TargetAMD Symposium

Quality Controls set-up during the Development Phases of a Biological-based Medicinal Product:

WAS Gene Therapy Protocol

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Definitions

GTMP:"…active substance that contains or consists of a recombinant nucleic acid used in…view to regulate, repair, replace, add or delete a genetic sequence".

Biological medicinal products:"… the active substance of which is a biological substance.

Biological substance:"…produced by or extracted from a biological source ..".

Dir. 2001/83/EC , amd. by Dir. 2009/120/EC

Biologics: can be defined…by reference to their method of manufacture. ***Guidelines for Good Manufacturing Practices for Medicinal Products for Human & Veterinary Use. Eudralex Volume 4***

ICH documents for Biologics

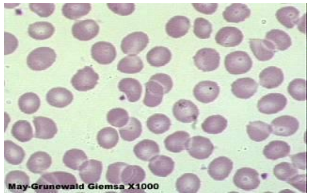
- Q5 A: Viral Safety
- Q5 B: Genetic Stability
- Q5 C: Product Stability
- Q5 D: Cell Substrates
- Q5 E: Comparability
- Q6 B: Specification
- M4 / M2: CTD / e-CTD
- Q7: GMP for APIs
- Q8: Pharmaceutical development
- Q9: Quality Risk Management
- Q10: Pharmaceutical quality system
- Q11: Development & Manufacture of Drug Substances

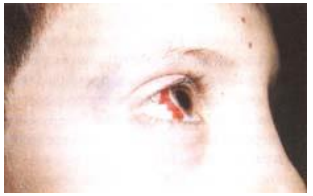
Wiskott-Aldrich Syndrome

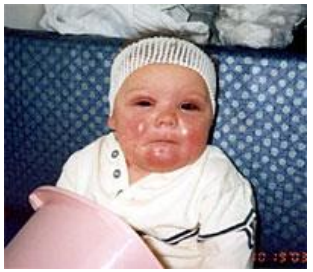
- **Rare X-linked PID** (1-10/million births total)
- **Due to lack of WASP in blood cells**

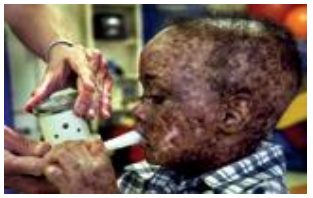
  cytoskeletal regulator via Arp2/ 3 complex activation
- **Complex symptoms**
  - Recurrent infections
  - Bleeding
  - Eczema
  - Auto-immune disorders
  - Increased incidence of malignancies
- **Treated by allogeneic BMT**

Genotypically id: 83%; related HLA-mismatched:50%

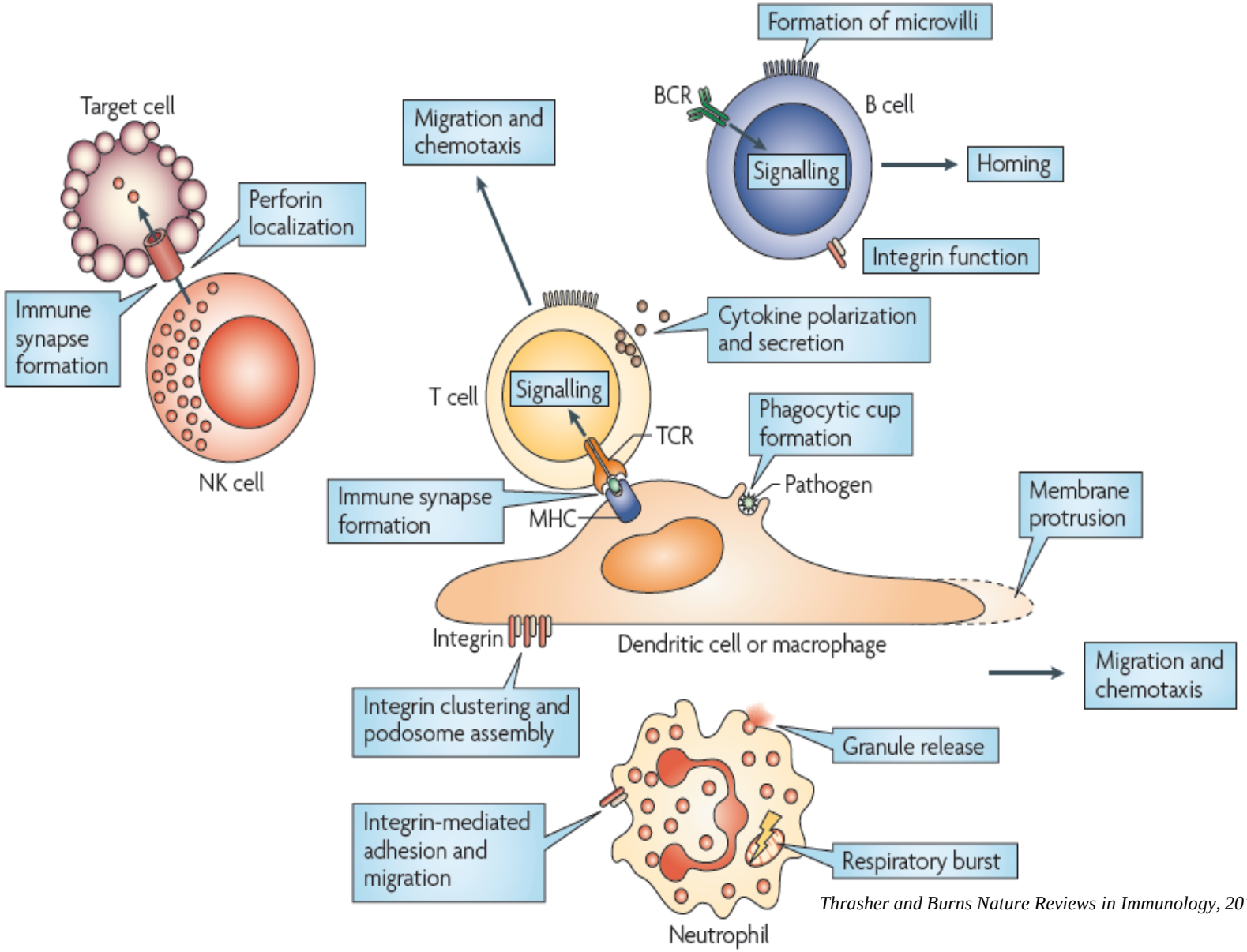








WASP Deficiency in the Immune System

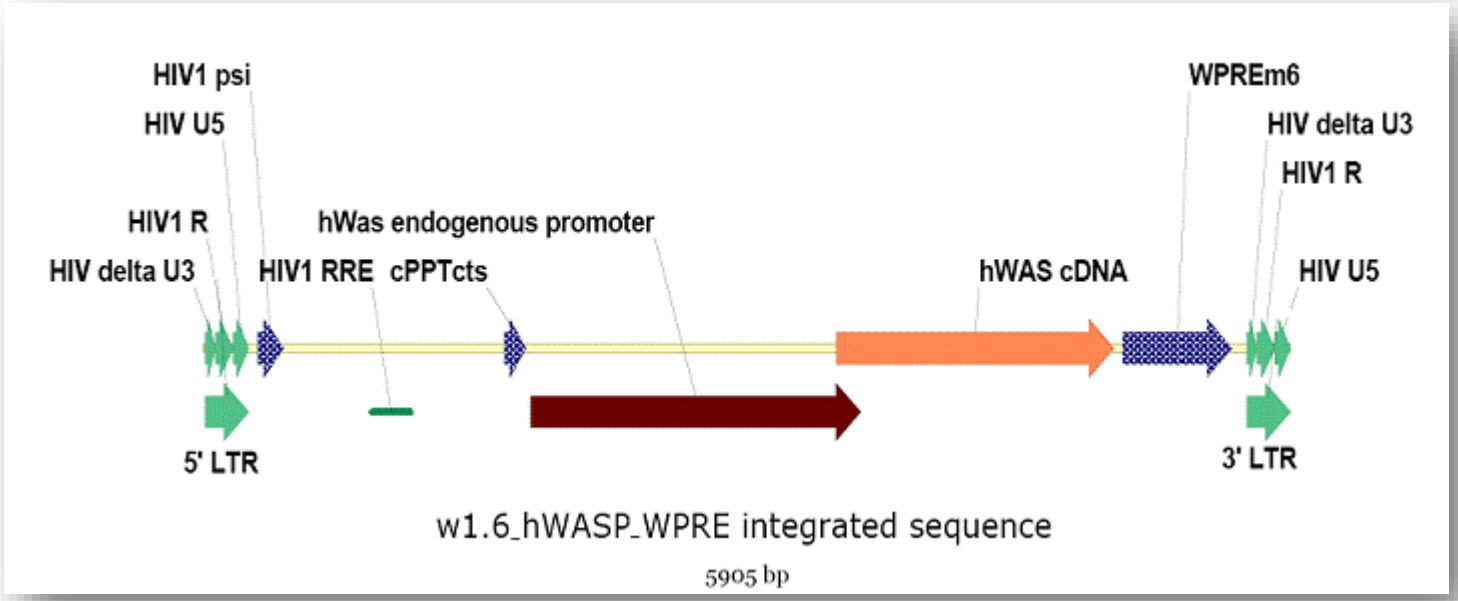


Thrasher and Burns Nature Reviews in Immunology, 2010

WAS Lentiviral Vector for enhanced safety

- The WAS vector is an advanced-generation replication-defective self-inactivating lentiviral vector derived from rHIV1 and pseudotyped with VSVg.

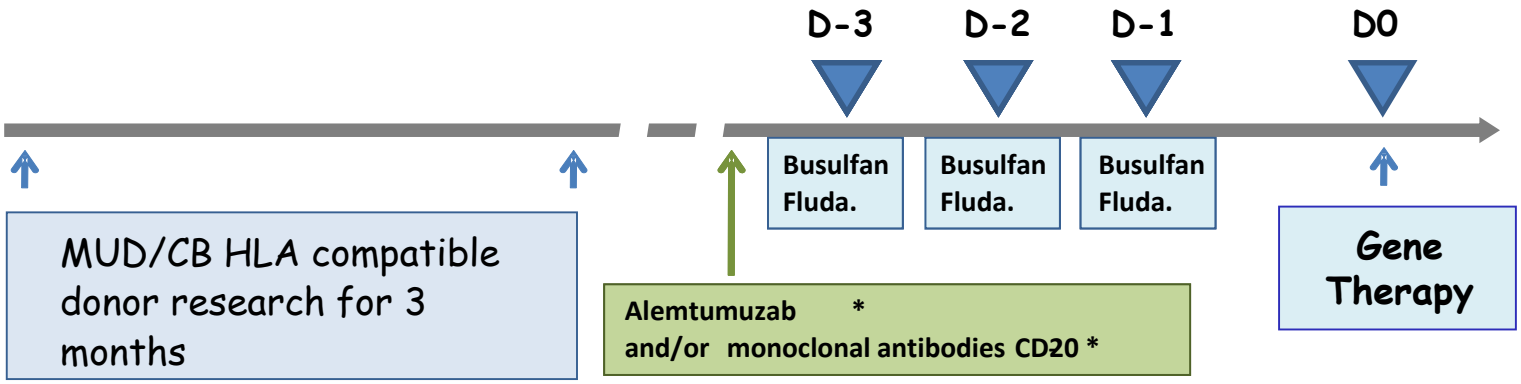
- It encodes for the human WAS cDNA driven by its endogenous 1.6 kb promoter.



Developed in collaboration with A. Thrasher, A. Aiuti, MG Roncarolo, L. Naldini

Clinical Trial for WAS: Phase I/II Pilot study

- Same vector and miscelleanous reagents used in three centers
- Severe WAS patients (score 3-5) with no HLA-geno-id BM or 10/10 or 9/10 Ag HLA-matched unrelated donor or cord blood unavailable
- Primary objectives:
  - safety and sustained engraftment of WASP+ transduced cells,
  - reconstitution of immunity
  - correction of thrombocytopenia
- Secondary objectives: to improve patient health (immunity and bleeding).



Clinical Studies

Applicable laws

Dir. 2001/20/EC, amend. Regulation No. 596/2009

Dir. 2005/28 /EC, Note for guidance on GCP, CPMP/ICH/135/95, CPMP/ICH/291/95 - ICH E6&E8

Principles

Pre-requesite:

Benefits >risks, Patients understanding & consent, Safeguards for physical & mental integrity, Insurance

Sponsor responsibility:

Trial conduct, investigator & institution, **QC (must be already set for both efficacy and safety evaluation before protocol starting)**, data collection stds, written protocol, investigator's brochure

GCP & Study design: ICH E6 & E8 Guidelines

Lentiviral -Transduced CD34+cells Product characterization/Product Testing

Multiple preclinical steps must be defined and validated, the product should be characterized

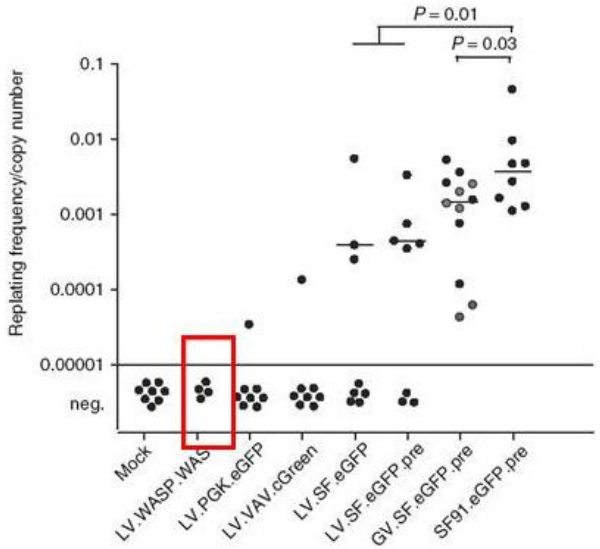
- Define critical product attributes
- Define and control all components (cell types) present in the product
- Establish appropriate product specifications
  - Ensure the safety and consistency of product lots
  - Base acceptance criteria on experience
  - Agree with regulatory standards

Lentiviral -Transduced CD34+cells Product characterization/Product Testing Quality issues I

- **Non-clinical studies must allow to bring the first evidences of efficacy and safety through development of the most appropriate analytical tools adapted to the product under evaluation**
- Starting material tested/documented for viral, TSE safety
- Excipients, reagents and structural components qualified
- Product consistent and well characterised
- Active moiety identified and quantified
  - **Manufacturing process validated and Potency assay available**
  - **Comparability issues**

Preclinical Safety data related to the selected WASP-LV vector

- Non-replicative, RCL-free
- Absence of vector toxicity *in vitro* on target cells
- Good safety profile from in vivo safety studies in mice
- No evidence of oncogenicity in IVIM assay
- Expected pattern of genomic integration



Modlich *et al.* Mol Ther 2009

Lentiviral -Transduced CD34+cells Product characterization/Product Testing Quality issues II

Control & validation of the manufacturing process

- **Critical steps & limits**
- Control via **relevant markers** (mRNA, protein expression..)

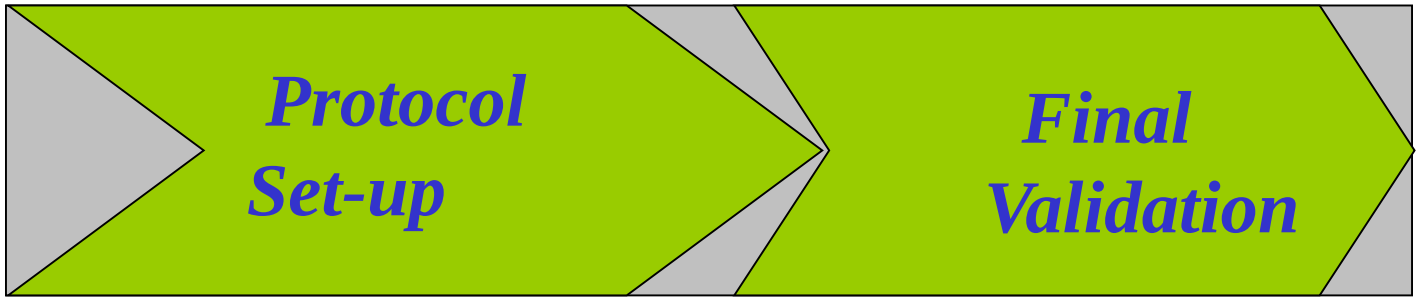
Purity

- Maximise active moiety
- Eliminate and control undesired components
- **Minimum requirement: consistency**

Tumourigenicity

- Limit amount of …(dedifferentiated cells, **VCN**)
- Demonstrate genotypic/phenotypic stability during process

Gene Therapy Protocols Set-up
Quality issues



Viral batch tests: research & clinical grade

Viral titer, quality, potency

Pre-Runs:

***Production Process design, PTA Media, Cytokines, bags**

***QC testing determination and validation**

***Specifications drawn up**

Target cells for GT:

Cell lines, CD34 CTRL, Patient CD34

Final corrected populations:

T-lymphocytes, DC, NK, B cells, granulocytes, Platelets

Hematopoïetic potential of GM cells:

Clonogenic progenitors

LTC-IC

Efficacy of GT (Potency tests):

cell viability

cell number/dose

transduction rate

VCN (Q-PCR)

WASP Intra-cytoplasmic stainning

Western Blot

In vitro prediction of efficacy

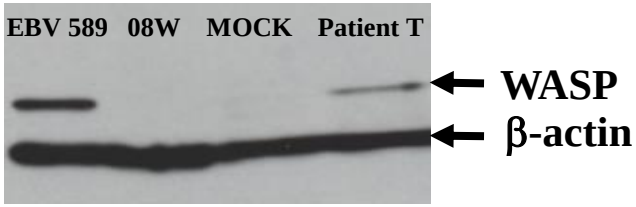
WASP restoration after Gene Transfer

Potency assays

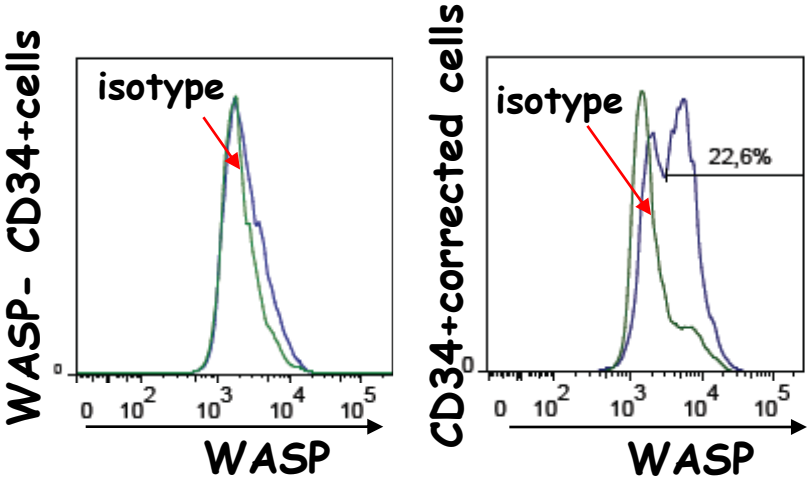
✓Quantitative or qualitative biological assay

✓Predict safety/efficacy of the biologics under evaluation

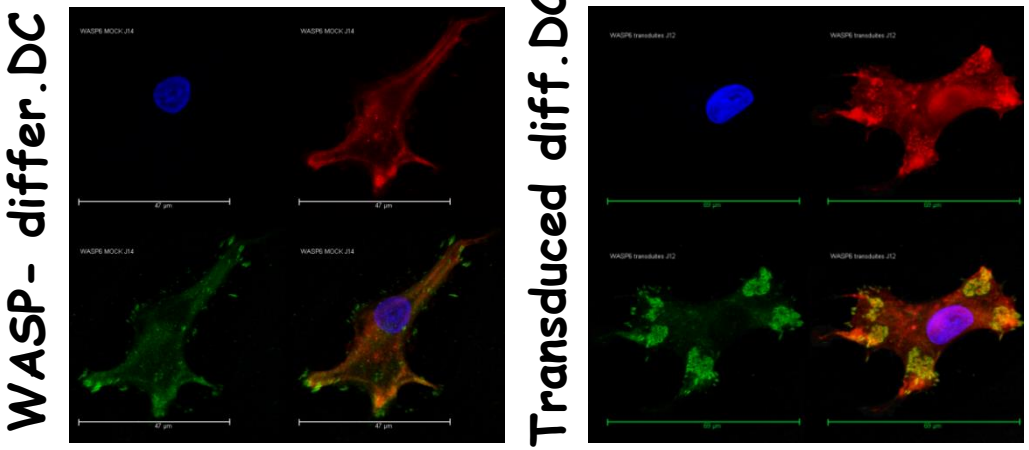
Western blot analysis



Intracellular immunostaining

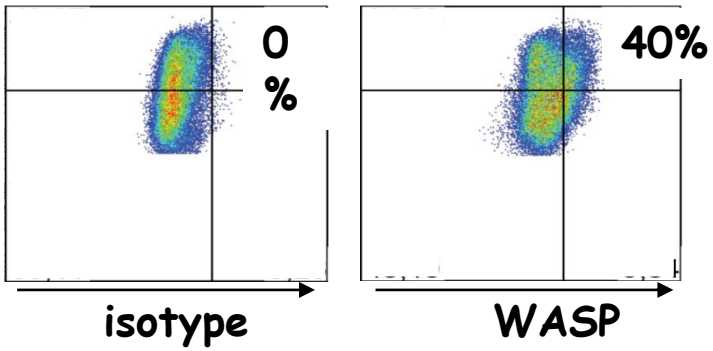


Functional recovery (DC podosomes)
Restored Dendritic cells cytoskeletal dynamics



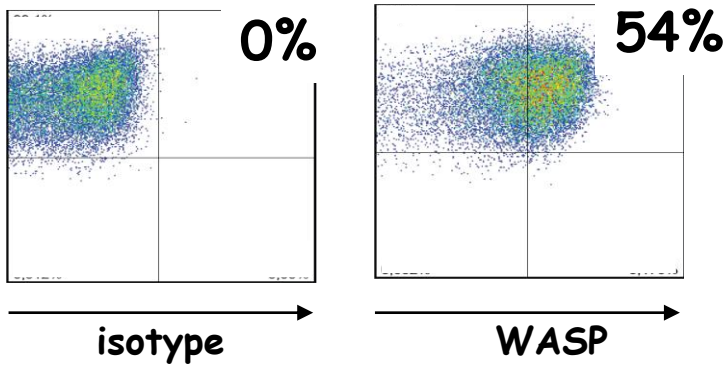
WAS Gene Therapy Clinical Trial
Characteristics of Genetically Modified CD34+cells

Patient FR01

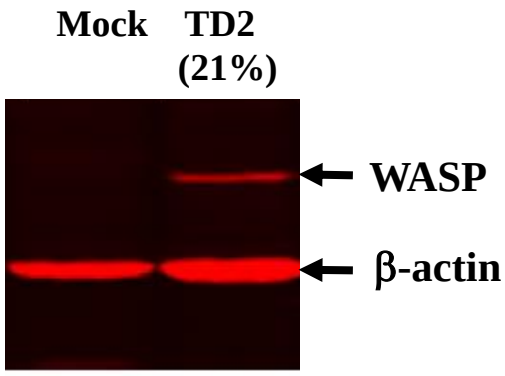


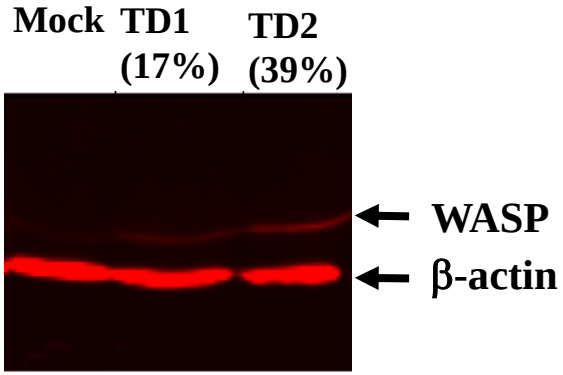
$CD34^+ \times 10^6/kg$	11
VCN in CD34	1.3

Patient FR02



$CD34^+ \times 10^6/kg$	10.8
VCN in CD34	1.2





QC used to release to product

Conclusion

All the patients show a clear clinical benefit

Kinetics of mature WASP+ blood cells generation is tightly dependent on:

- **the VCN**
- **the number of CD34 infused**
- **their own selective advantage**

Efforts are ongoing to improve and standardise the transduction procedure

Importance of reliable QC methods for product characterization since the clinical benefit is directly dependant on the « quality » of the genetically modified progenitor cells

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