LMU

Hematopoietic stem cell gene therapy for patients with Wiskott-Aldrich Syndrome

Christoph Klein
Dr. von Hauner Children‘s Hospital
Ludwig-Maximilians University Munich

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Wiskott Aldrich Syndrom

Recurrent life-threatening infections

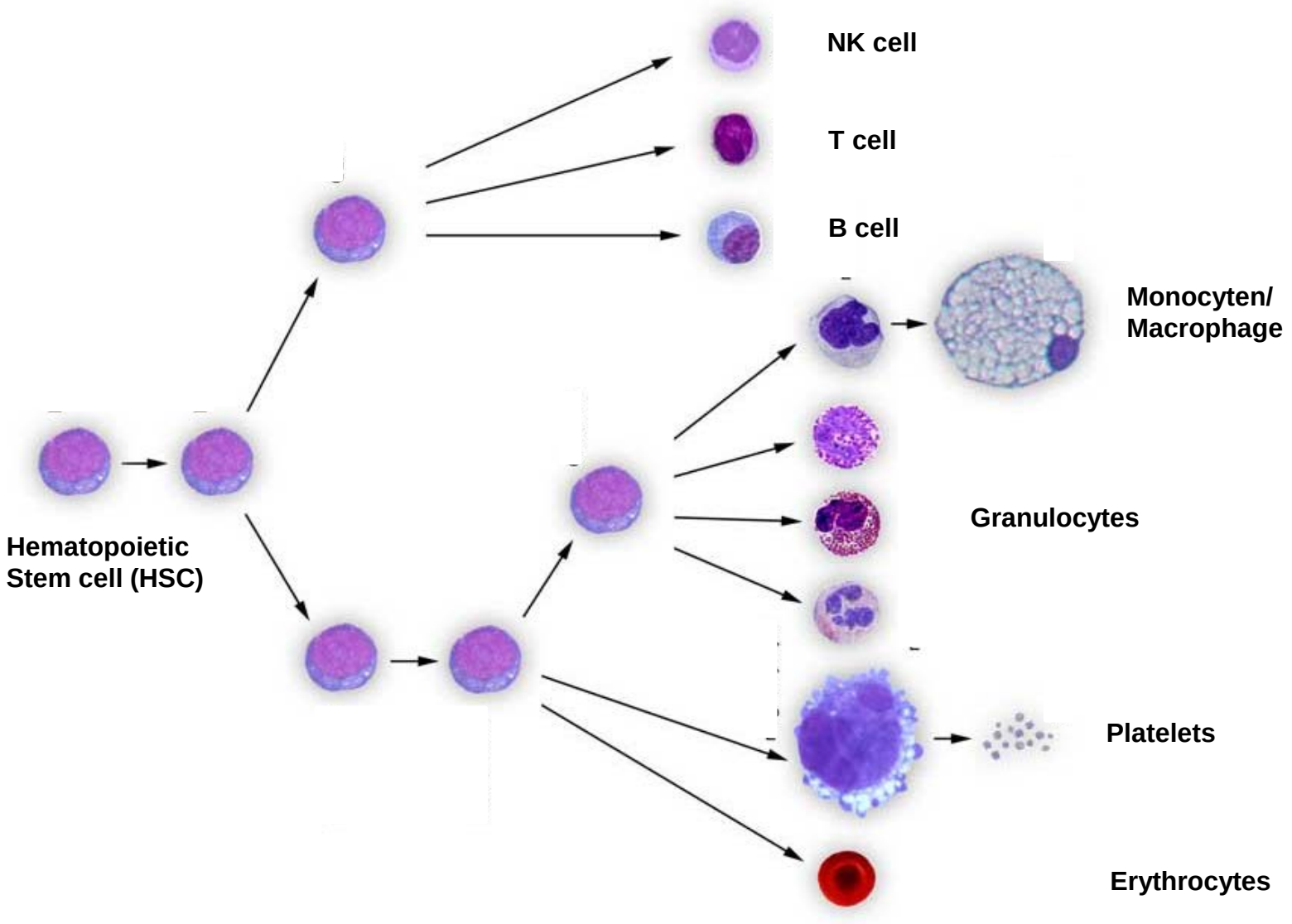
Autoimmunity

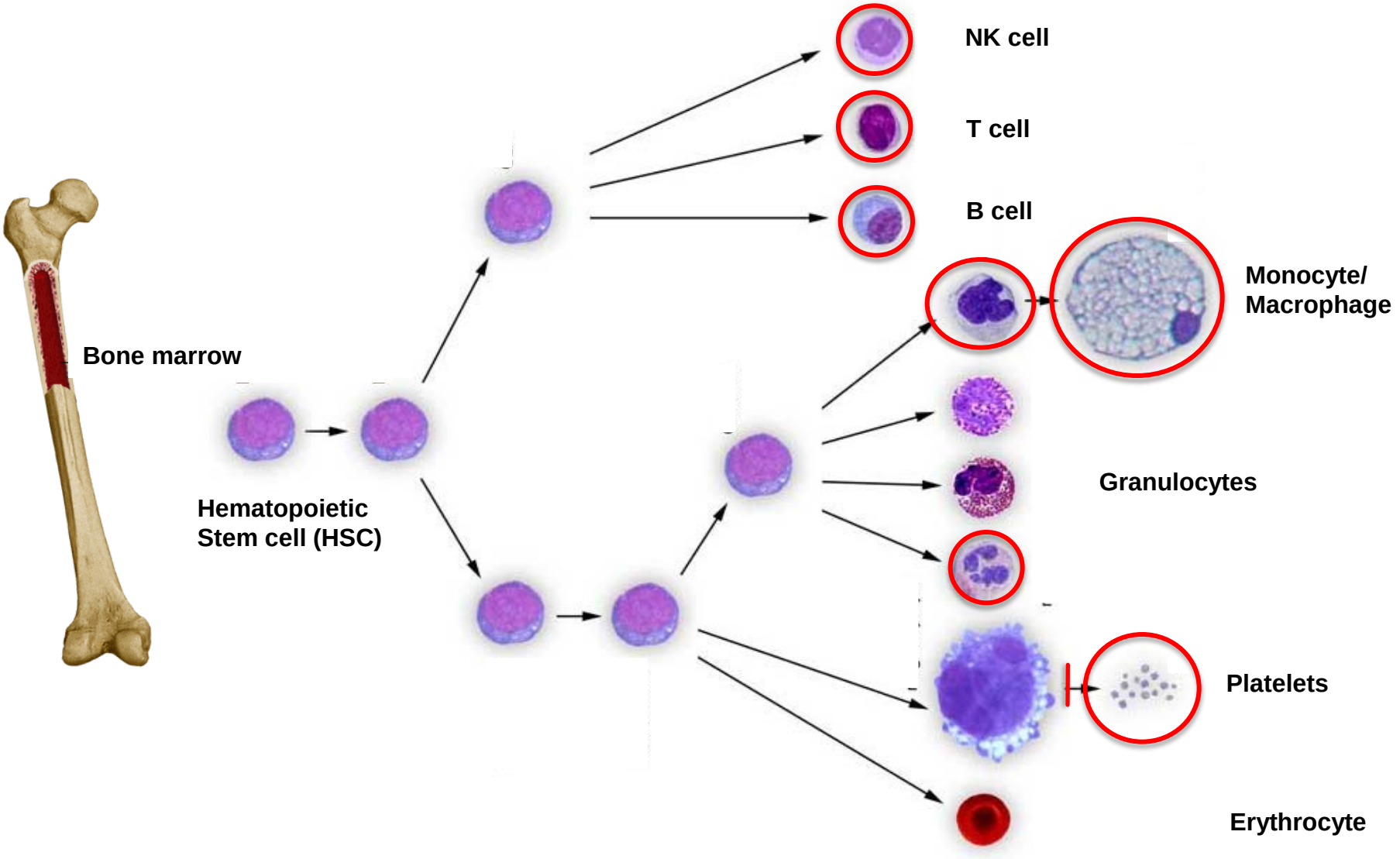
Eczema

Bleeding

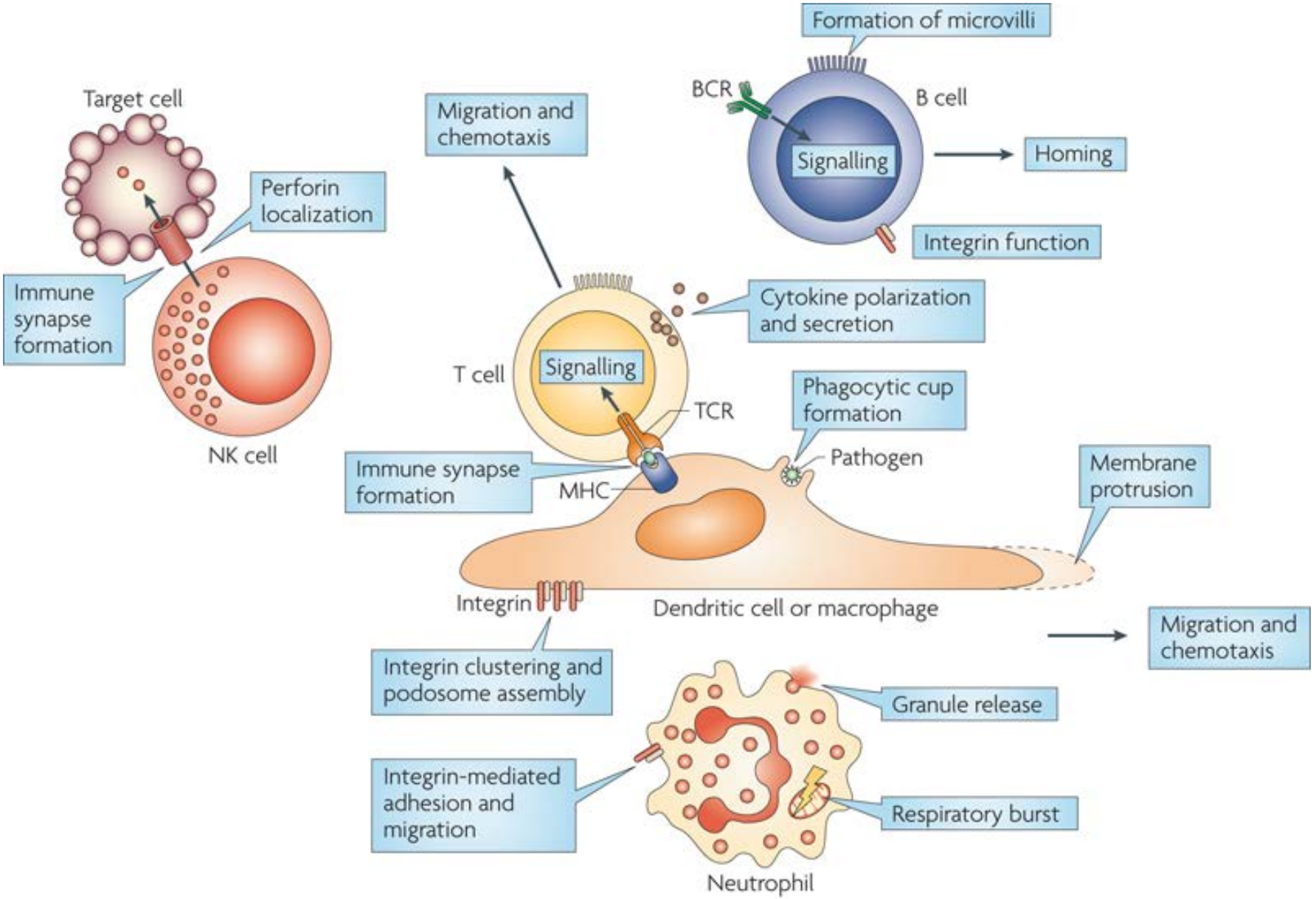
Leukemia/Lymphoma

Life expectancy <20 years


NK cell
T cell
B cell
Monocyten/
Macrophage
Granulocytes
Hematopoietic
Stem cell (HSC)
Platelets
Erythrocytes

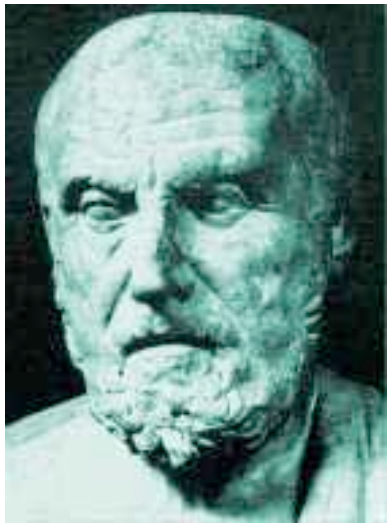

NK cell
T cell
B cell
Monocyte/
Macrophage
Bone marrow
Granulocytes
Hematopoietic
Stem cell (HSC)
Platelets
Erythrocyte

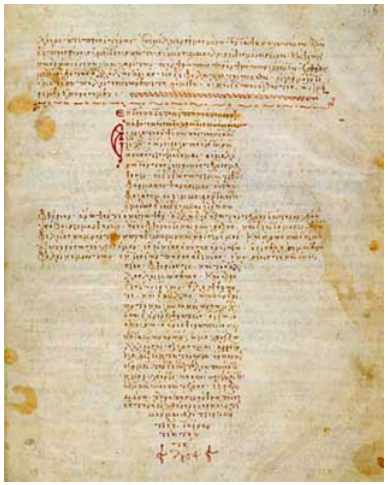
Wiskott-Aldrich Syndrom (WAS)



Thrasher and Burns , Nat Rev Immunol. 2010

Ethical Aspects





Risk-benefit analysis

Alternative therapeutic options

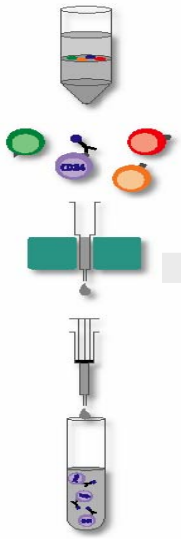
Experimental therapy in non-autonomous children

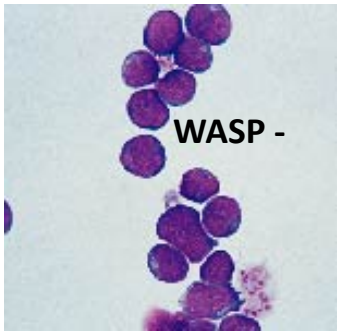
Clinical gene therapy protocol for WAS

Purification of HSC

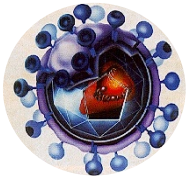
Transduction using
GALV-retroviral vectors

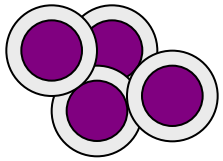
Busulfan
8 mg/kg



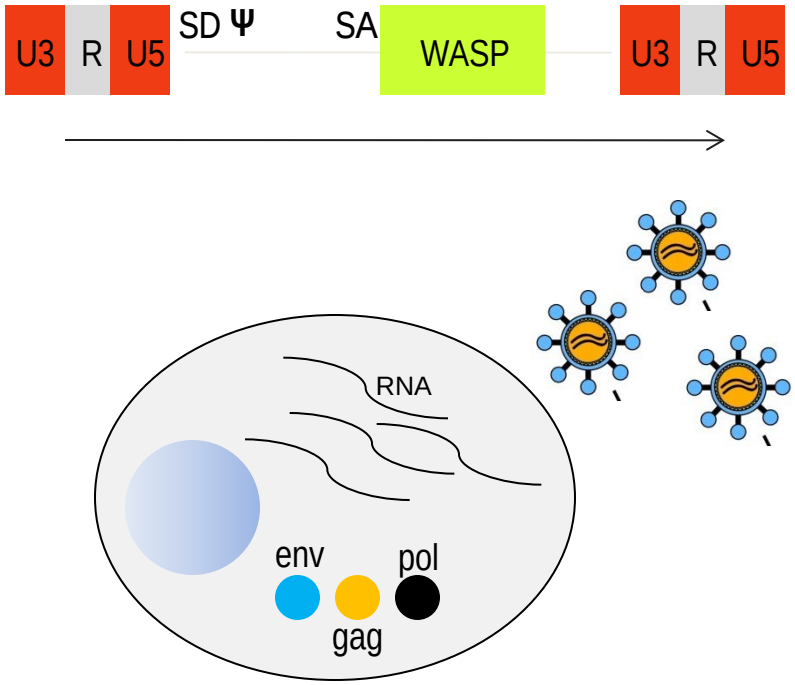


CMMP-WASP



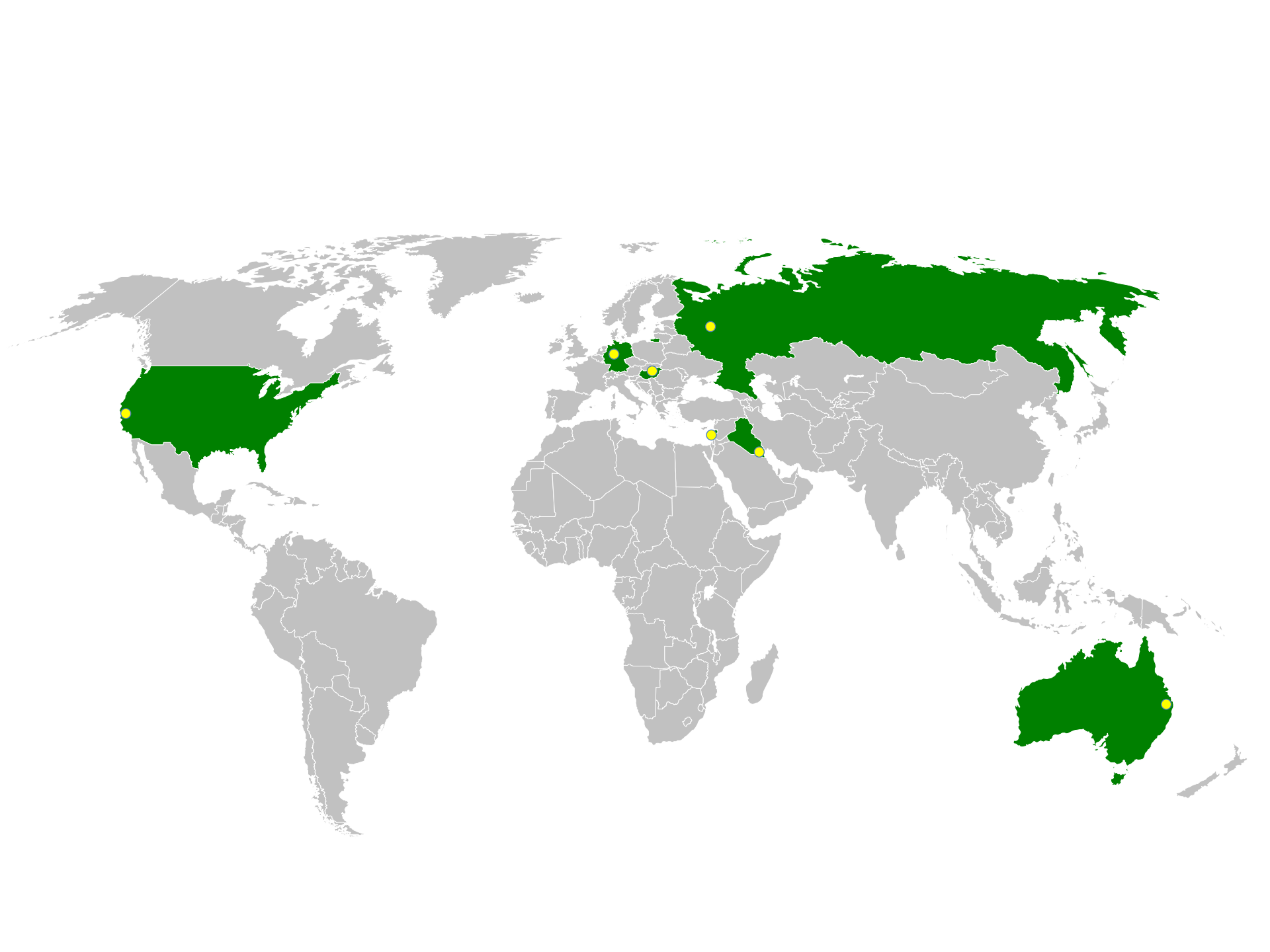


Retroviral vector production platform

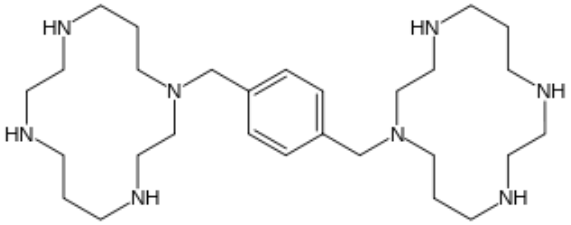


Synopsis of WAS patients treated by HSC gene therapy

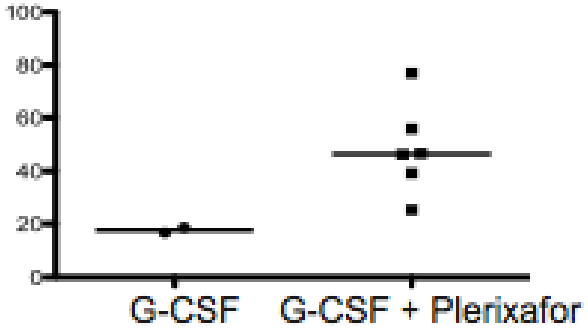
	Age	Mutation	CD34+ cells
WAS1	3 yrs	IVS6+1 G>T	10.0×10^6/kg
WAS2	3 yrs	Arg86His	11.1×10^6/kg
WAS3	3 yrs	Glu133His	2.5×10^6/kg
WAS4	4 yrs	Arg34X	31.6×10^6/kg
WAS5	12 yrs	Glu31Lys	20.9×10^6/kg
WAS6	4 yrs	Asp259fs	22×10^6/kg
WAS7	3 yrs	IVS3-1 G>T	14.8×10^6/kg
WAS8	3 yrs	Ala134Thr	20.9×10^6/kg
WAS9	2 yrs	Val303fsX4	25.5×10^6/kg
WAS10	14 yrs	His30del	11.4×10^6/kg



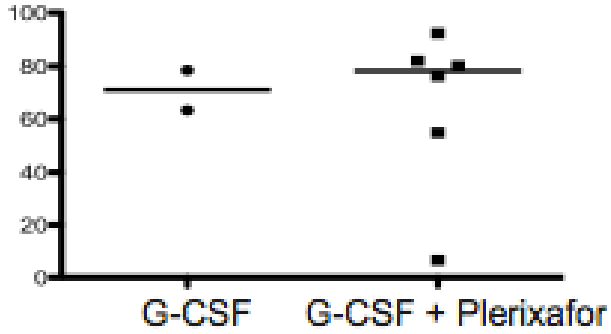
CXCR4 antagonist (Plerixafor®) facilitates myeloid engraftment



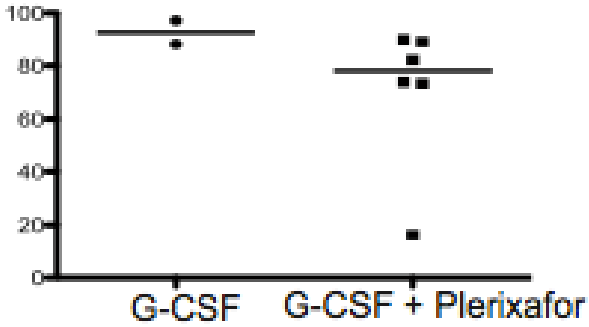
Monocytes



CD4 T cells

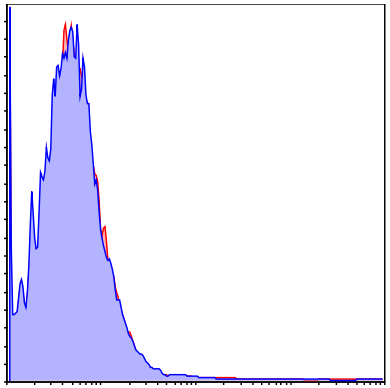


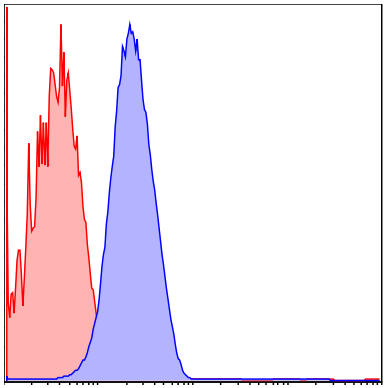
CD8 T cells

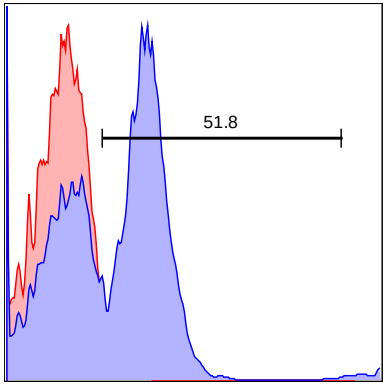


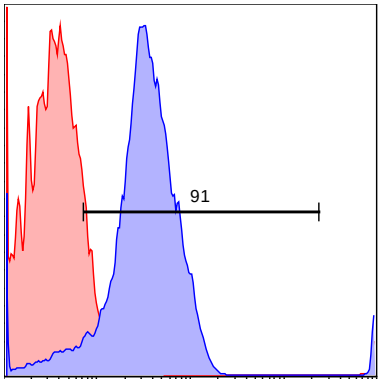
Expression of WASp in platelets

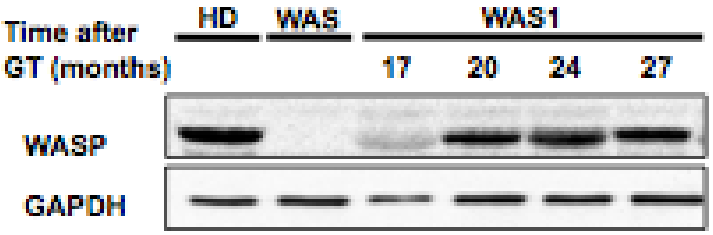
WAS | Healthy donor | WAS1 post GT | WAS2 post GT

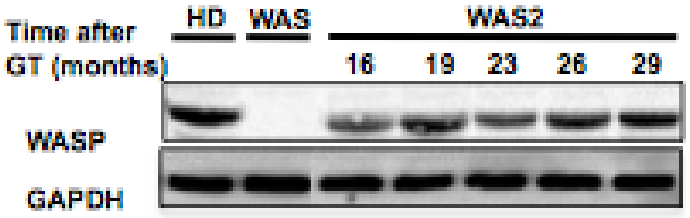




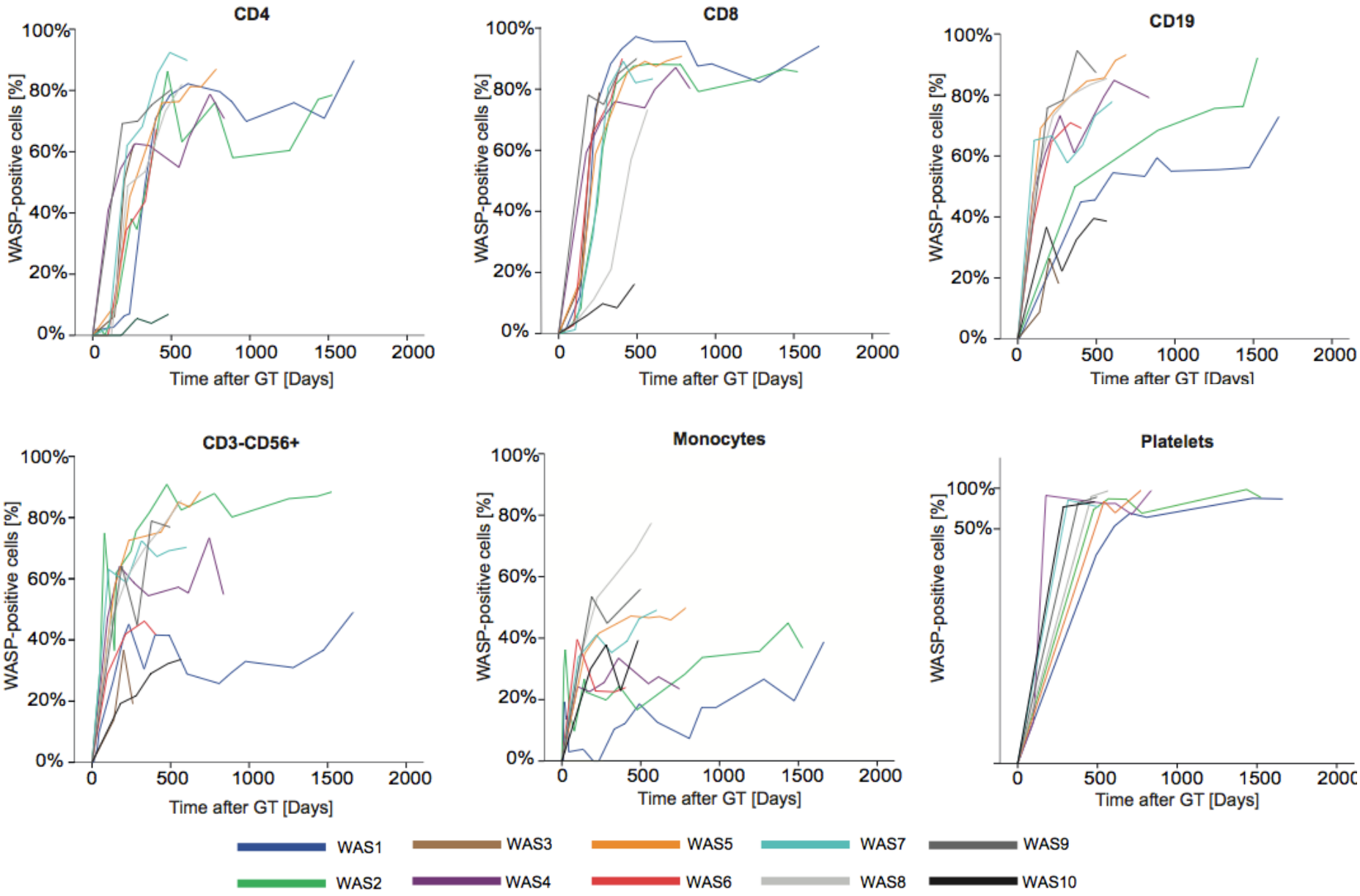




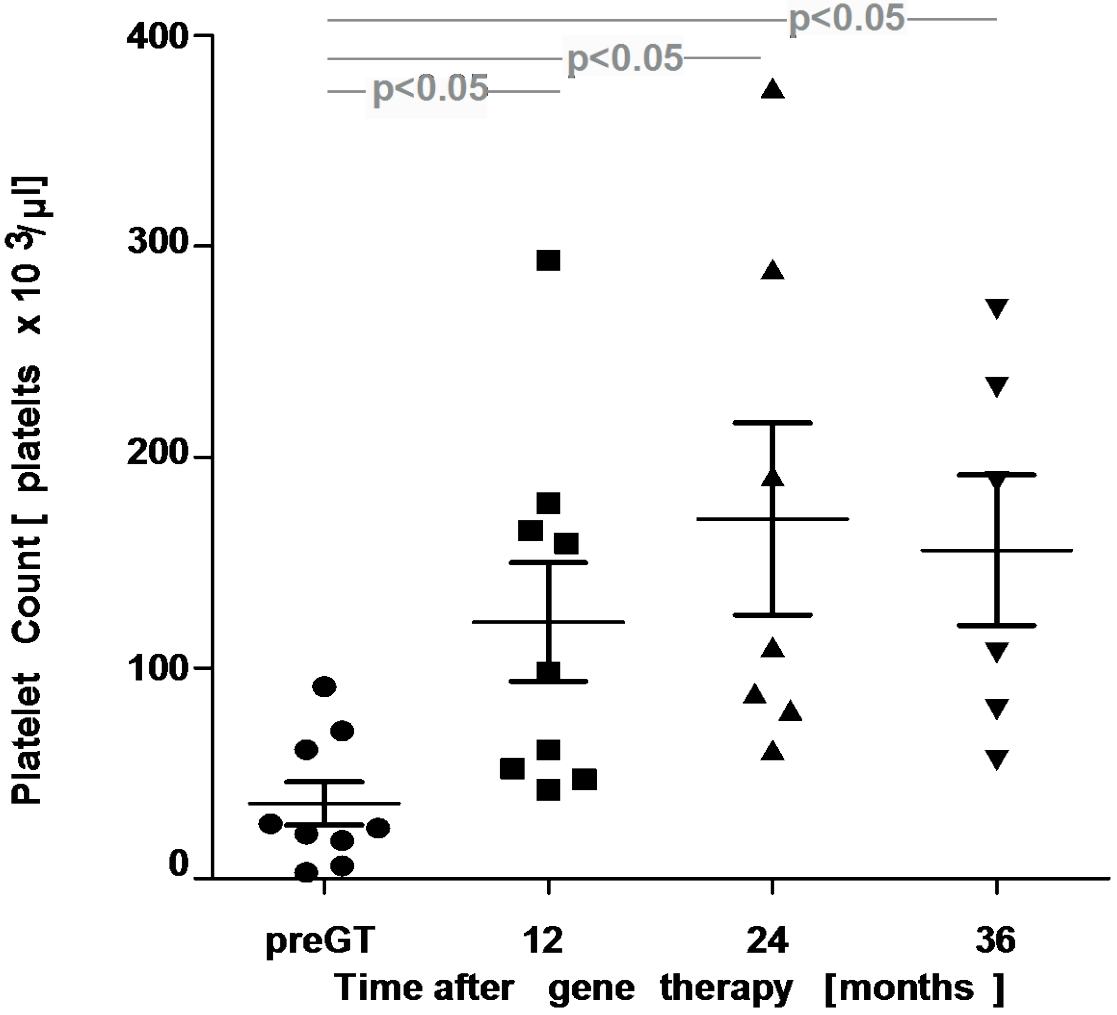




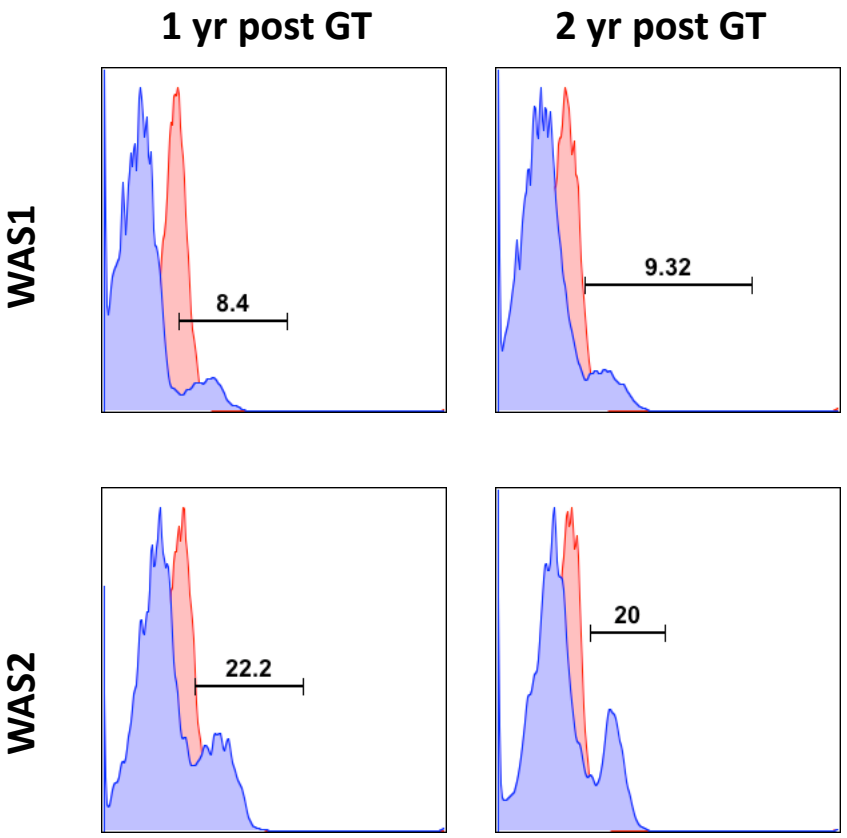
Reconstitution of WASP expression in peripheral blood cells

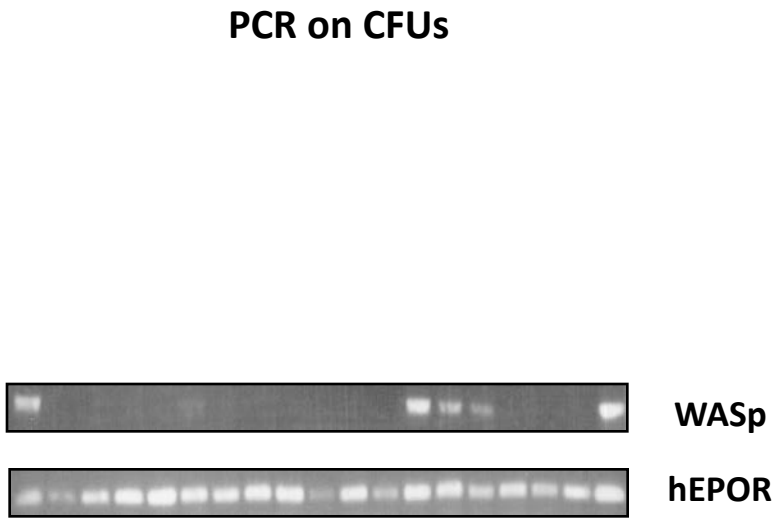


Reconstitution of thrombocytopenia

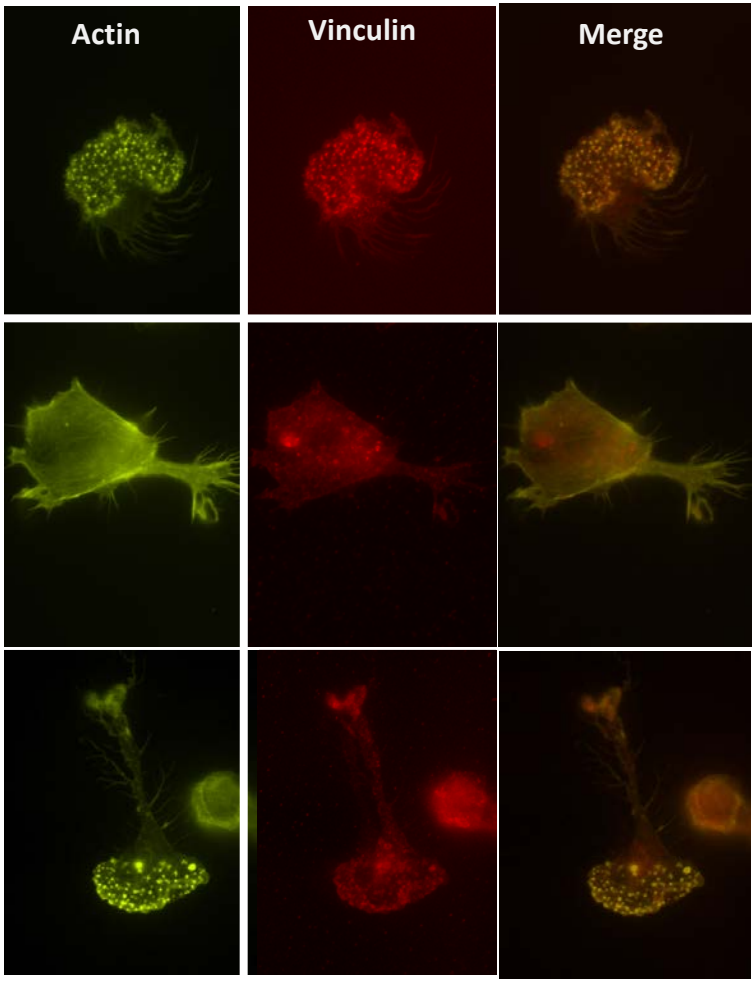


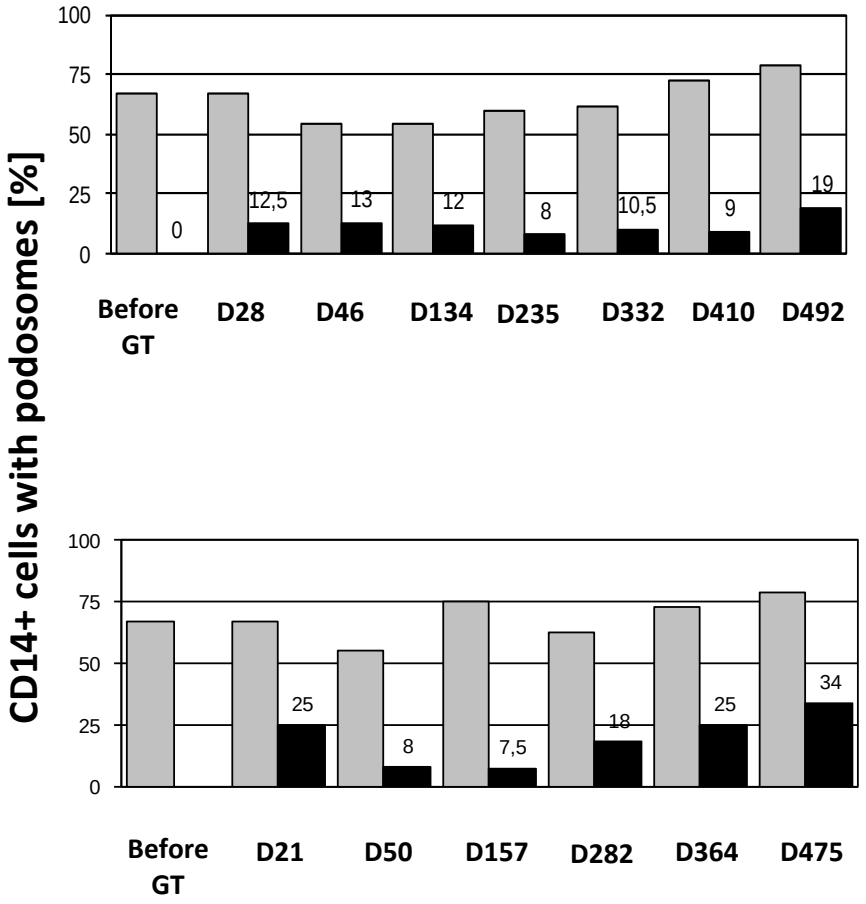
Expression of WASp in CD34+ cells



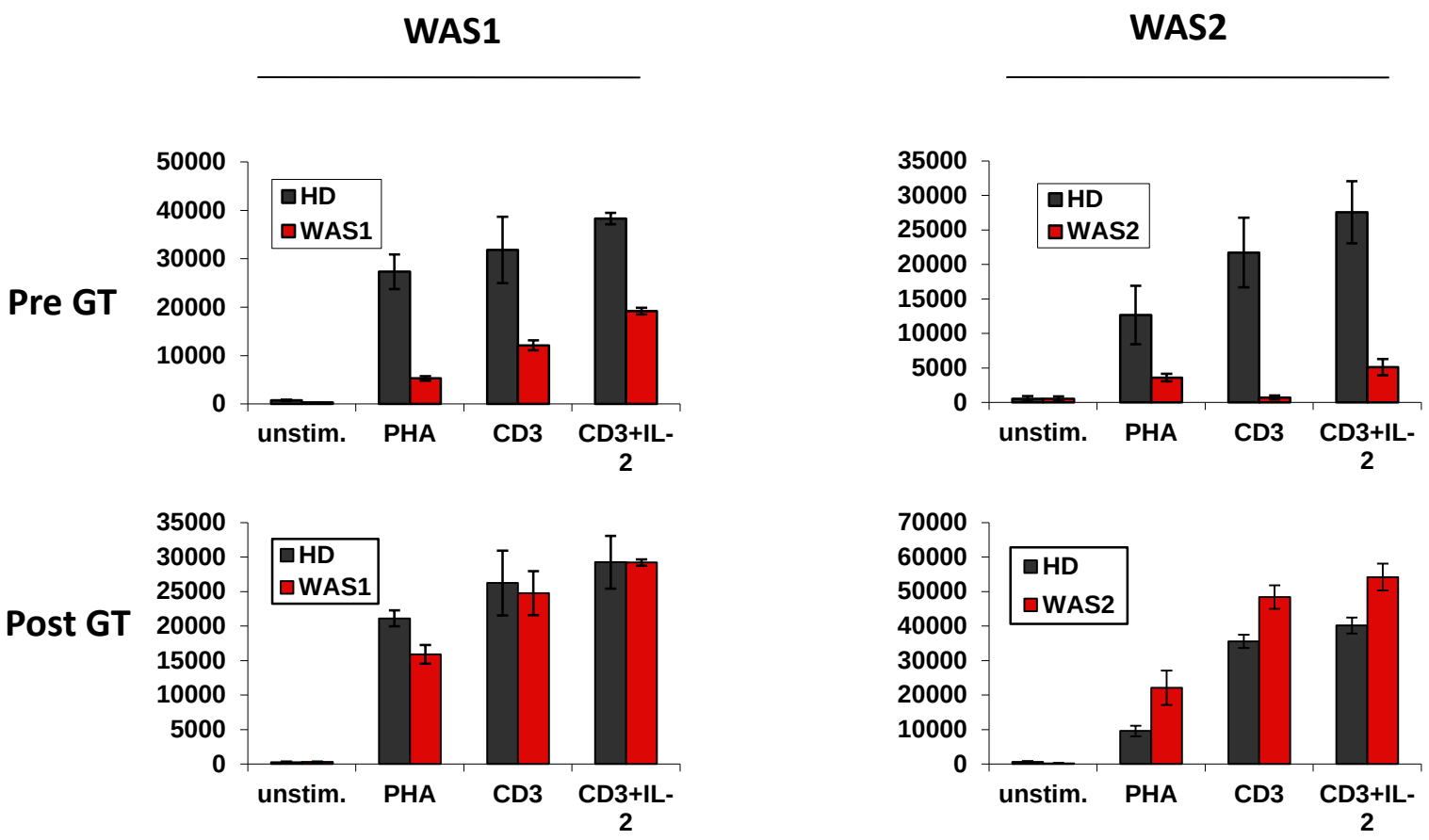


Restored podosome formation in monocytes

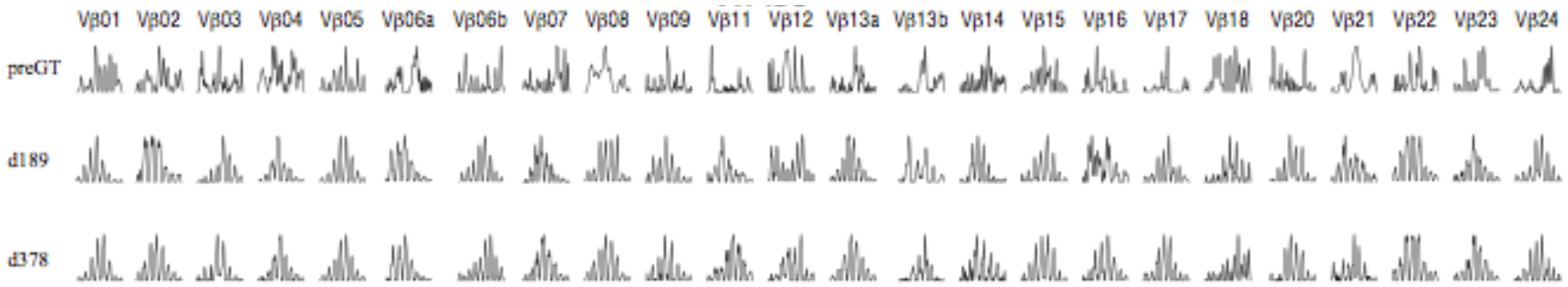




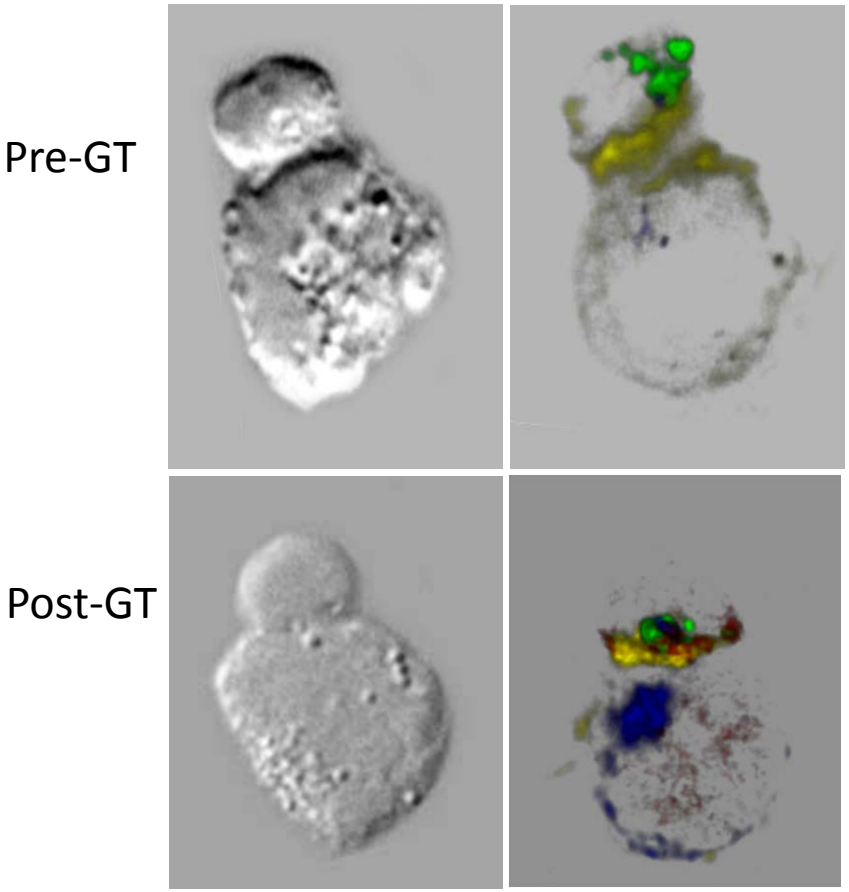
Reconstitution of T-cell proliferation

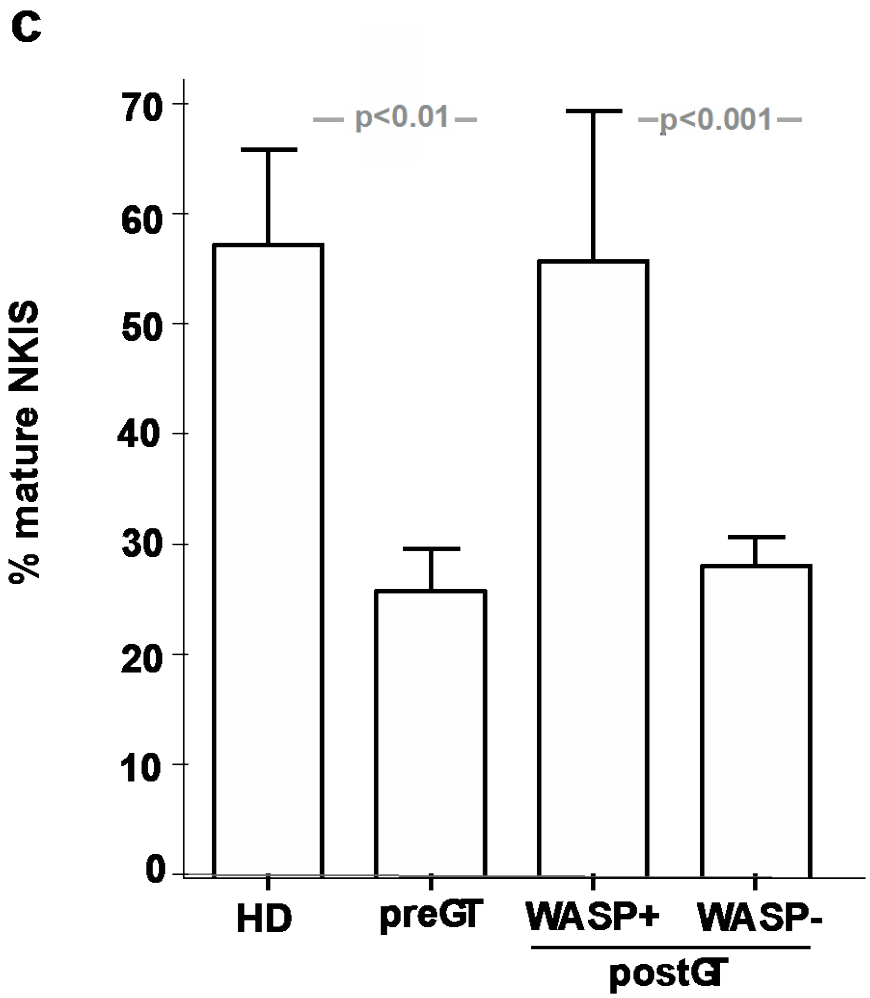


Distribution of TCR Vβ repertoire

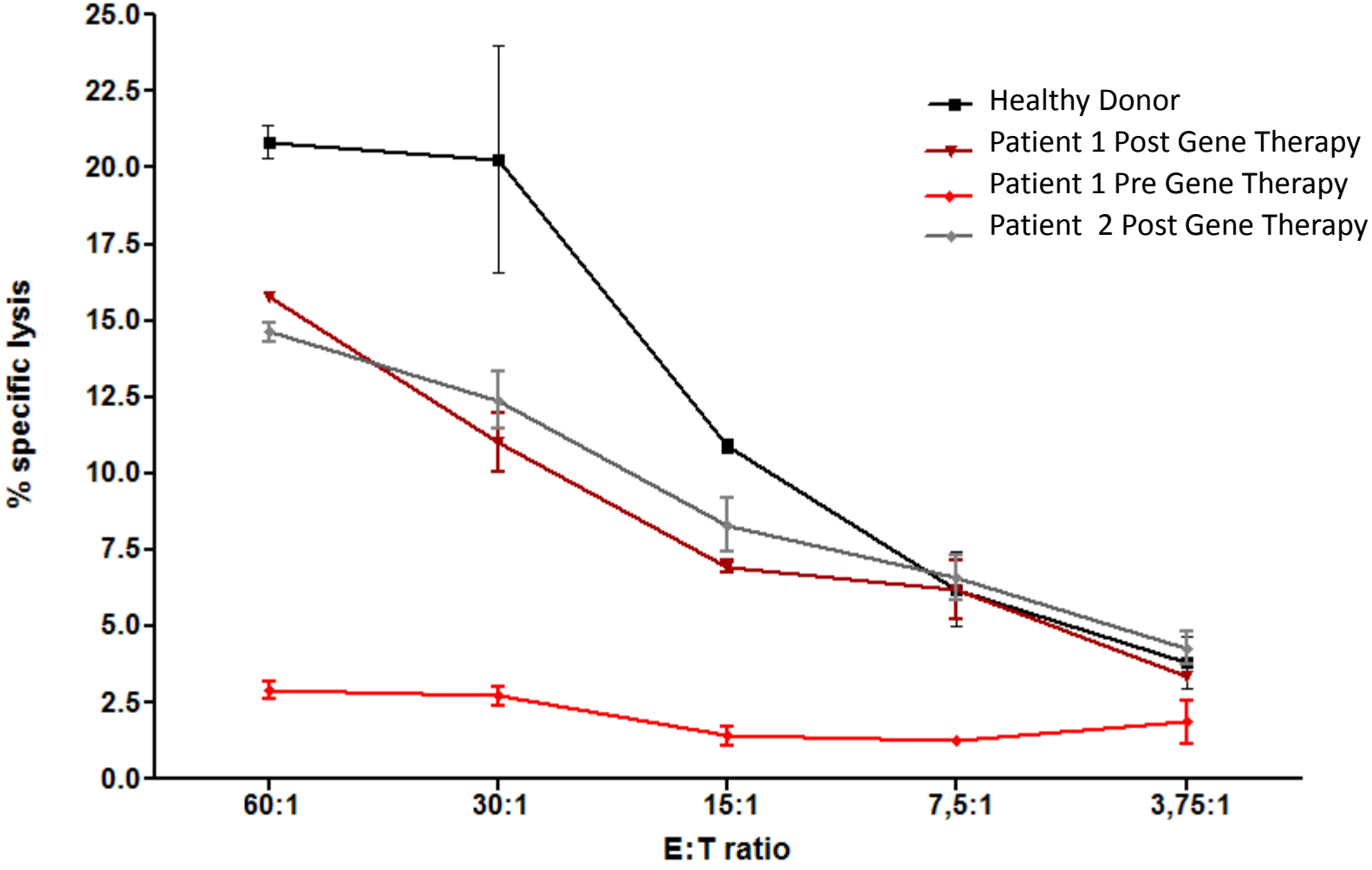


Reconstitution of NK cell synapse

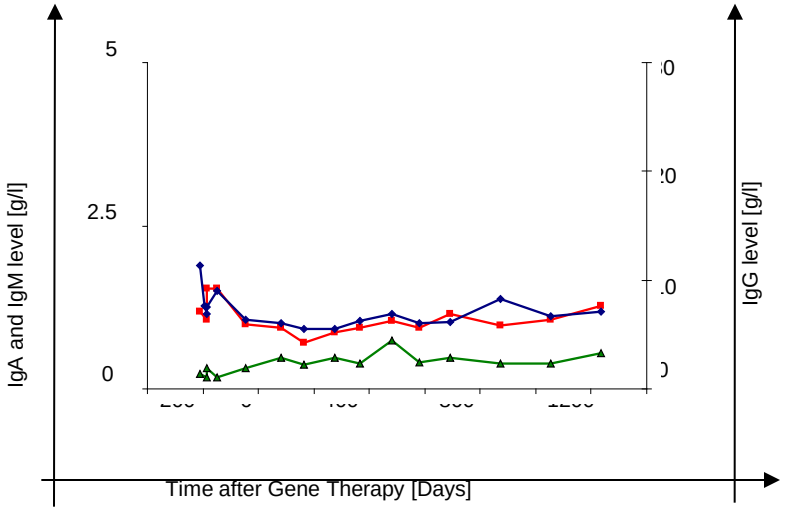


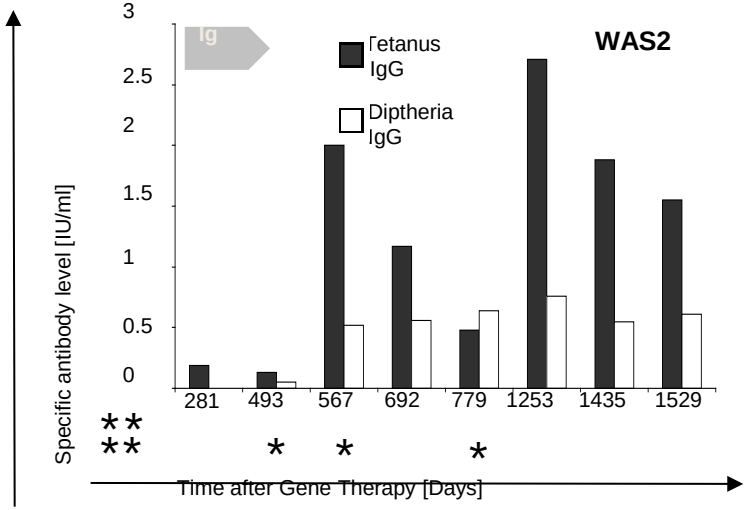


Reconstitution of NK cell lytic capacity

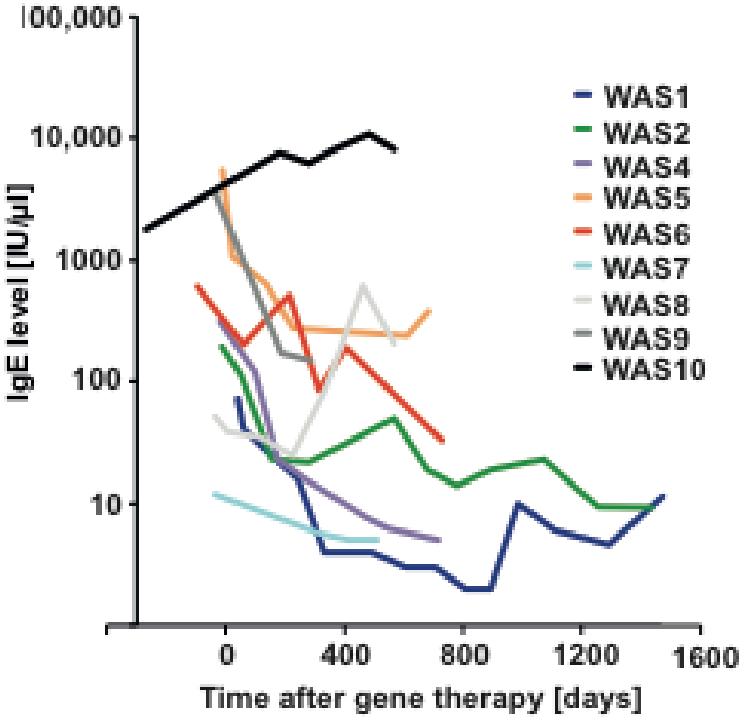


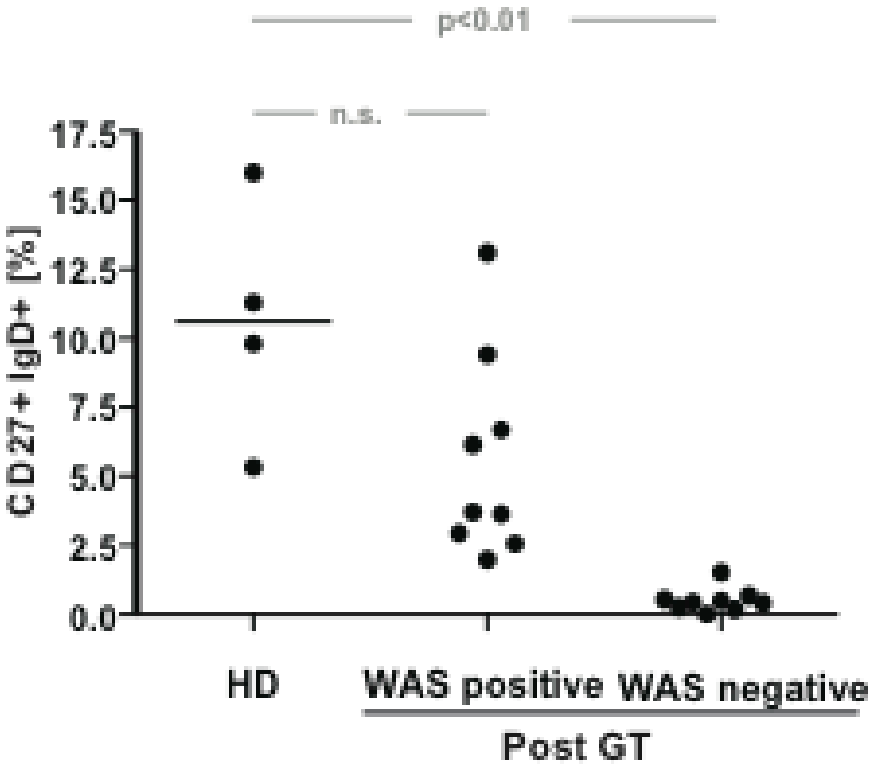
Reconstitution of B cells





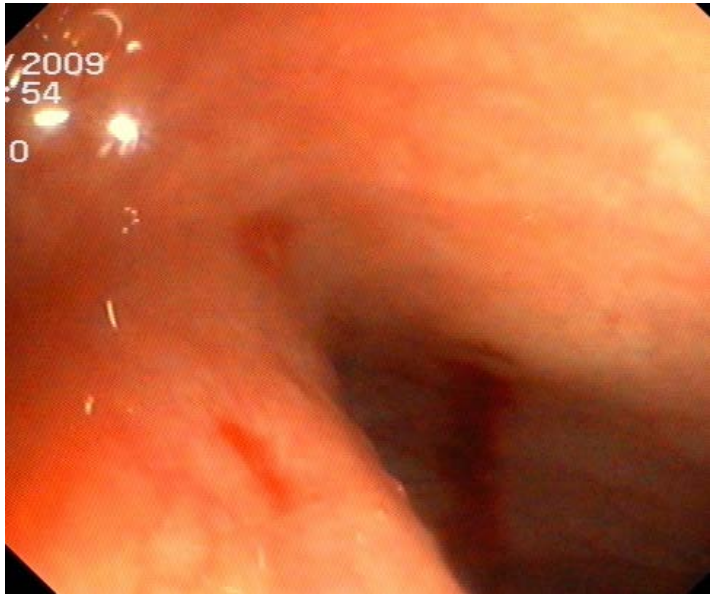
Reconstitution of B cells



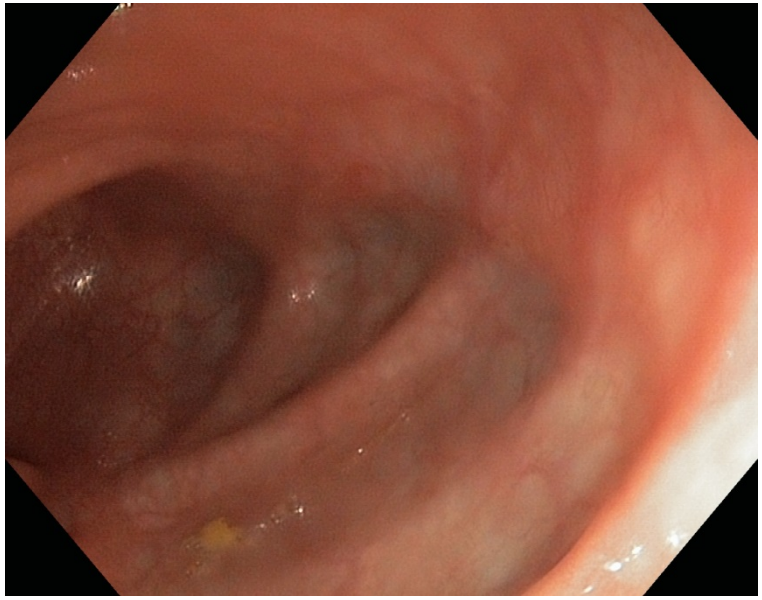


Colitis

Pre GT



Post GT



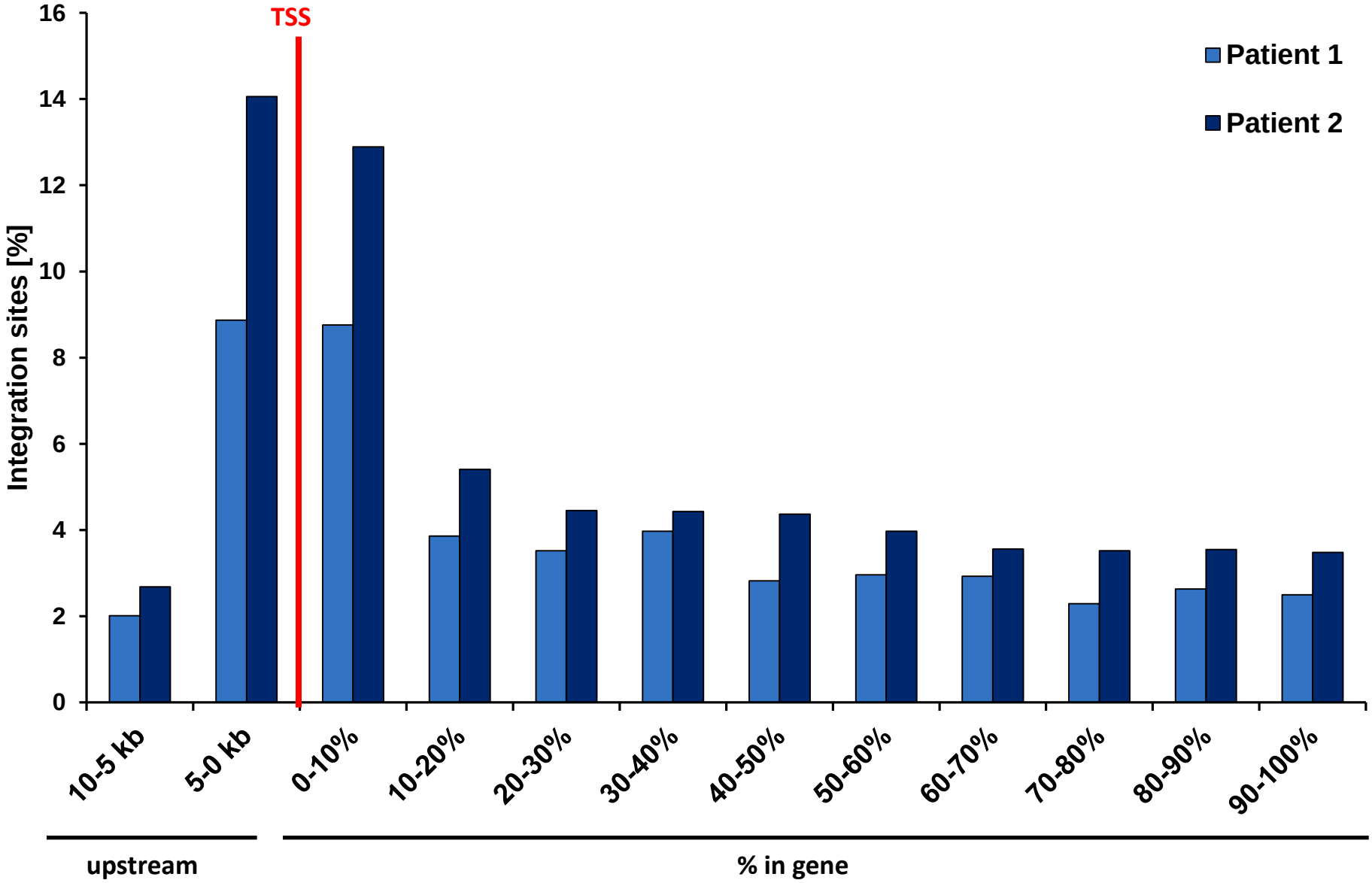
Eczema

Pre GT

Day 157 post GT

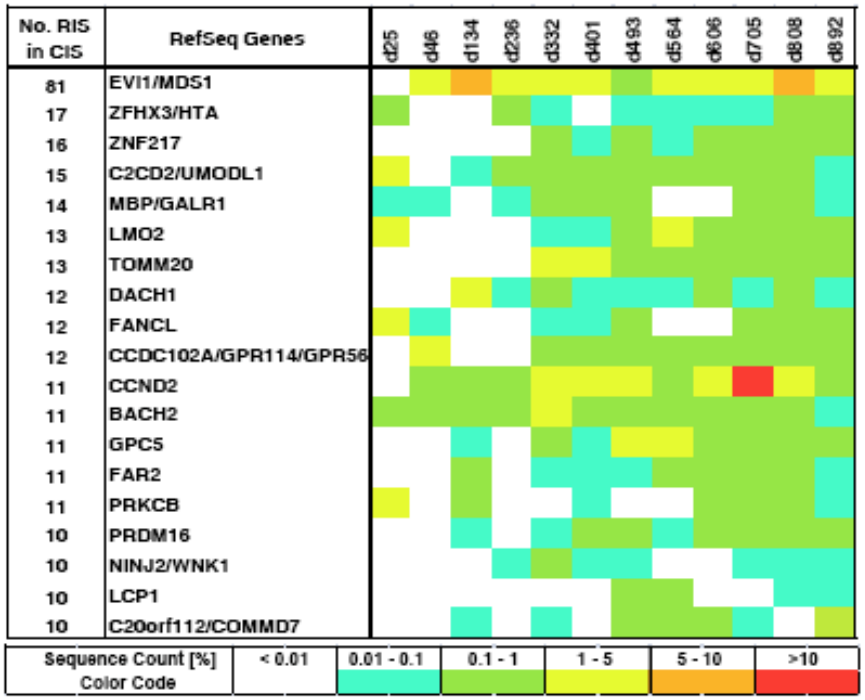
Day 283 post GT

Integration sites (LAM-PCR Analysis)



Comprehensive annotation of retroviral insertion sites

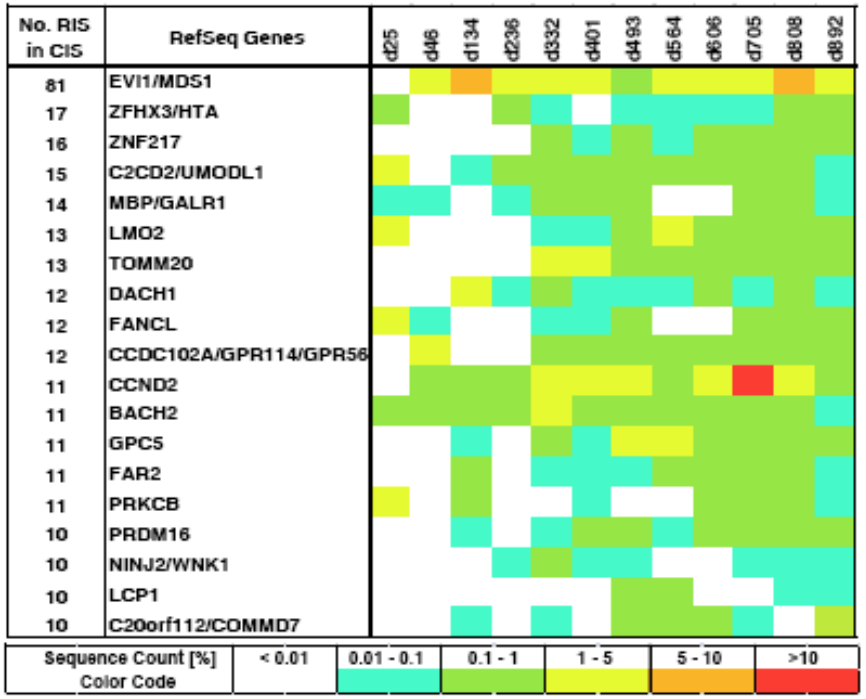
WAS1

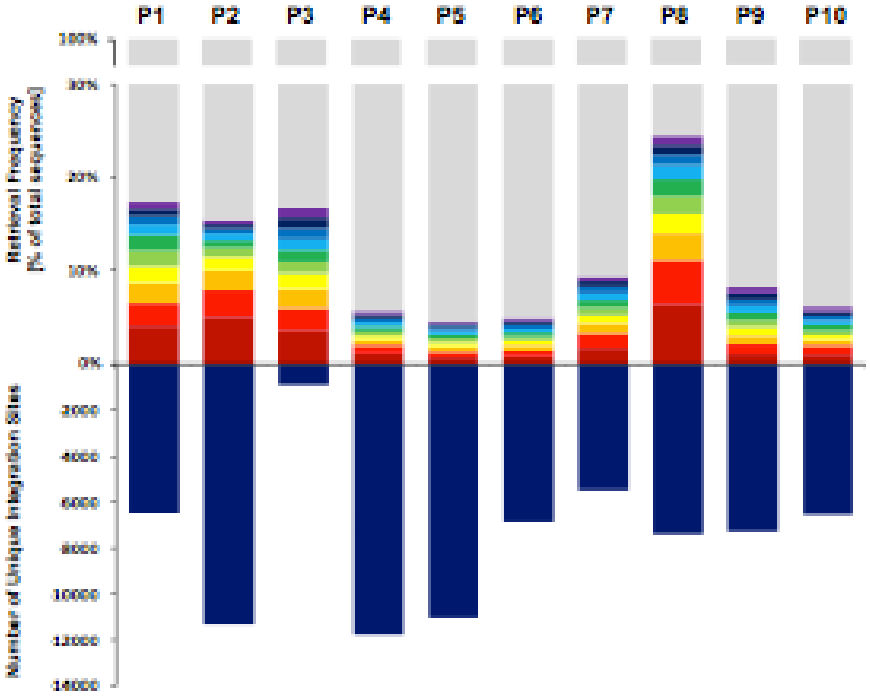


Comprehensive annotation of retroviral insertion sites

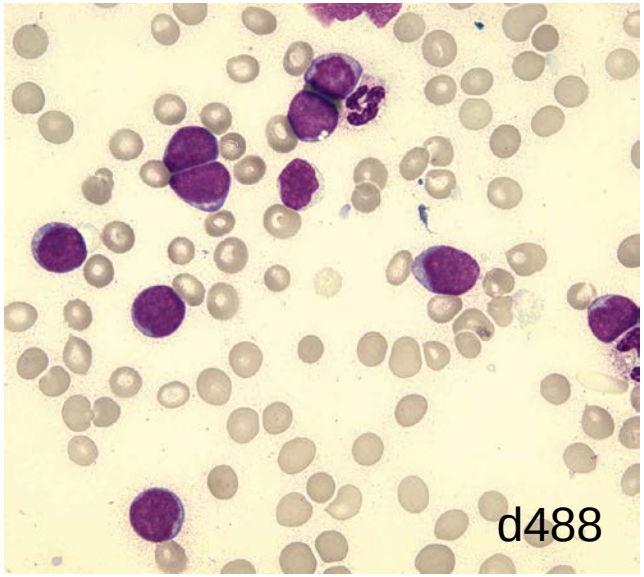
WAS1

WAS1-10





1. SAE: Patient WAS6



4.5 year old boy

Gene therapy June 2009

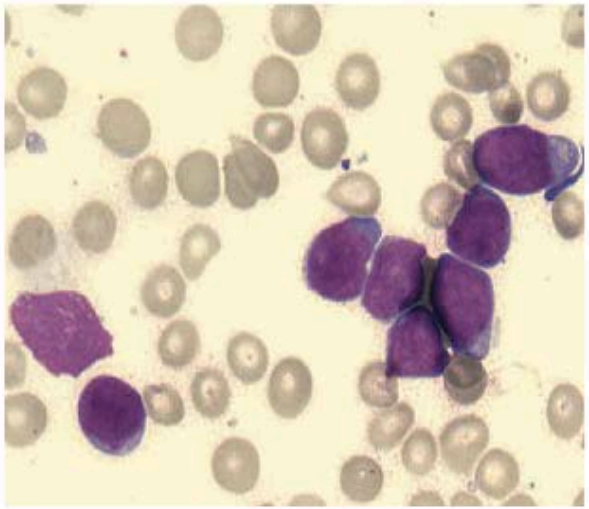
Very good recovery and clinical improvement

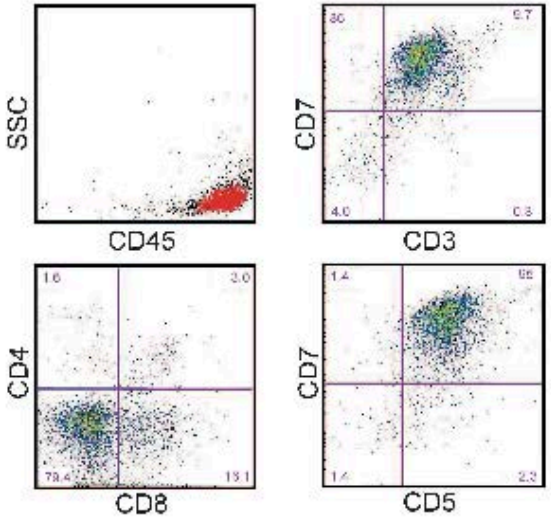
Unremarkable hematopoies up to day +405

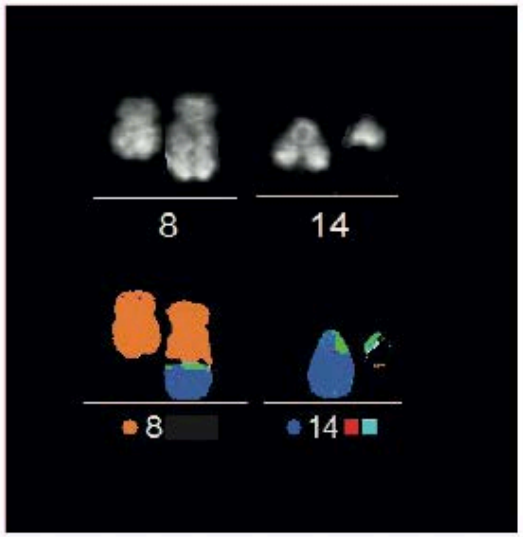
Normal blood count day +458

Brief history of fever and bronchitis

T-Cell Acute Lymphoblastic Leukemia



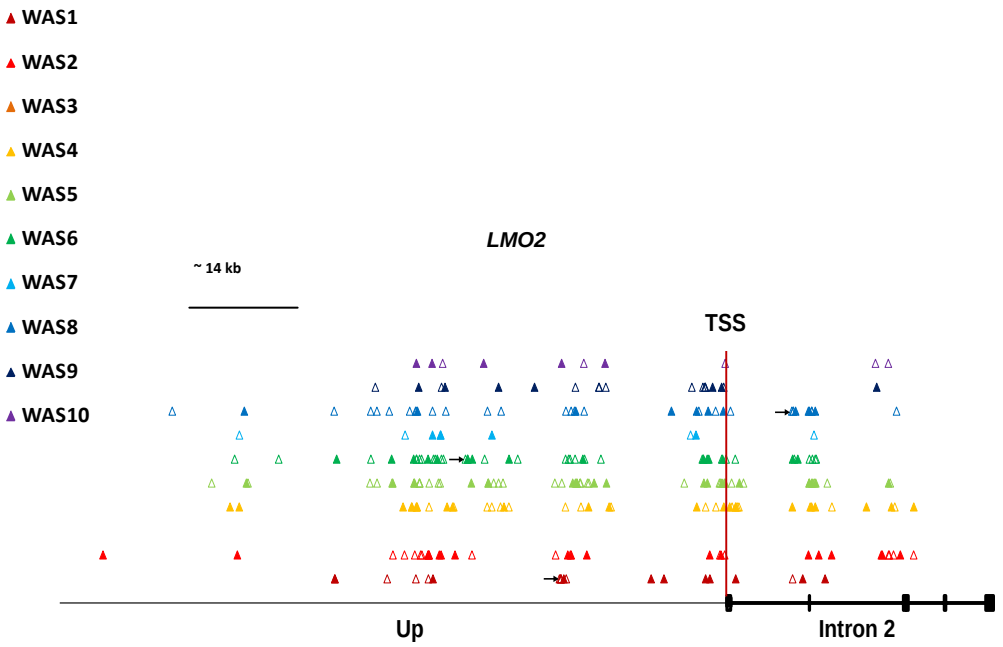


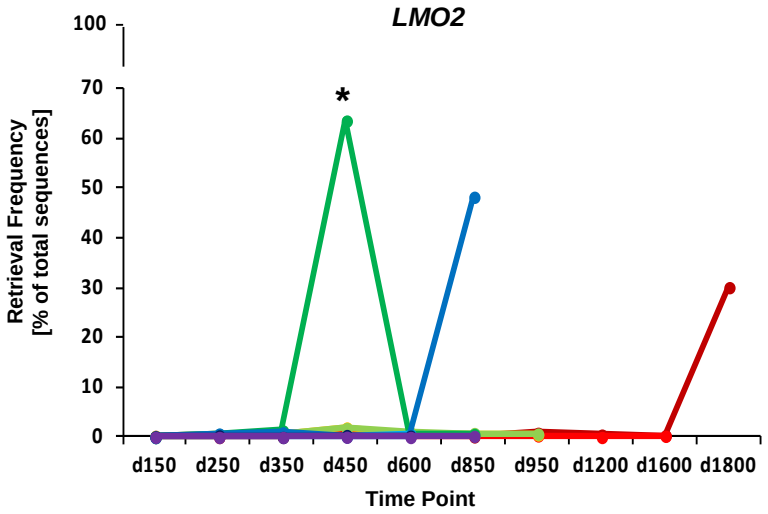


Clinical Summary of HSC-GT-associated Leukemia

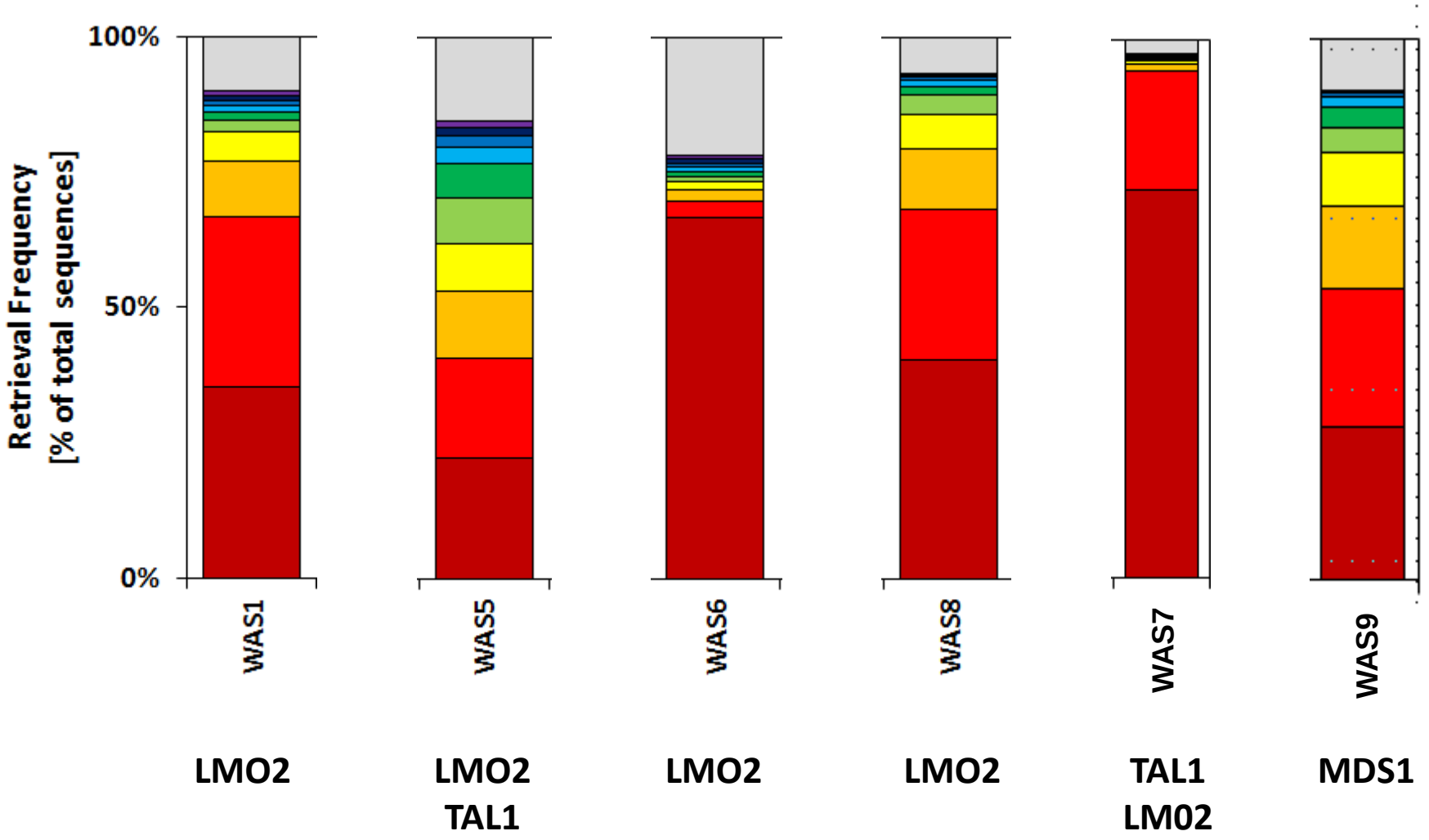
Patient	Leukemia	Time to diagnosis post GT [d]	Pred response	CNS	Therapy
WAS8	T-ALL	427	poor	pos	Chemo/Radio -> 2nd AML -> HSCT
WAS6	T-ALL	489	poor	neg	Chemo/HSCT
WAS5	T-ALL	1074	good	neg/pos	Chemo/HSCT/ Death after Tx
WAS1	T-ALL	1812	good	neg	Chemo -> 2nd AML-> HSCT
WAS7	T-ALL	1105	poor	Neg	Chemo/HSCT
WAS9	MDS/AML	1165	N/A	Neg	Chemo/HSCT

LMO2 integrations

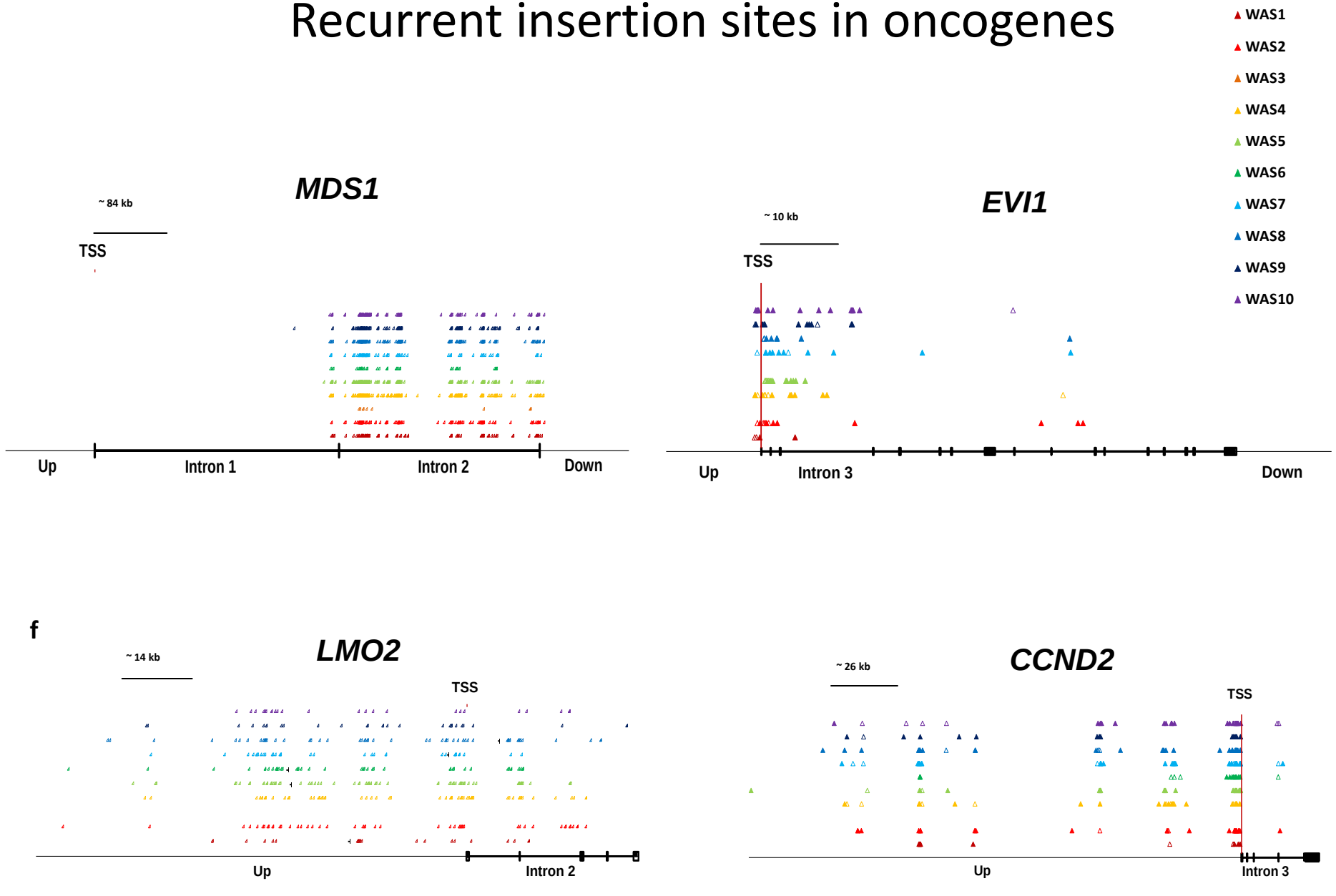




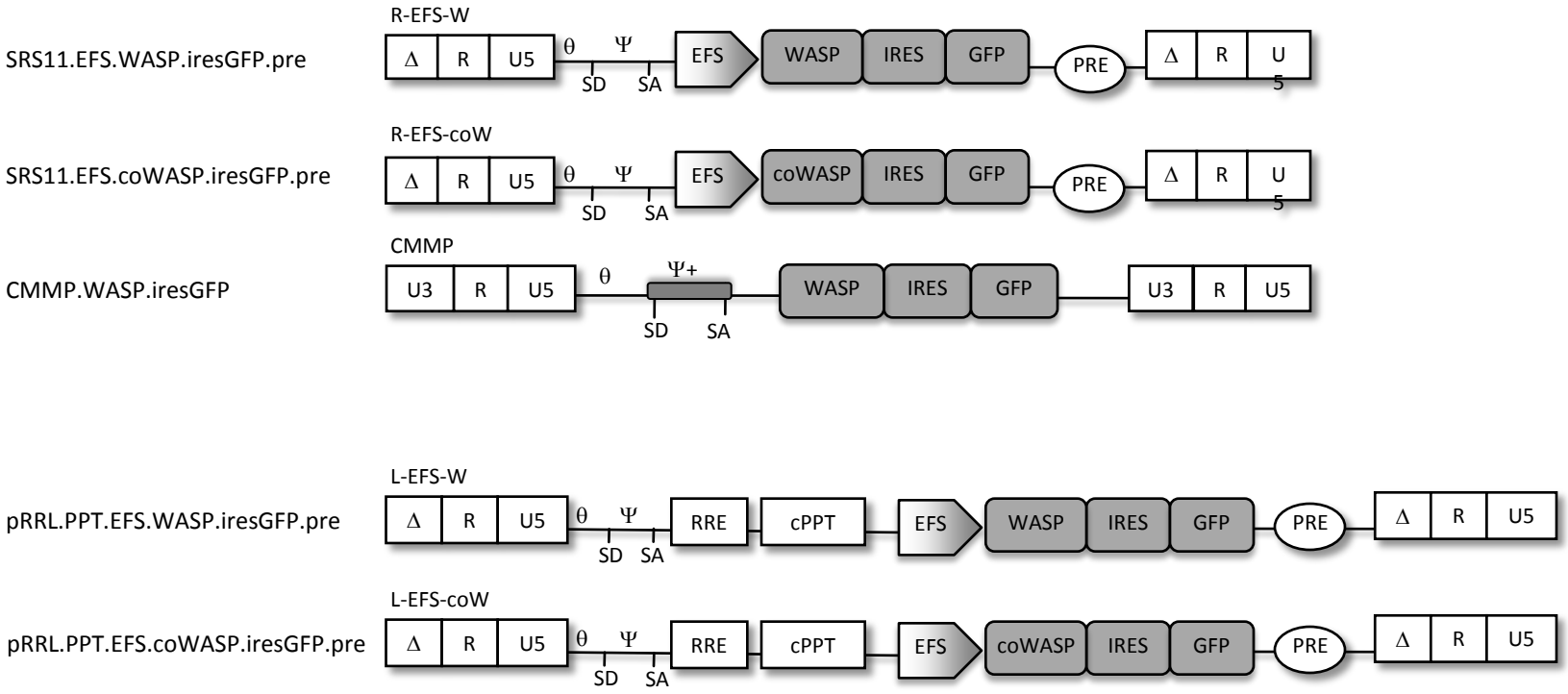
Insertion site analysis at leukemia diagnosis



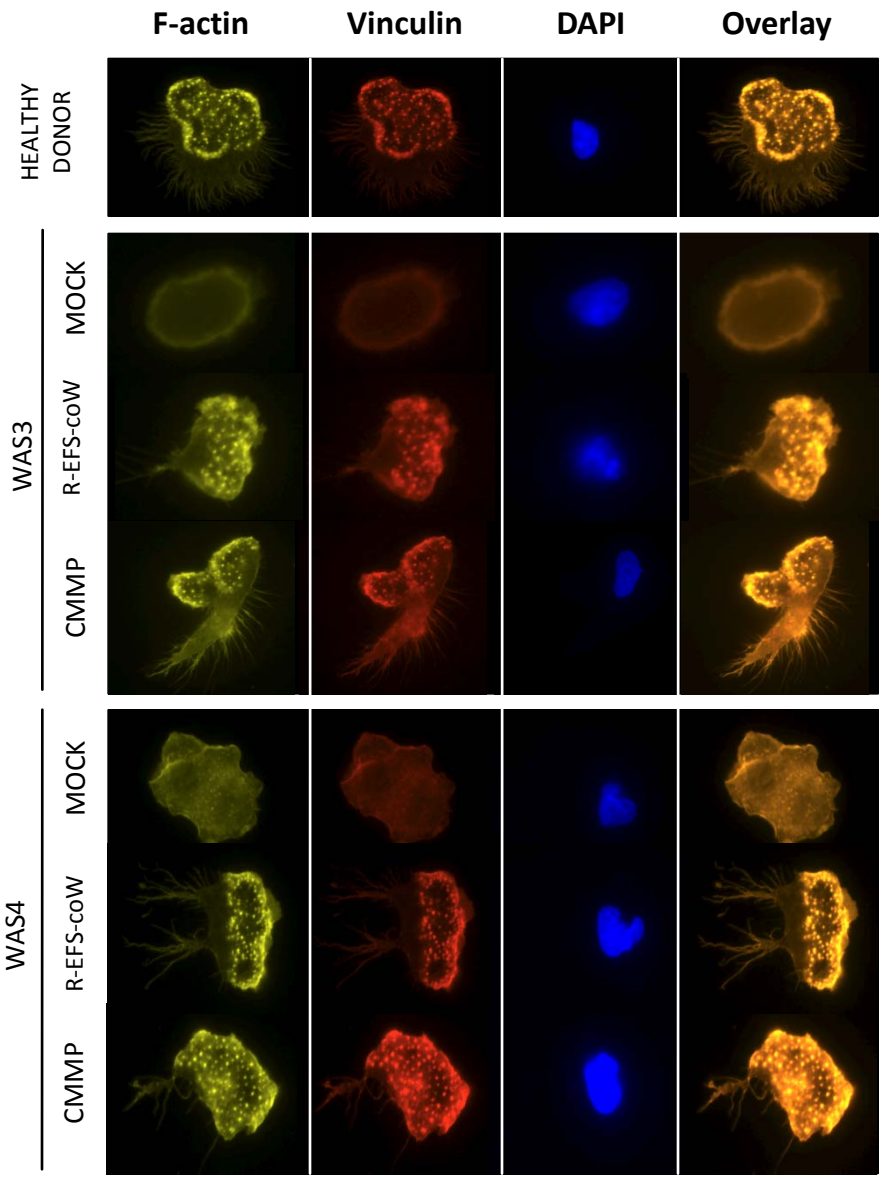
Recurrent insertion sites in oncogenes

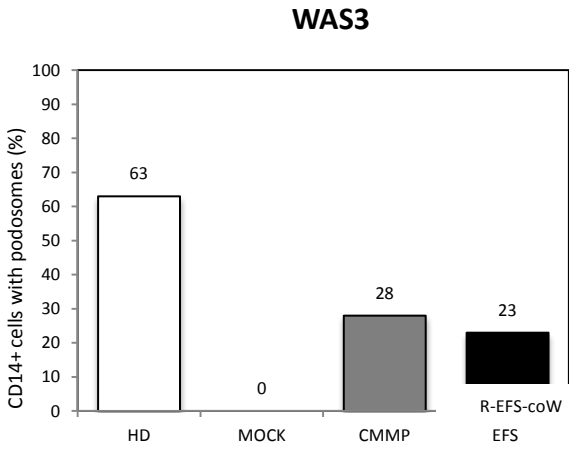


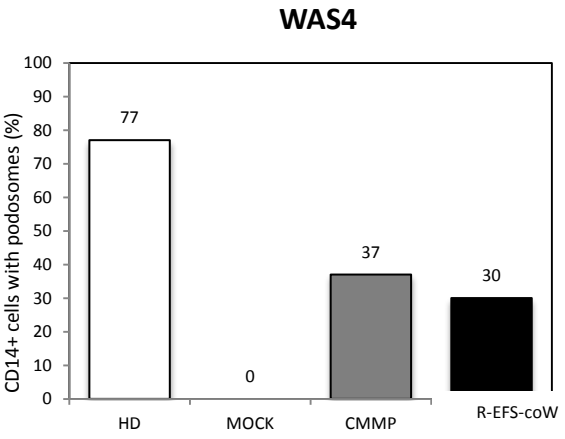
Novel vectors



Functional reconstitution of human myeloid cells







Conclusions

- Transgenic WASP is stably expressed in relevant hematopoietic lineages upon HSC gene therapy
- Leukocytes and platelets are functionally corrected
- Immunodeficiency, bleeding diathesis and autoimmunity resolve
- Initally polyclonal hematopoiesis with insertions in multiple loci
- 6/10 patients developed acute leukemia (T-ALL and AML)
- Novel genetic engineering tools are needed

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