

Protocol #1007-1052:  
Pilot and Feasibility Study of  
Hematopoietic Stem Cell Gene  
Transfer for Wiskott-Aldrich Syndrome

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# Outline of presentation

- ❖ Background about Wiskott-Aldrich syndrome (WAS)
- ❖ Preclinical safety and efficacy data
- ❖ Outline of clinical protocol
- ❖ Responses to specific reviewer questions

# Background about Wiskott-Aldrich syndrome (WAS)

- X-linked disorder due to mutations in the WAS gene
- WAS expression is restricted to hematopoietic cells and deficiency results in a classic triad of thrombocytopenia, eczema and immunodeficiency
- males with WAS die prematurely, typically of hemorrhage, overwhelming infection, autoimmunity or malignancy
- survival without definitive treatment is worst in those who lack protein expression

# Mutations in the WAS gene result in several phenotypes including classic WAS

**TABLE I.** Clinical phenotypes associated with mutations of the *WASP* gene

	WAS	XLT	IXLT	XLN
Phenotype				
Thrombocytopenia	+	+	(+)	-
Small platelets	+	+	+	-
Eczema	+/+/+/+/+	-/+	-	-
Immune deficiency	+/++	-/(+)	-	-
Infections	+/++	-/(+)	-	+*
Autoimmunity and/or malignancies	Frequent	Possible	-	-
Congenital neutropenia	-	-	-	+
Disease scores	3, 4, or 5	1, 2, or (5)†	<1	0
<i>WASP</i> mutations	Nonsense; frame shift caused by deletions, insertions; splicing defects	Missense (exons 1-3); inframe deletions or insertions	Missense	Missense in Cdc42-binding site
<i>WASP</i> expression	Absent or truncated	Present, reduced quantity	Present, normal quantity	Present
Treatment				
IVIG	Yes	No (with exceptions)	No	No
HSCT	Yes at an early age	Might be considered if there is a sibling donor	No	?
Splenectomy	No	Might be considered‡	No	No

*IXLT*, Intermittent XLT; *HSCT*, hematopoietic stem cell transplantation; *XLN*, X-linked neutropenia.

\*Infections typical for neutropenia.

†Patients with XLT with a score of 1 or 2 might progress to a score of 5. Incidence of autoimmunity and malignancies are less in XLT than in WAS.

‡Splenectomy results in increased platelet numbers and reduced bleeding but causes a marked increase in sepsis, requiring continuous antibiotic prophylaxis.

# WAS clinical scoring system

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<i>Score</i>	<i>Symptoms</i>
0	None
0.5	Intermittent thrombocytopenia
1	Isolated thrombocytopenia and small platelets
2	Microthrombocytopenia + localized eczema responding to standard therapy and/or occasional upper respiratory infections
2.5	Microthrombocytopenia + persistent eczema responding to therapy or frequent infections severe enough to require antibiotic therapy
3	Microthrombocytopenia and persistent eczema and frequent infections
4	As in 3 but persistent difficult to treat eczema requiring continuous corticoid administration and occasionally oral antibiotics for superinfection of eczema, and/or life-threatening infections such as abscesses, pneumonia, meningitis, sepsis and recurrent herpes simplex infection
5	Patients with WAS /XLT + auto-immunity or malignancy

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# Rationale for gene transfer in WAS

- Allogeneic HSCT is curative for WAS but survival is not uniformly good
- Subgroups of WAS patients are at high risk for poor outcome
  - older age
  - those lacking well matched unrelated donors
- GVHD causes morbidity and mortality that limit the efficacy of standard allogeneic HSCT
- Ex vivo gene transfer has been shown to result in multilineage engraftment in other immunodeficiency diseases (X-linked SCID, ADA-SCID, CGD)

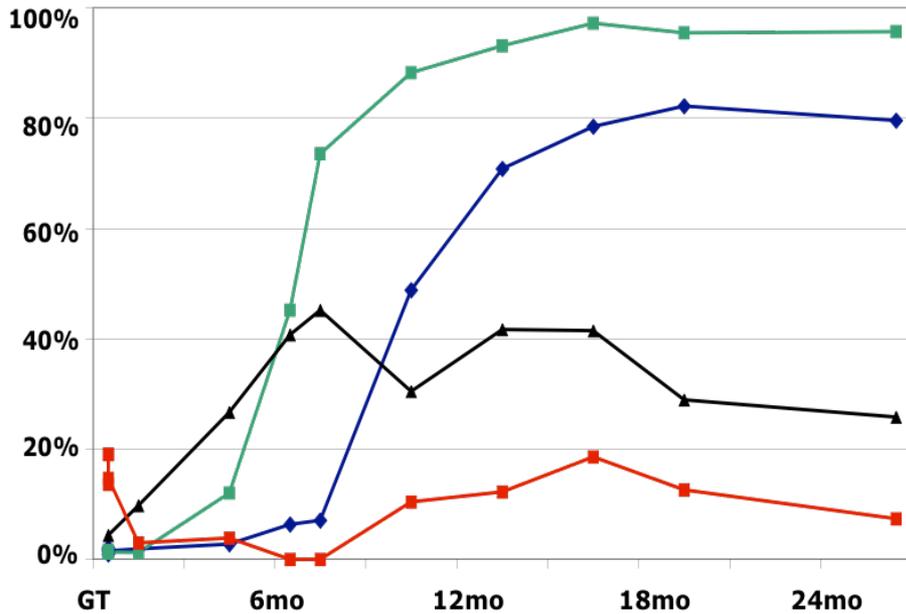
# Clinical data

10 pediatric WAS patients have undergone autologous transplantation of CD34+ cells treated ex vivo with a gammaretroviral vector in Hannover Germany after 8 mg/kg busulfan conditioning.

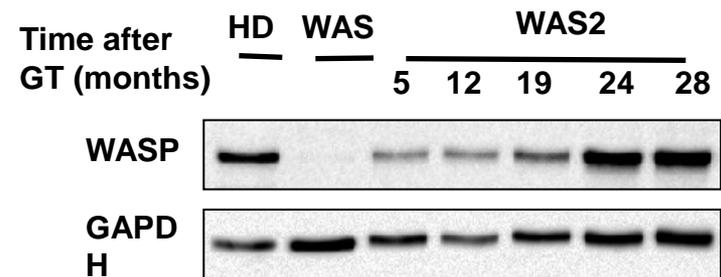
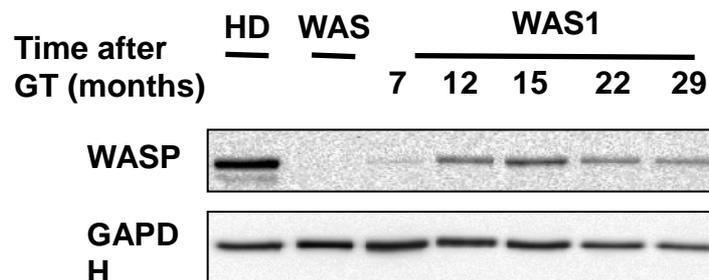
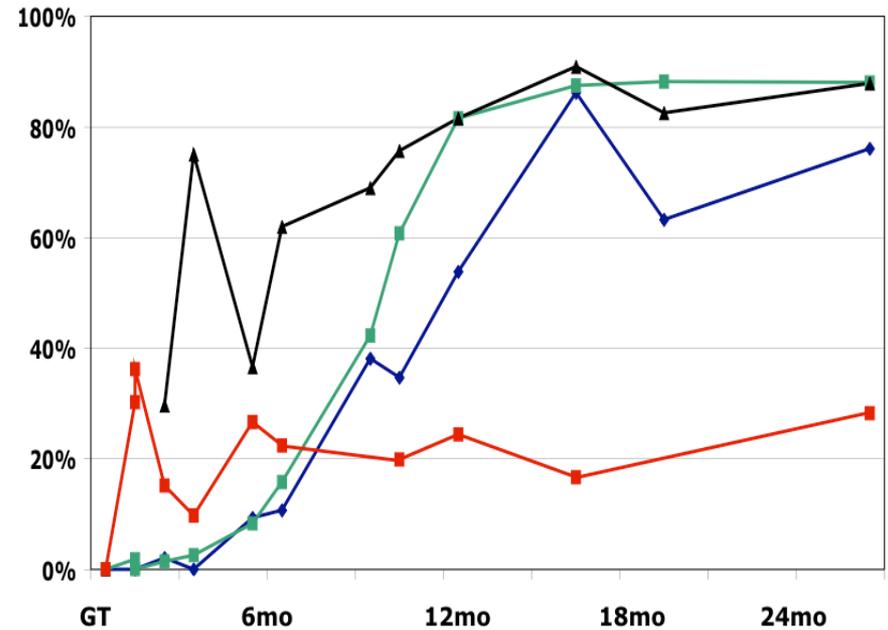
Of these 10 patients, 2 have been reported in abstract presentation (Boztug et. al. ASH meeting #502, 2007) and have longer follow-up

# Lineage specific engraftment and protein expression in 2 WAS patients undergoing GT with gammaretroviral vector

◆ CD4 ■ CD8 ▲ NK ■ Monocytes



◆ CD4 ■ CD8 ▲ NK ■ Monocytes



# Clinical data

Since then 8 additional patients have been treated in Hannover.

	WAS3	WAS4	WAS5	WAS6	WAS7	WAS8	WAS9	WAS10
WASP mutation	Glu133His	Arg34X	Glu31Lys	Asp259fs	IVS3-1 G>T	Ala134Thr	Val303fsX4	His30del
Therapy date	July 2008	Feb 2009	Mar 2009	June 2009	Sept 2009	Oct 2009	Nov 2009	Dec 2009
Age at therapy	4	3	2	12	5	5	2	14
Number of cells in transplant (/kg bw)	2.49*10 <sup>6</sup>	31.6*10 <sup>6</sup>	20.86*10 <sup>6</sup>	22.0*10 <sup>6</sup>	14.8*10 <sup>6</sup>	20.9*10 <sup>6</sup>	25.5*10 <sup>6</sup>	11.4*10 <sup>6</sup>
Outcome	Failure – allo HSCT transplant	Doing well	Doing well	Doing well	Doing well	Doing well	Doing well	Doing well

# Summary of clinical data from Hannover trial

In summary, 10 children with WAS have undergone ex vivo gene transfer of autologous CD34+ cells.

9/10 are doing well with safety and efficacy analysis ongoing.

This vector is a gammaretroviral vector with intact LTR.

Because of concerns for insertional mutagenesis in other trials using this type of vector (X-SCID, CGD), we propose a trial in patients with WAS using a 3rd generation lentiviral vector

# Proposed protocol

Pilot and feasibility study, open labeled, non-randomized, single center in up to 5 patients with WAS

Single infusion of autologous CD34+ bone marrow cells transduced with the lentiviral vector (w1.6\_hWASP\_WPRE (VSVg))

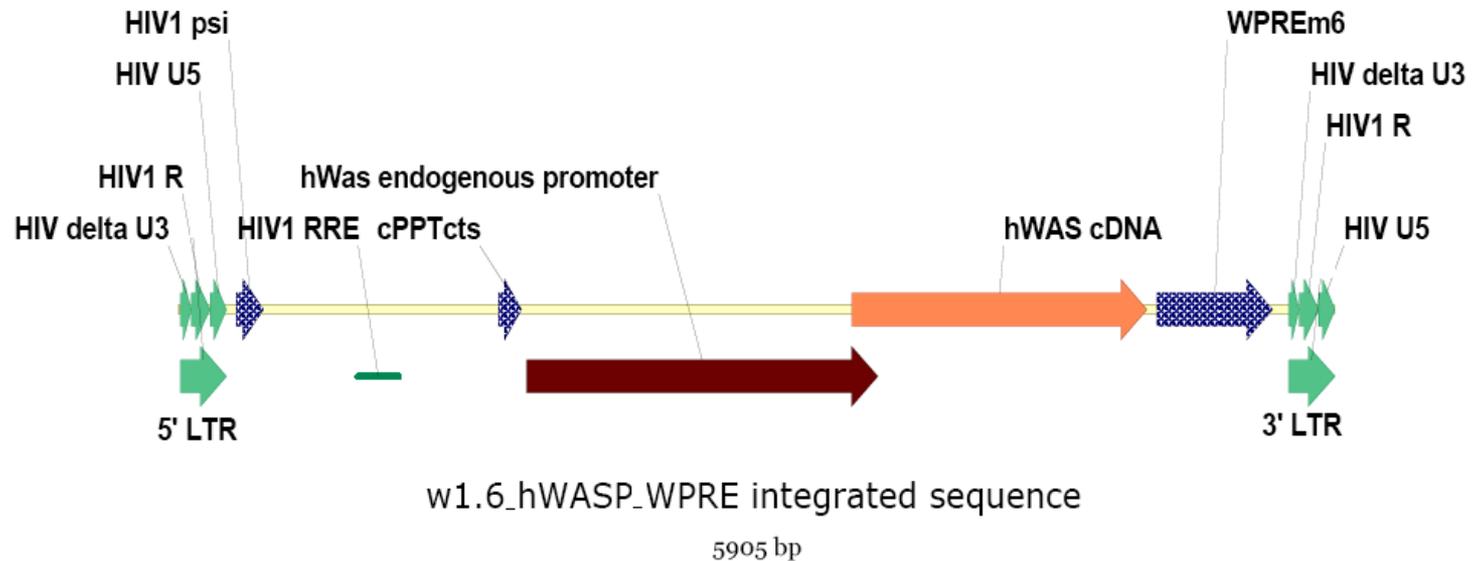
Vector is a 3rd generation replication-defective self-inactivating lentiviral vector with mutated woodchuck post-transcriptional regulatory element (WPRE) and VSV envelope

WAS human cDNA is driven by endogenous human 1.6 kB promoter

# Vector Schema

Produced at Genethon (Evry, France)

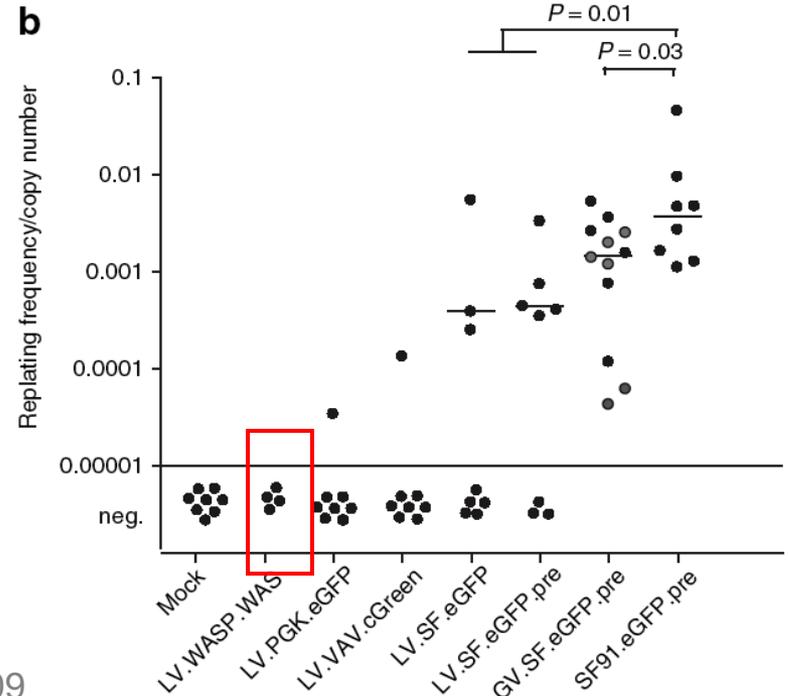
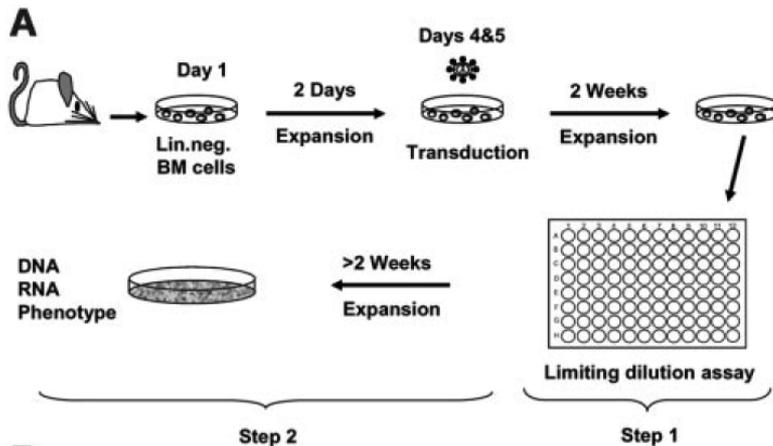
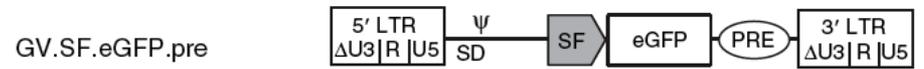
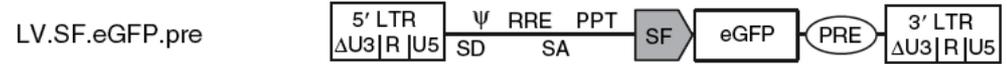
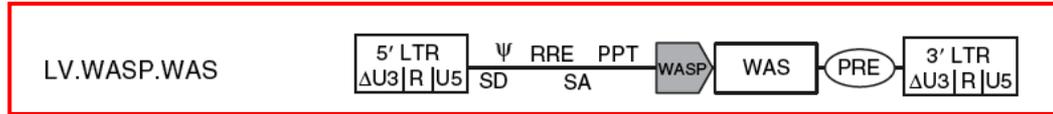
Extensively purified, replication incompetent (no RCL)



Sufficient vector to treat 15 patients:  
5 patients in London and 5 patients in Paris will be treated  
with the same vector in parallel trials.

# Preclinical safety: In vitro immortalization assay

In vitro immortalization assay (Baum lab) was used to compare genotoxicity between the clinical vector and vectors with the SFFV viral promoter/enhancer placed internally or in LTR

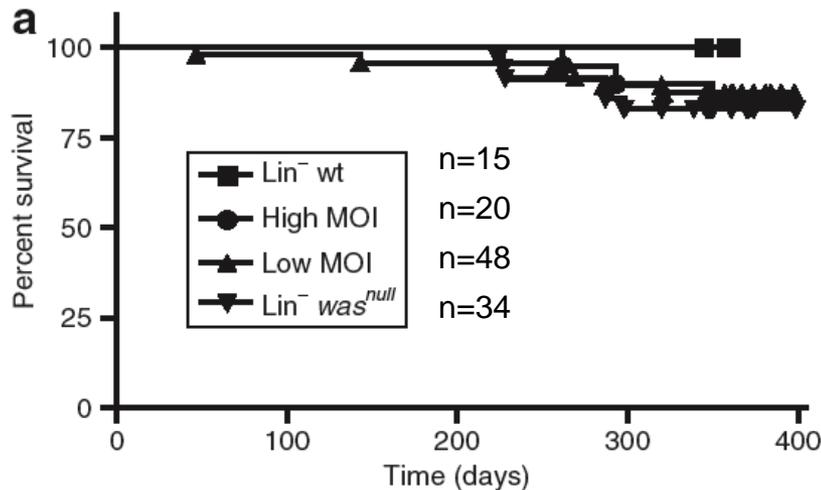
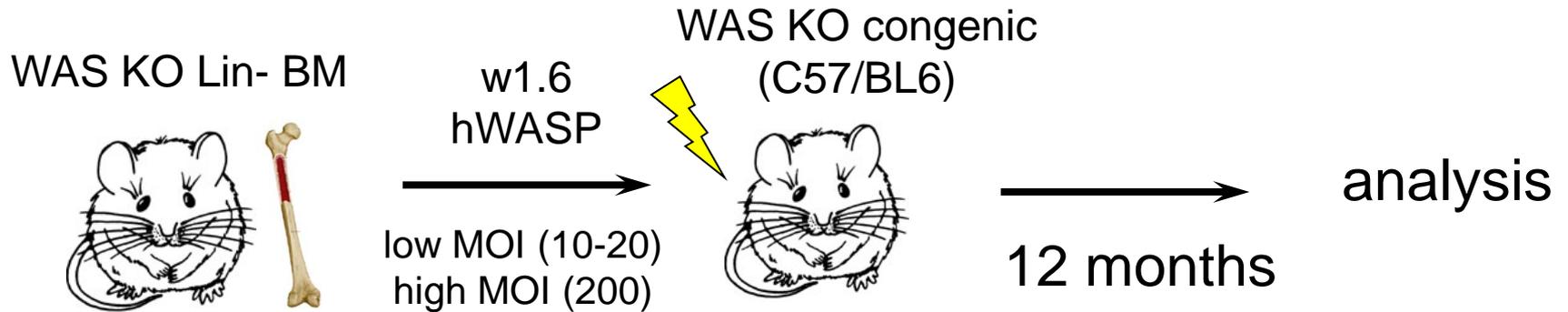


# Preclinical safety: In vivo Studies

Safety was investigated in mouse models:

1. Primary engraftment in C57/BL6 for long-term safety (12-16 months)
2. Secondary engraftment in 129Sv for genotoxicity (primary 4 months, secondary 6 months)

# Preclinical safety (Primary recipients)



In 68 vector-treated mice, only 4 lymphomas were found, all HOST

**No donor-derived tumors were found.**

# Preclinical safety (Secondary recipients)



From each group (WT, WAS KO, WA 1x, WA 2x), 11-16 secondary engrafted animals were created

$2 \times 10^6$  unfractionated BM per mouse to maximize expansion and detection of clonal dominance

Of 32 secondary recipients, 3 host tumors arose.

**No donor-derived tumors were seen.**

# Preclinical efficacy

## In WAS cells

\* **Expression in B-LCL and CD34+ in vitro (flow)**

\* **Expression in CD34+ in vitro (Western)**

Expression in DC in vitro (q-RT-PCR)

Functional reconstitution in vitro

T cells proliferation and IL2 production

Dendritic cells cytoskeletal dynamics

## Functional reconstitution in vivo in WKO mice

\* **T cell proliferation**

\* **T cell cytokine production**

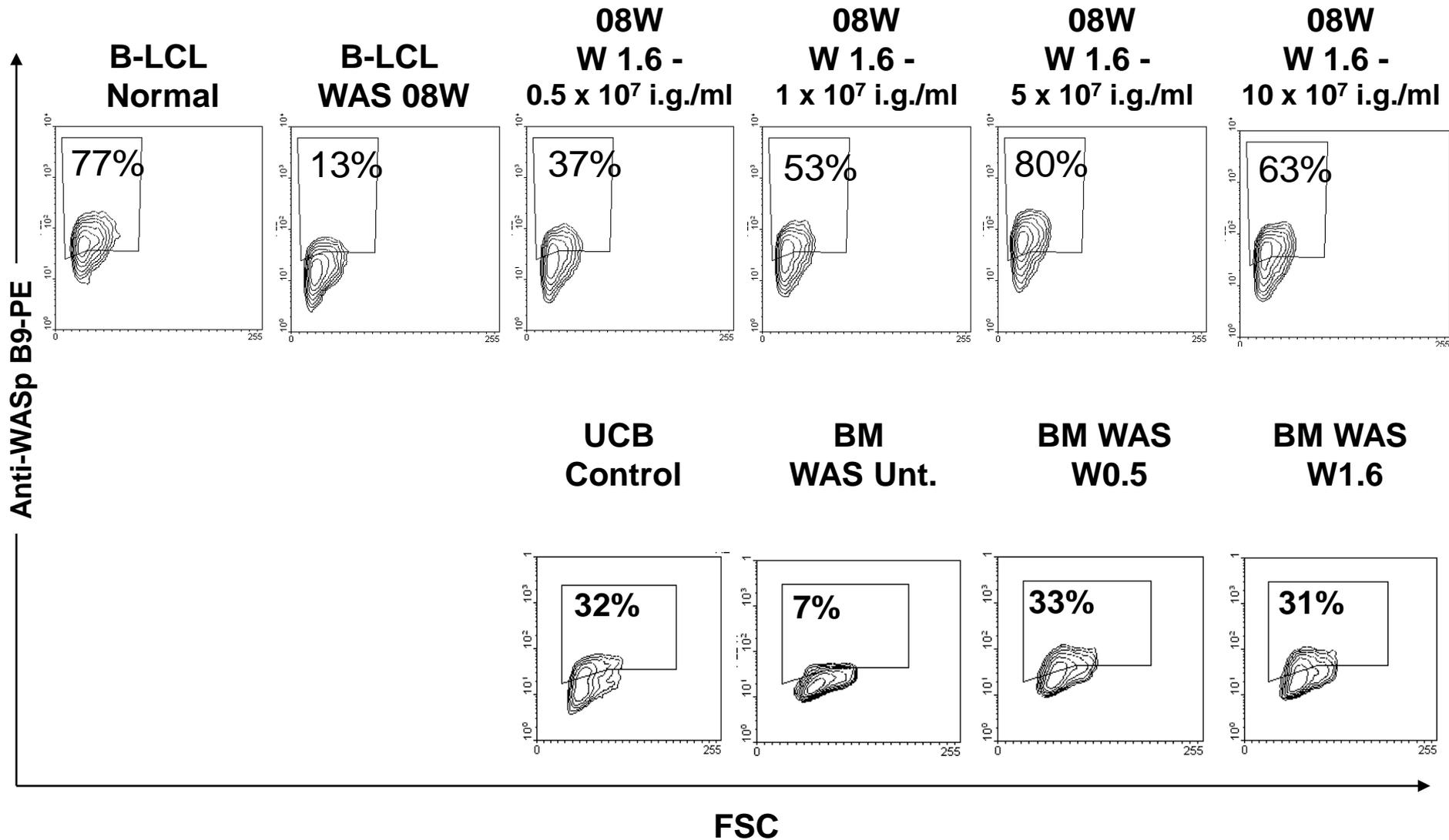
Dendritic cell podosomes

\* **B cell migration to CXCL13**

Antibody production to pneumococcus

\* **Amelioration of colitis**

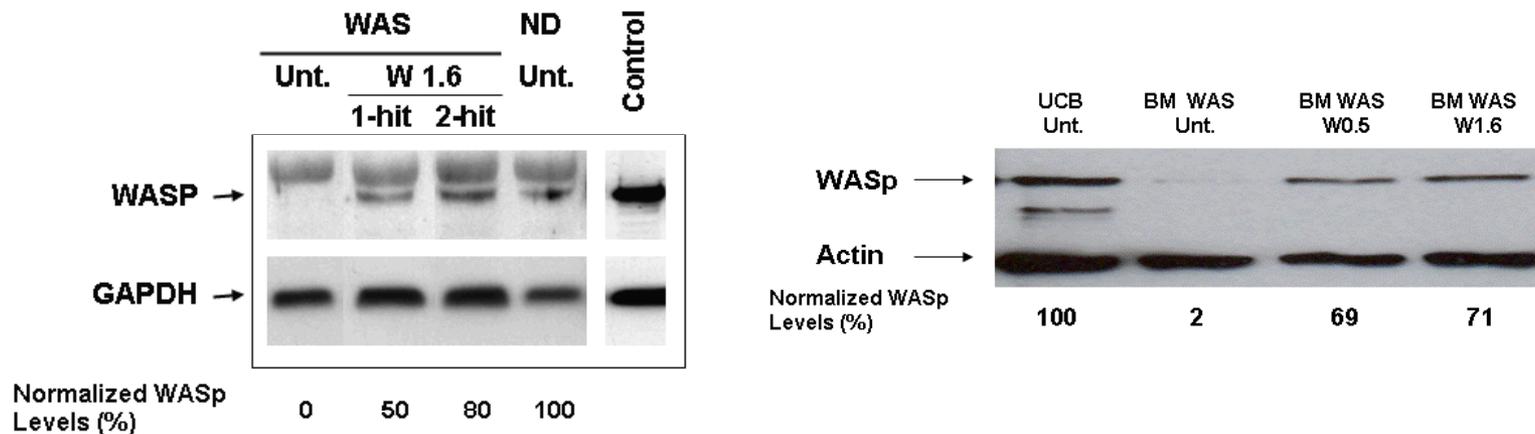
# Expression by flow in B-LCL and CD34+ cells



# Expression by Western blot in BM CD34+ cells

Figure 7

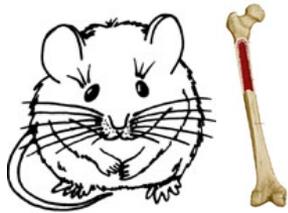
A



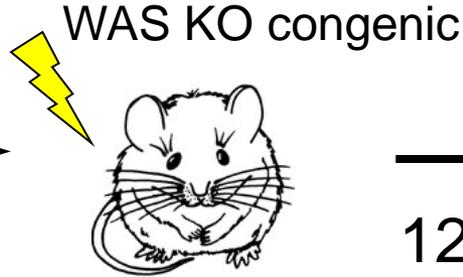
Conclusion: WAS transgene protein expression from the w1.6 vector results in 50-80% expression of WAS protein in CD34 cells by flow and by immunoblot

# *In vivo* functional reconstitution experiments T cell proliferation

WAS KO Lin<sup>-</sup> BM

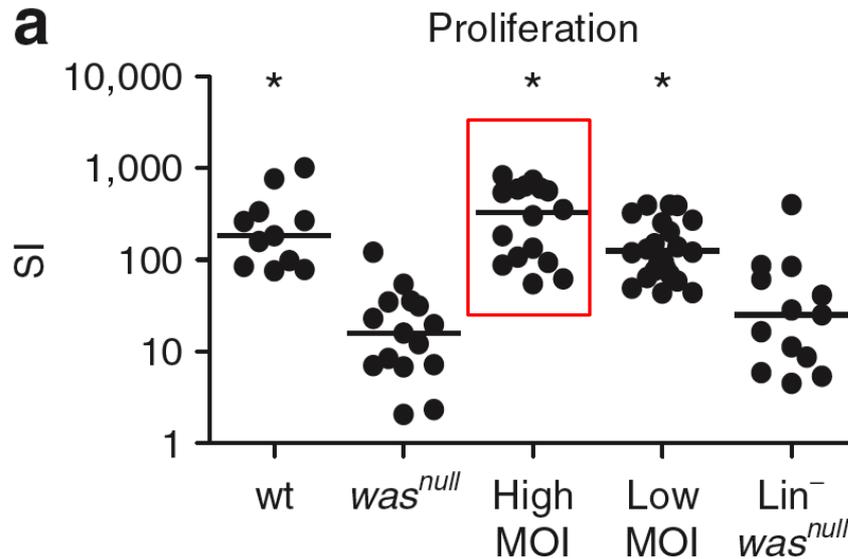


w1.6  
hWASP  
low = 10-20  
high = 200

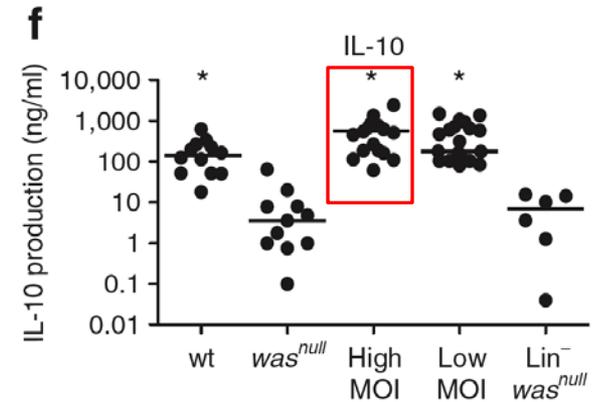
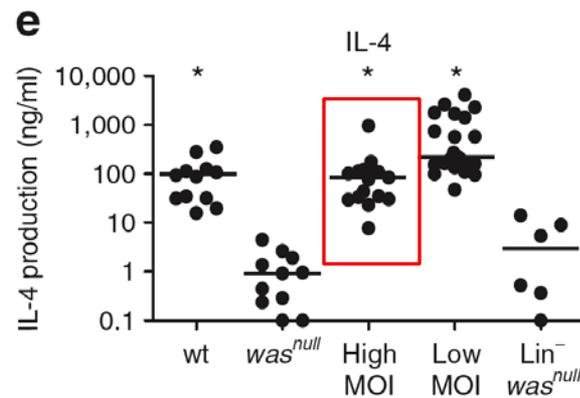
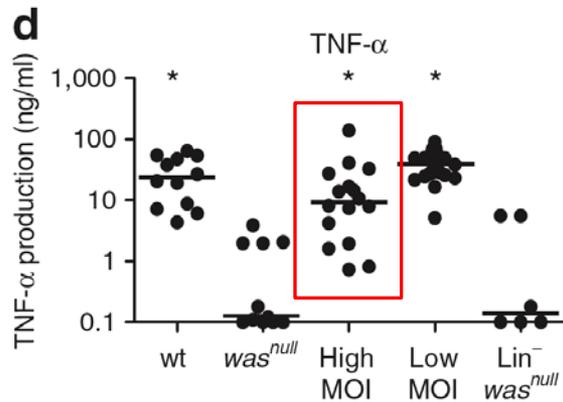
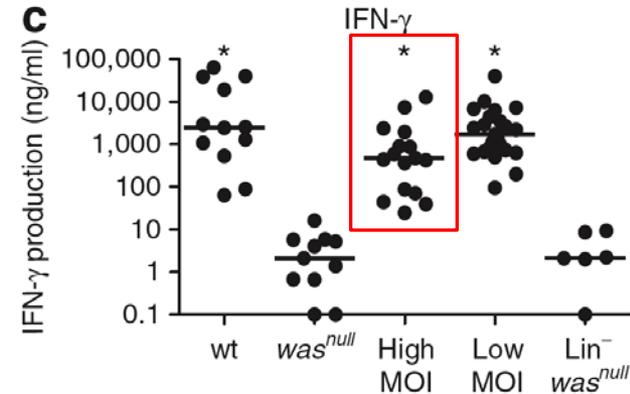
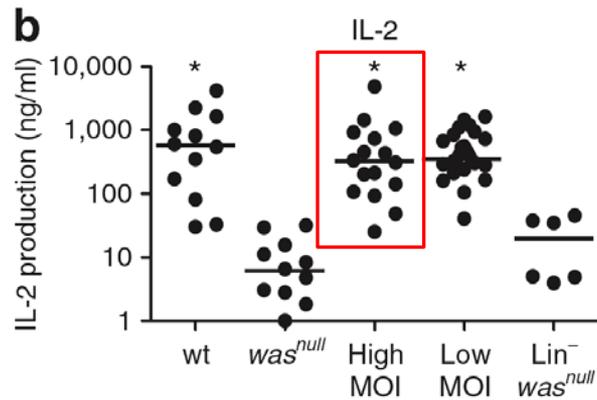


12 months

analysis



# *In vivo* functional reconstitution experiments Th1/Th2 cytokine production

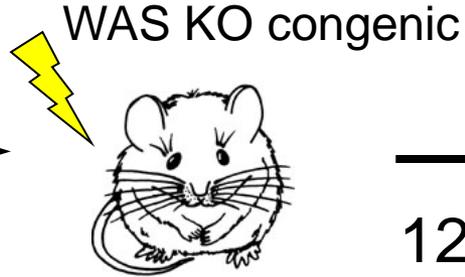


# *In vivo* functional reconstitution experiments B cell migration

WAS KO Lin- BM

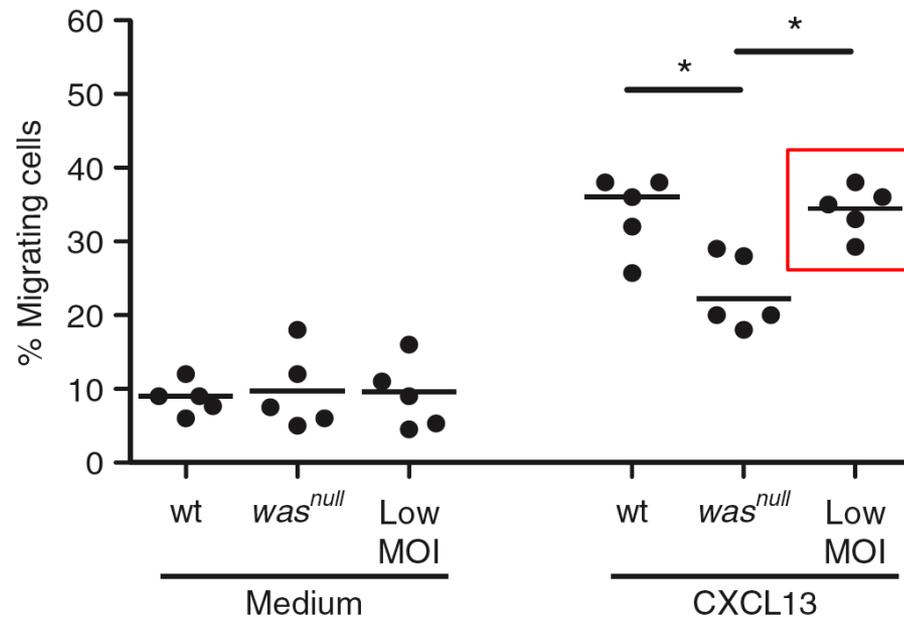


w1.6  
hWASP  
low = 10-20



12 months

analysis



# In vivo functional reconstitution experiments Amelioration of colitis

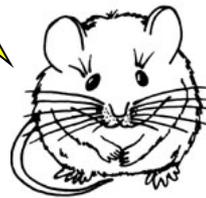
129/Sv WAS KO  
Lin- BM



w1.6  
hWASP



129/Sv WAS ♀ KO



4-8.5 mos

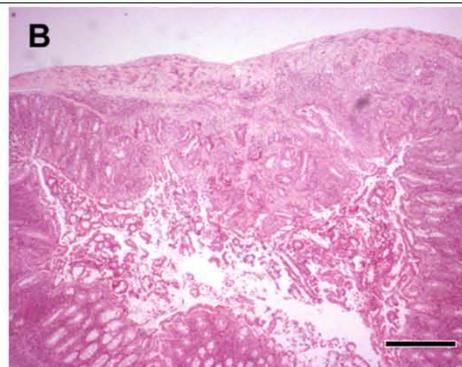
analysis

Lin- WT  
GFP-LV

Lin- WKO  
GFP-LV

Lin- WKO  
WAS-LV

Lin- WKO  
WAS-LV mut6



# Review of the clinical protocol

- ❖ Accrual
- ❖ Objectives & Endpoints
- ❖ Study Population
- ❖ Treatment Schema

# Accrual

The 5 subjects will be enrolled over 5 years.

10 subjects will be enrolled in parallel trials using the same vector in Paris, France and London, UK.

European trials will not be part of the IND for this trial.

# Objectives

## Primary objectives:

1. To safely administer a lentiviral gene therapy vector encoding the human WAS cDNA in patients with WAS
2. To achieve engraftment of WASP-expressing transduced T cells

## Secondary objectives:

1. Sustained engraftment of WASP-expressing transduced cells
2. Clinical effect in terms of augmented immunity and reduction in bleeding
3. Molecular characterization of gene transfer

# Endpoints

Primary endpoints:

1. Safety of infusion of transduced cells as rescue of hematopoiesis after conditioning (hematopoietic recovery as assessed by absolute neutrophil count (ANC) above  $0.5 \times 10^9/L$  for three consecutive days, achieved within 6 weeks post-infusion
2. Engraftment of genetically corrected T cells in peripheral blood (as assessed by evidence of vector sequences in  $>1\%$  of CD3+ T cells) at 6 months.

# Endpoints

## Secondary and Exploratory endpoints

Lineage specific gene marking and WAS protein expression

Immunologic reconstitution

Total and specific IgG production

T cell proliferation to mitogen, anti-CD3 and antigen

Correction of thrombocytopenia

Molecular analysis

Copy number

Lineage specific insertional analysis

T cell clonality

Clinical follow-up

Exploratory (TREC and extended phenotyping by flow cytometry)

# Subject eligibility

## Eligibility criteria:

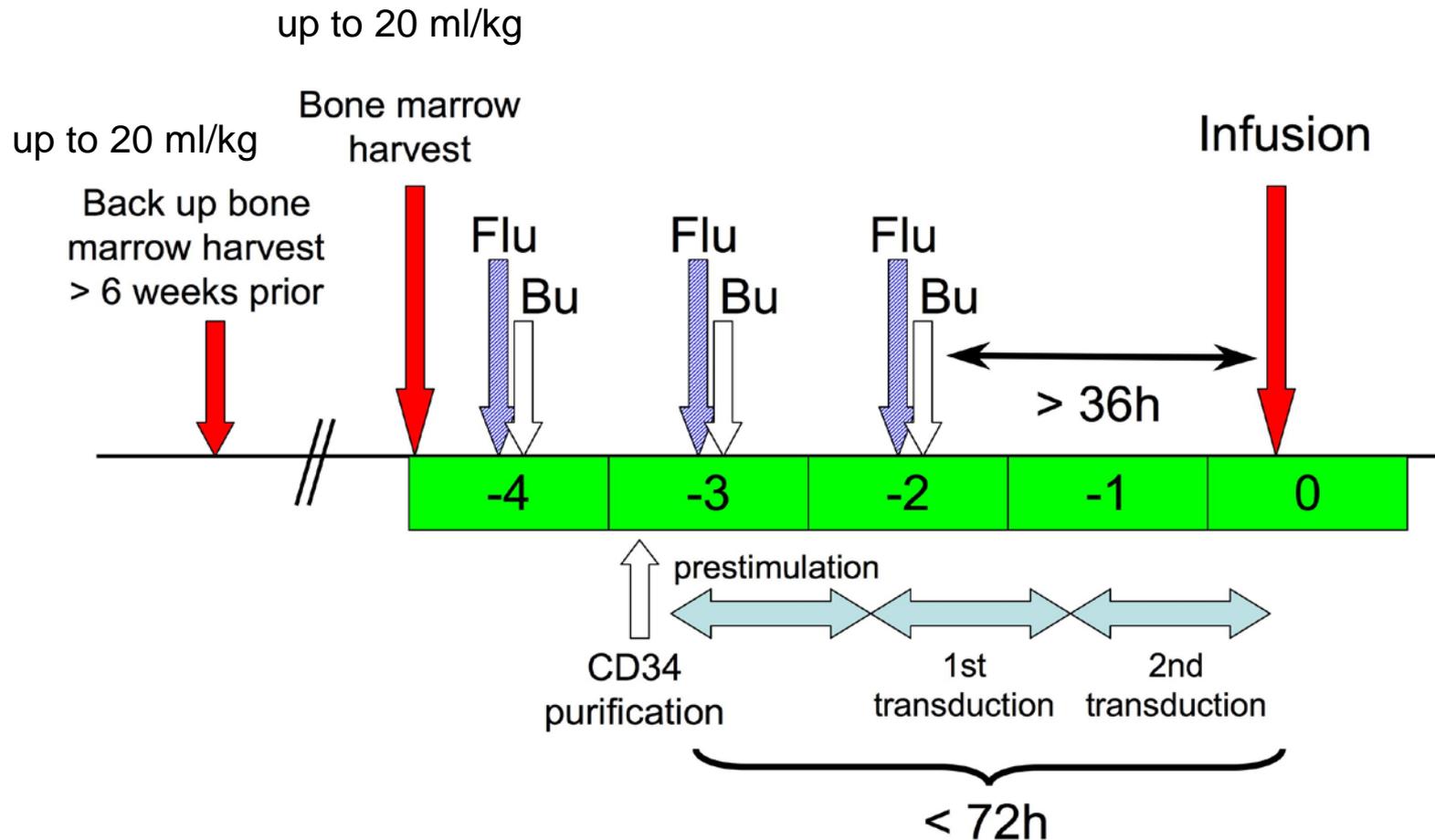
1. Confirmed molecular diagnosis by DNA sequencing and either
  - a. absence of the WAS protein by flow cytometry OR
  - b. clinical score 3-5
  
2. For subjects <5 years of age:
  - a. Lack of HLA genotypically identical bone marrow donor
  
  - b. Lack of a 9/10 or 10/10 molecularly HLA-matched unrelated donor after 3 months of searching.
  
  - c. Lack of a 6/6 molecularly HLA-matched cord blood donor\* of adequate cell number after 3 months of searching
  
3. For subjects 5 years of age or older
  - a. Lack of HLA-genotypically identical bone marrow donor

# Subject eligibility

## Eligibility criteria:

4. Subjects who have undergone allogeneic transplant previously must additionally have:
  - a. Failure defined as <5% donor T cell engraftment and
  - b. Contraindication to re-use the same donor due to severe GVHD or non-availability
5. Parental/guardian/patient signed informed consent
6. Willingness to return for follow-up during the 5 year study period

# Treatment schema\*



fludarabine  $40 \text{ mg/m}^2 \times 3 = 120 \text{ mg/m}^2$   
busulfan  $4 \text{ mg/kg} \times 3 = 12 \text{ mg/kg}$

\*approved by MHRA in UK

# Responses to RAC review questions

- Preclinical in vitro and in vivo data
- Enrollment and eligibility
- Transduction and clinical protocol
- Trial management
- Informed consent

# Responses to RAC review questions

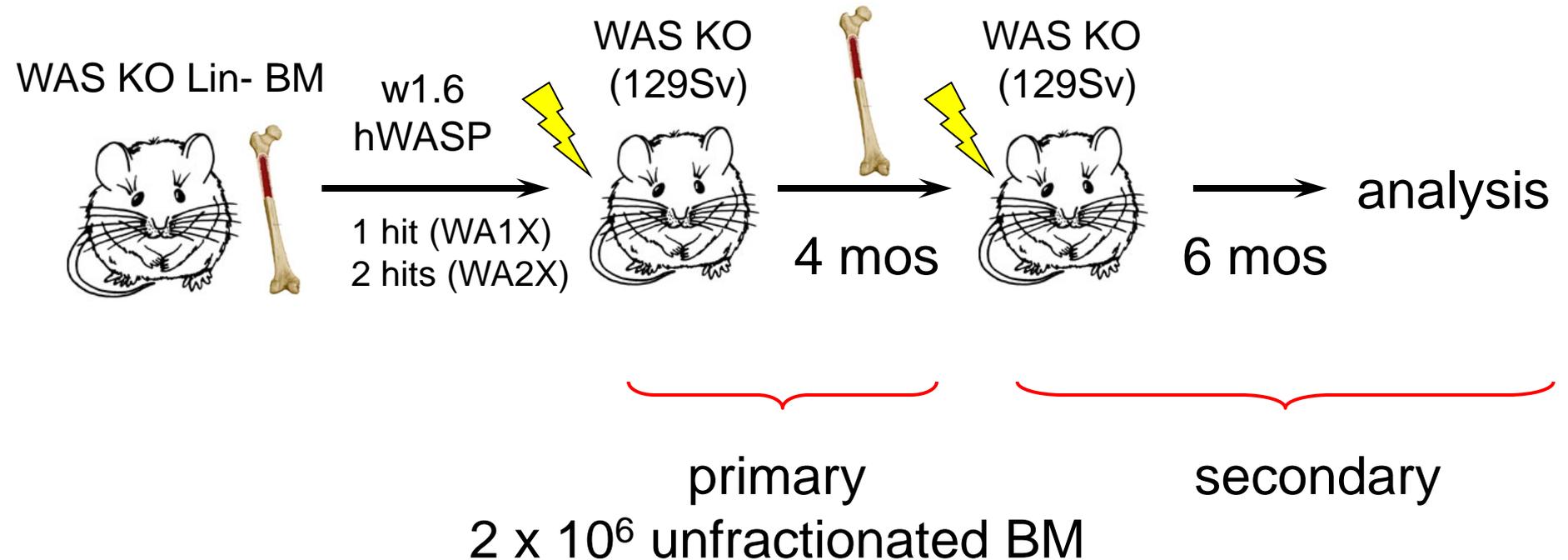
- Preclinical in vitro and in vivo data
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*The data provided in the updated letter (Table I; taken from Marangoni and Charrier, 2009) show only modest improvements in B cell, platelet and granulocyte numbers, but no effect on T cells in vector transduced-HSCT-engrafted mice...[Are] mice not a good model for testing the efficacy of the therapy?*

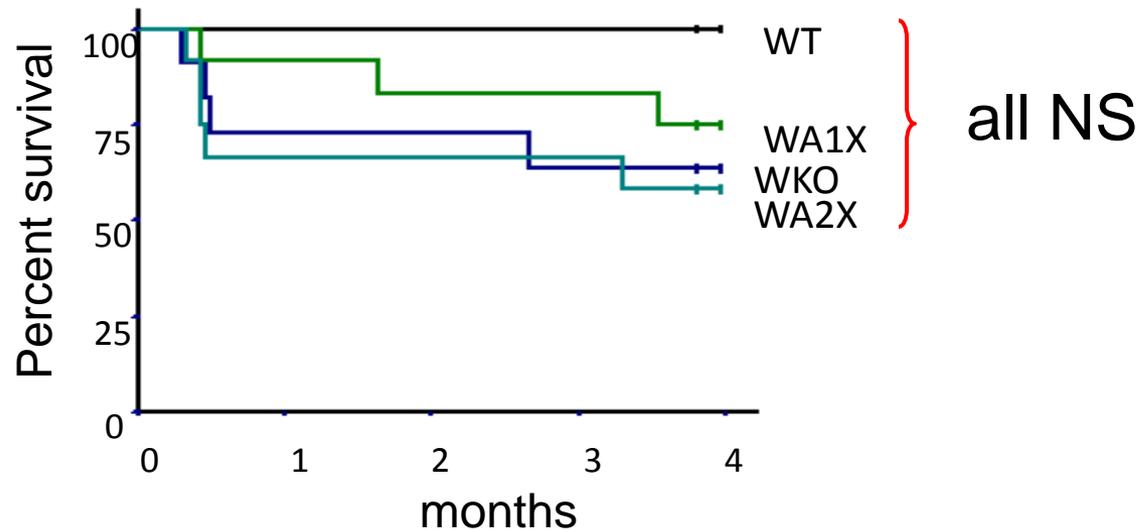
Defect in humans	Reference	Present in mouse model	Corrected in mouse model	Reference
T cell proliferation	Molina 1993	Yes	Yes	Marangoni 2009
Th1/Th2 cytokine production	Molina 1993	Yes	Yes	Marangoni 2009
B cell migration	Westerberg 2005	Yes	Yes	Marangoni 2009
Antibody to pneumococcus	Ochs 1980	Yes	Yes	Bosticardi ASGT 2009
DC podosome formation	Burns 2001	Yes	Yes	Zanta-Boussif 2009

Mice are good model for functional reconstitution after GT.

# Questions on mouse studies (Secondary transplants for genotoxicity analysis)



*No Kaplan-Meier curves are presented for primary recipients and the endpoints for the secondary graft protocols were not different from mice that received untransduced grafts.*



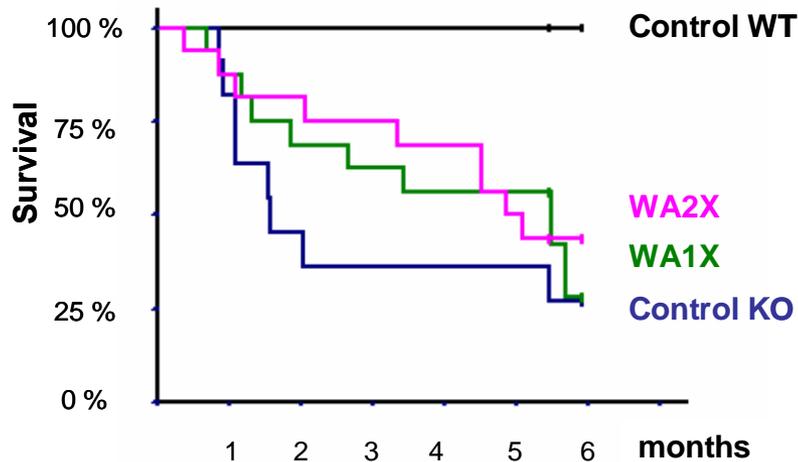
Primary recipients were sacrificed at 4 months to generate secondary recipients for genotoxicity studies. Therefore the Kaplan-Meier curves are not indicative of any effect of gene therapy.

*Additionally, the western blots of the bone marrow extracts [from secondary transplant recipients] were not entirely convincing -- there were bands in the control KO animal and it looked like only 1/3 animals that received the WASp transgene expressed the gene (p. 48).*

We agree with the reviewer that these particular blots were not optimally described or presented. Control KO animals do have a non-specific band that is also present in mouse S20, which is included twice in this blot. Therefore in this blot only mouse S19 is positive.

Of 8 secondary recipient 1X mice assayed, 6/8 had expression by Western blot (75%).

*The investigators state that there was poorer engraftment in the WA 2x-engrafted mice and yet they have slightly better survival (although not statistically significant).*



The differences in survival are not significant.

This experiment, was designed to elicit genotoxicity. The poor engraftment of gene modified cells in some of the mice in the WA 2x arm was discovered in retrospect.

The study was not designed to examine efficacy in this model, which was demonstrated instead in the previously presented primary transplant experiments.

# Responses to RAC review questions

- Preclinical in vitro and in vivo data
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*How likely is it that a patient with expression of endogenous WAS protein will be included in this study?*

Most patients enrolled are anticipated to be WAS protein null.

Report of genotype, phenotype and clinical score on 227 WAS/XLT families with 262 affected members, 248 with clinical score available, of those 179 had protein analysis

Score	Total	WAS protein neg	WAS protein pos
3	21	17	4
4	27	23	4
5	37	32	5

Only 15% of 85 patients with score 3-5 were protein positive.  
~1 of 5 enrolled would have endogenous WAS protein.

# Eligibility issues with regard to genotype and phenotype

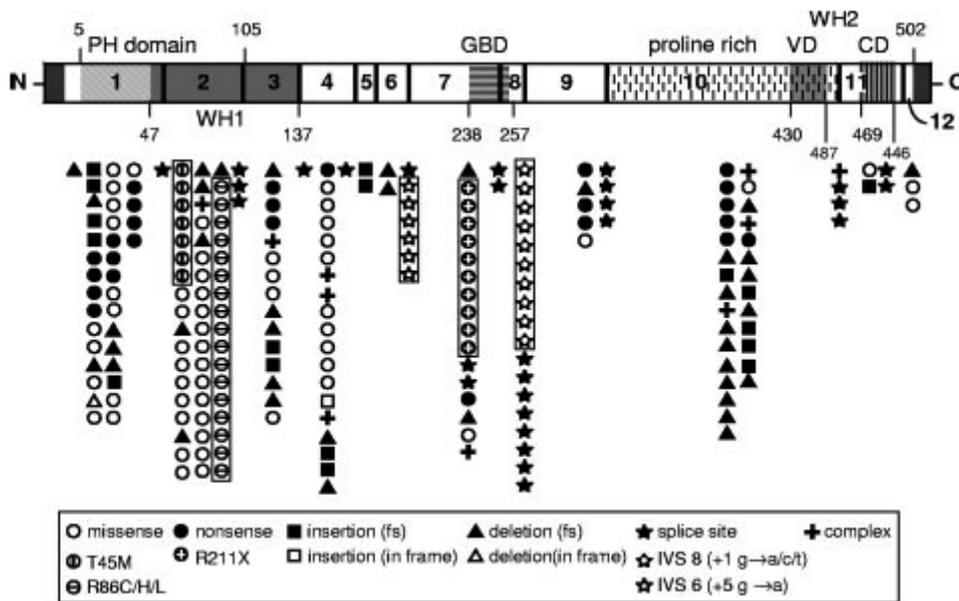
*Will the investigators perform genotyping and WAS protein expression analysis on patients prior to their inclusion in the trial or will only clinical criteria be used?*

Yes, genotyping and WAS protein expression analysis will be performed on all potential subjects.

Patients with high clinical score who express WAS protein should be included in the trial, especially those with the more common missense genotypes in exons 1 and 2.

*Are the investigators concerned that the transgene expression will downregulate endogenous protein expression in these patients?*

## WIP binding



Many exon 1 & 2 mutations disrupt WIP-WASP interaction

These mutants are expressed but are unstable and degraded

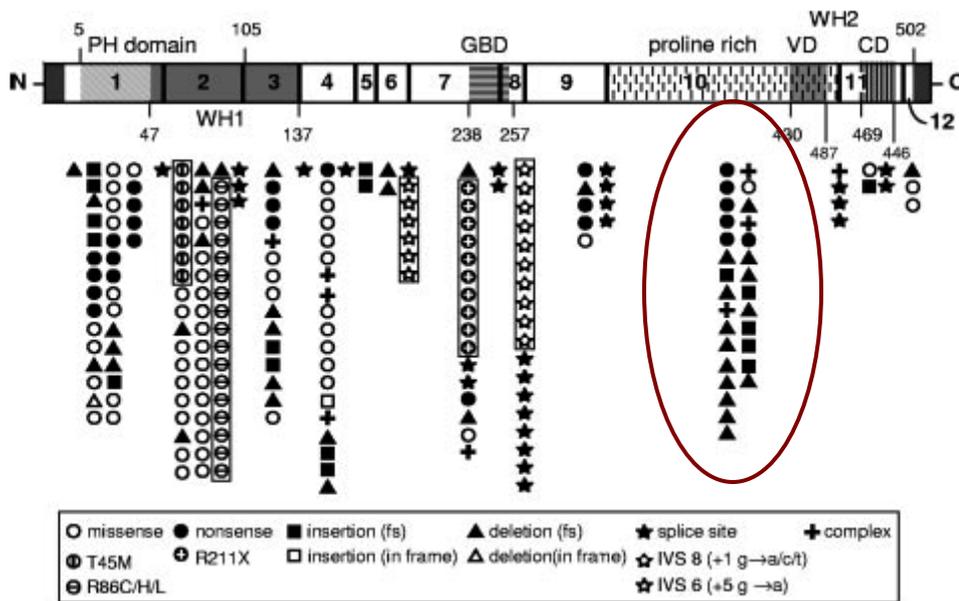
Transgenic wild-type protein could compete for WIP and endogenous (mutant) protein levels would fall

This would be advantageous to wild-type protein, and desired.

Thus we predict that these patients would benefit from the protocol.

*Is there any evidence for a dominant-negative effect of truncated or mutant WAS protein that would preclude the inclusion of patients with endogenous WAS expression?*

## VCA region



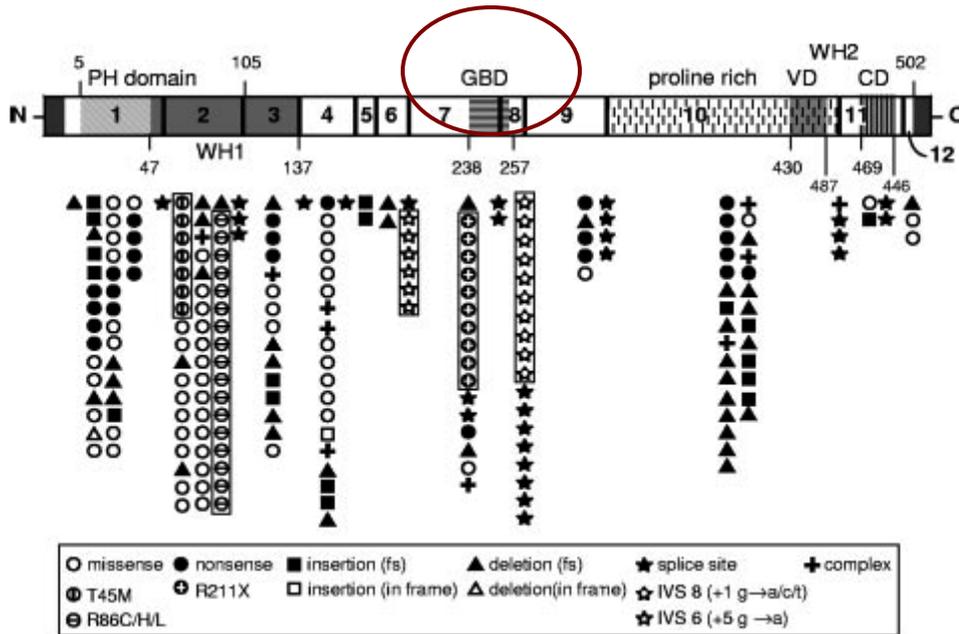
Mutants truncating the VCA region (aa423-502) have DN activity in mice and murine T cells in vitro

WAS pts with ins/del in exon 10 can have frameshifts resulting in similar truncations

Less than half of VCA deletion patients express protein (6/14)

WAS patients expressing potentially DN mutants may exist and are expected to be rare

*Is there any evidence for a dominant-negative effect of truncated or mutant WAS protein that would preclude the inclusion of patients with endogenous WAS expression?*



Devriendt et al Nat Genet 2001,  
Jin et al Blood 2004, Ancliff et al  
Blood 2006

Patients with activating point mutations in the GTPase binding domain (GBD) have been described in which mutant WAS has dominant activity

Such patients would generally not qualify as they do not have a WAS clinical phenotype

We will exclude these patients

*Please provide justification for the criteria wherein availability of a 9/10 adult donor excludes participation, but a 5/6 cord blood unit of sufficient cell dosage does not.*

We do not consider a 5/6 cord blood transplant to be equivalent in risk to a 9/10 adult donor transplant for :

1. the limited published experience for cord blood in WAS
  - primarily case reports
  - large surveys have 0-3 patients reported respectively

Author	Year	Number of transplants	Number of cord blood transplants
Filipovich	2001	170	0
Antoine	2003	1082 (SCID and nonSCID)	8 (0.7%)
Ozsahin	2008	96	3

*Please provide justification for the criteria wherein availability of a 9/10 adult donor excludes participation, but a 5/6 cord blood unit of sufficient cell dosage does not.*

We do not consider a 5/6 cord blood transplant to be equivalent in risk to a 9/10 adult donor transplant for :

2. the far greater experience with mismatched unrelated donor BMT for WAS, with equivalent survival for 9/10 vs. 10/10

Survey of 187 transplants for WAS with median f/u 57 months

60 fully matched unrelated donor recipients

51/60 (85% alive)

18 1-antigen mismatched unrelated donor recipients

17/18 (94% alive)

*Please provide justification for the criteria wherein availability of a 9/10 adult donor excludes participation, but a 5/6 cord blood unit of sufficient cell dosage does not.*

We do not consider a 5/6 cord blood transplant to be equivalent in risk to a 9/10 adult donor transplant for :

3. the high rate of viral reactivation after cord blood transplant, particularly problematic for immunocompromised WAS patients

COBLT study: prospective analysis of 191 patients with pediatric hematologic malignancy undergoing cord blood transplantation

severe life-threatening or fatal infection	N (%)	Bacterial	Fungal	Viral
at least 1	166 (87%)	77%	33%	61%
2 or more infections	77% 762 episodes	48%	11%	34%

# Responses to RAC review questions

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*Is there precedent for this [conditioning] regimen and does it pose an increased risk for incomplete cytoablation and possible persistence of autoreactive cells?*

Results in previous trials using similar regimens:

41/41 CML recipients were 100% fully donor after receiving 120 mg/m<sup>2</sup> of fludarabine

In a survey of 187 WAS patients transplanted, 11 received 120-160 mg/m<sup>2</sup> of fludarabine in combination with busulfan/treosulfan.

- Only 1 patient of 11 failed to engraft (the sole recipient of haploidentical transplant)
- The remaining 10 had 76-100% donor T cell engraftment

Thus we believe this regimen will result in cytoablation in the majority of cases.

*Is it necessary to collect 20 ml/kg of unprocessed autologous marrow for a back-up or would a lower limit of this (e.g. 10 ml/kg) be sufficient for an adequate back-up and increase the yield of the second harvest allowing more subjects to proceed to gene transfer?*

Our goal in collecting up to 20 ml/kg is to ensure the back-up is adequate for reconstitution of hematopoiesis in case of failure of engraftment with gene corrected cells.

To ensure recovery of hematopoiesis between harvests we have required additional time (at least 6 weeks) compared to standard NMDP guidelines (4 weeks).

In younger recipients, < 20 ml/kg may be collected.

*The investigators state “The risk of secondary malignancy as a result of conditioning is likely to be very low based on experience with these regimens in non-malignant HSCT for inherited disease” (p. 16, also on p. 33). Are there any published data that can be used to support this statement?*

Disease	n	malignancies	ref
WAS	96	0	Ozsahin Blood 2008
WAS	170	1 AML	Filipovich Blood 2001
SCID	90	2 AML (reticular dysgenesis)	Neven Blood 2009
WAS	187	0 MDS or AML, 1 lymphoma	manuscript in preparation

The survey data above show the risk of secondary malignancy in WAS or other immunodeficiency patients treated with similar conditioning to be low.

*Given the strong need for correction in more than just T cells, why is engraftment in other compartments a secondary endpoint?*

In this pilot and feasibility study we restricted the primary endpoints as much as possible to safety endpoints.

Reasons to consider T cell engraftment primary:

1. Patients with severe T cell related complications (autoimmunity, malignancy, severe infection) are targeted
2. Even in setting of mixed/split chimerism after BMT, low myeloid reconstitution and low platelets can be addressed with other therapies (i.e. splenectomy)

# Responses to RAC review questions

- Preclinical in vitro and in vivo data
- Enrollment and eligibility
- Transduction and clinical protocol
- **Trial management**
- Informed consent

*No mention was made of how the parallel trials in Europe and this one in the US using the same vector and basic approach will cross-inform about Serious Adverse Events, such as insertional oncogenesis events. While the regulatory oversight will be done separately in each country, such sentinel events in any trial should be reported to the Sponsors and by them to their oversight entities.*

The Sponsor in the US (Dr. Williams) and Sponsor in Europe (Genethon) will cross-inform each other.

Each sponsor will directly inform the other of SAE and SUSAR determined to be probably or definitely related to the procedure.

Annual safety reports generated for regulatory agencies in one continent will be transmitted to the other sponsor.

# Responses to RAC review questions

- Preclinical in vitro and in vivo data
- Enrollment and eligibility
- Transduction and clinical protocol
- Trial management
- Informed consent

*There is no mention that this is a phase I trial or that it is first-in-human research.*

*The consent form should inform potential subjects about the results from the German trial of retroviral-mediated gene transfer for WAS, which are of direct relevance.*

*One risk that is not presented is that of partial immune reconstitution with resultant autoimmunity.*

This trial strictly speaking is not first-in-human as 10 pediatric patients with WAS have undergone gene transfer in Hannover Germany with a different vector, as reviewed in the introduction.

The consent has been modified to state that:

- 1) this is a pilot trial and what that means;
- 2) 10 patients with WAS have had gene transfer with a different vector;
- 3) this is the first trial in humans using this vector;
- 4) that partial reconstitution and autoimmunity is a risk