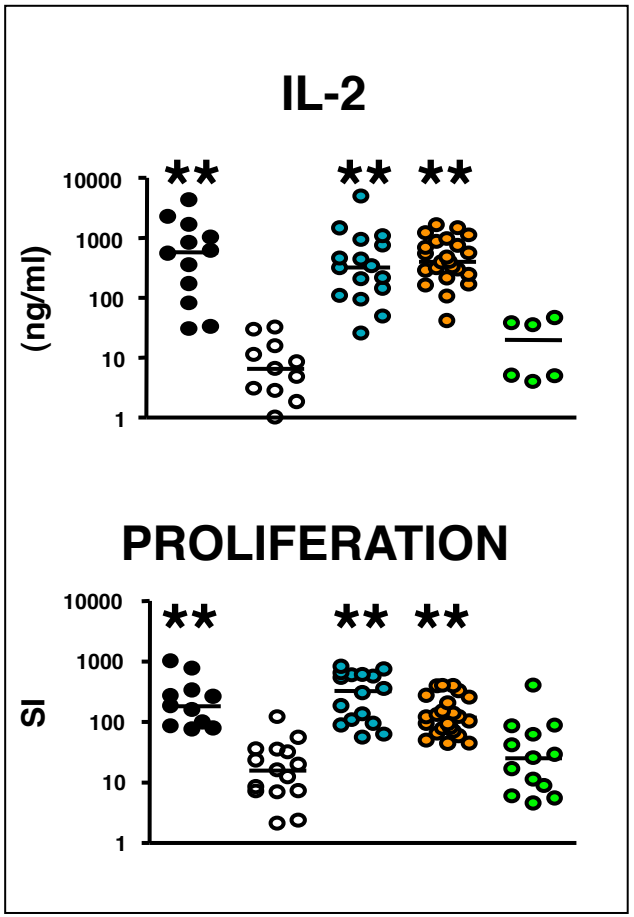
No long-term toxicity

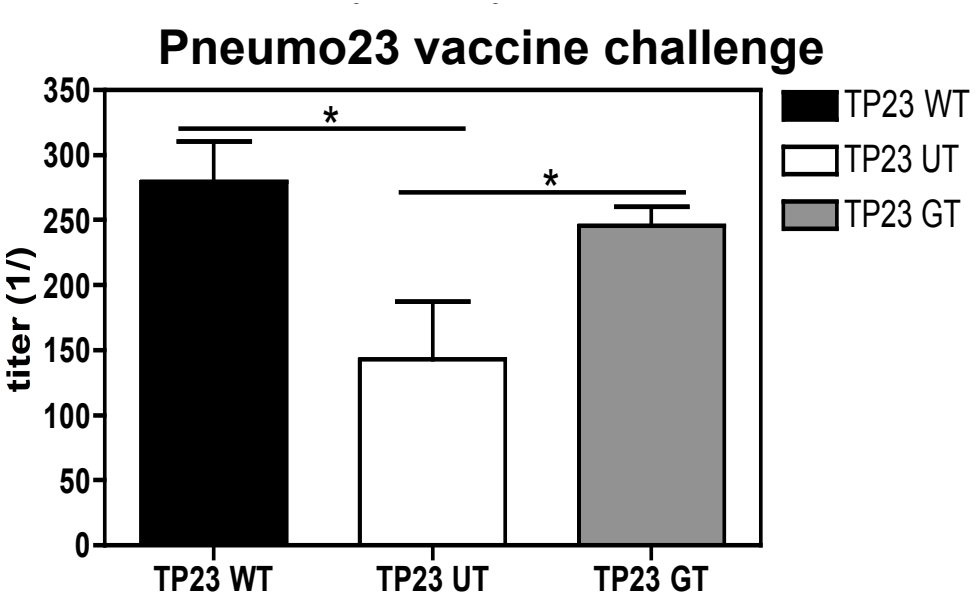
No vector derived tumors

No increase in tumor incidence

FUNCTIONAL CORRECTION OF T-CELLS AND B CELLS

T-cell functions





wt w1.6W high MOI Lin^{-} *Was*$^{-/-}$

Was$^{-/-}$ w1.6W low MOI

F. Marangoni, A. Villa, M. Bosticardo

SUMMARY OF TOXICITY AND SAFETY STUDIES (CD34+ cells)

In vitro

In vitro growth

Colony assay (CFC,LTC-IC)

Vector shedding

Vector integrations

In vivo

Biodistribution

Vector shedding

Germline transmission

$Rag2^{-/-}\gamma c^{-/-}$ (neonates)

The path to clinical trial in WAS

- Lack of toxicity
- Safety and efficacy in the animal model
- Selective advantage for gene corrected cells
- Efficient gene transfer in human CD34+ stem cells

A phase I/II clinical trial of haematopoietic stem cell gene therapy for the Wiskott-Aldrich Syndrome

Design: non-randomized, open label, single center

Population: 6 patients
-Severe WAS mutation or WAS clinical phenotype
-No HLA-identical sibling
-No HLA-matched UR BM or UCB donor (Pts <5 yrs)

Follow up: 3 years, then long-term safety protocol

Study objective: Evaluate the safety, biological activity and efficacy of GT

Financial sponsor: Fondazione Telethon
PI: A Aiuti, MG Roncarolo, Co-PI: Fabio Ciceri

Authorized by Ethical Committee (12/09) and
National authority (ISS) (03/10)

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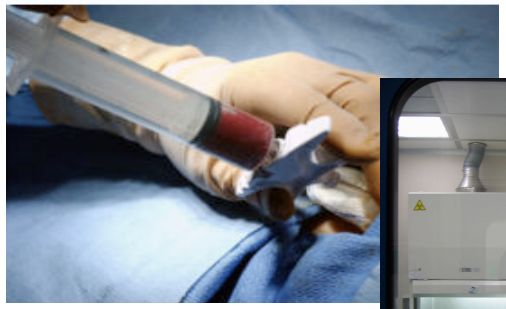
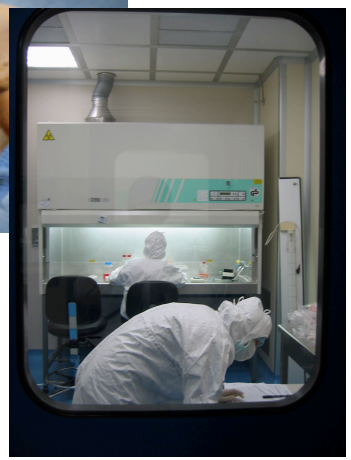
TELETHON INSTITUTE FOR GENE THERAPY

“Old” therapy approaches

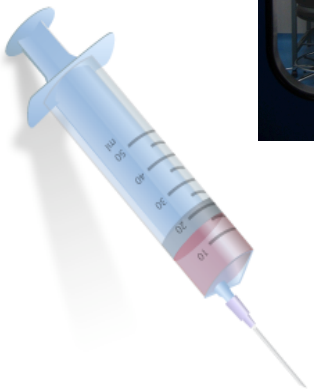
Gene/cell therapy for rare diseases

Very rare population!





“Personalised” therapy

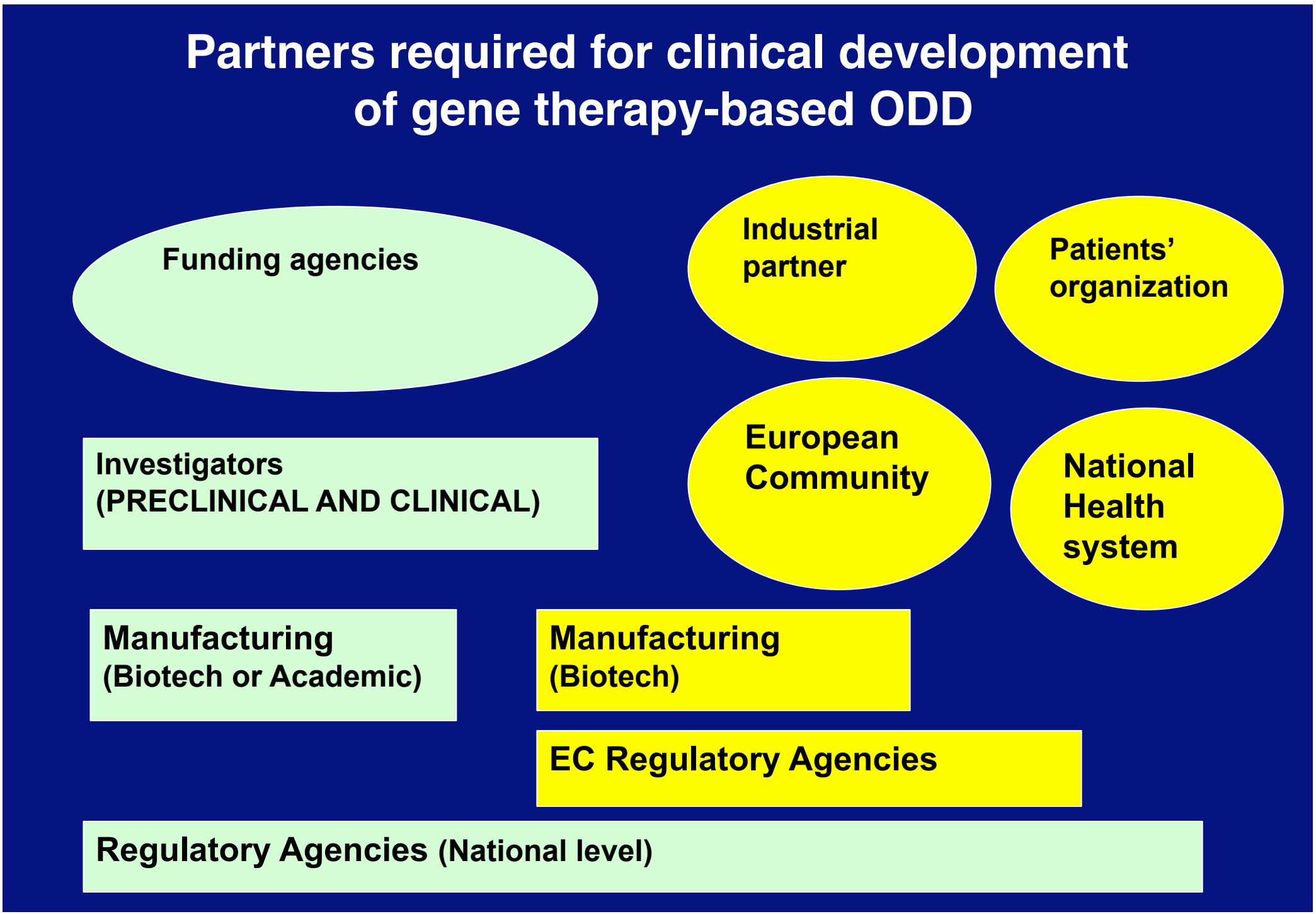


Single curative injection

BOTTLENECKS FOR MARKETING AUTHORIZATION of ODD GT PRODUCTS

- Often very rare populations
- Long-term safety
- Mainly academic-driven, high costs
- Limited interest for pharma company’s investment
- Manufacturing and standardization
- Rapidly evolving scientific field and regulation

Partners required for clinical development of gene therapy-based ODD



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EDITORIALS

Gene Therapy Fulfilling Its Promise

Donald B. Kohn, M.D., and Fabio Candotti, M.D.