A diversity of human hematopoietic differentiation programs identified through in vivo tracking of hematopoiesis in gene therapy patients

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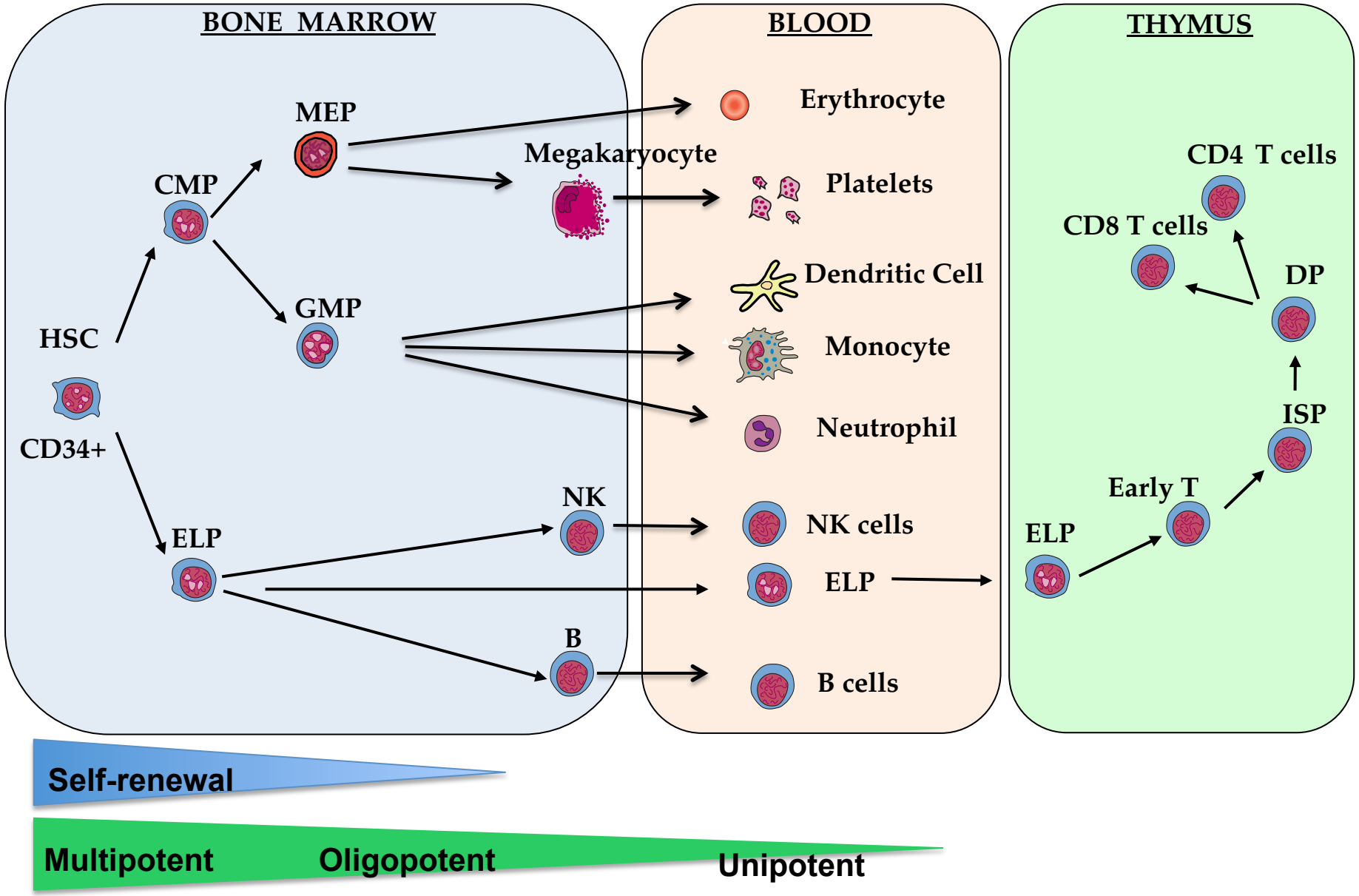
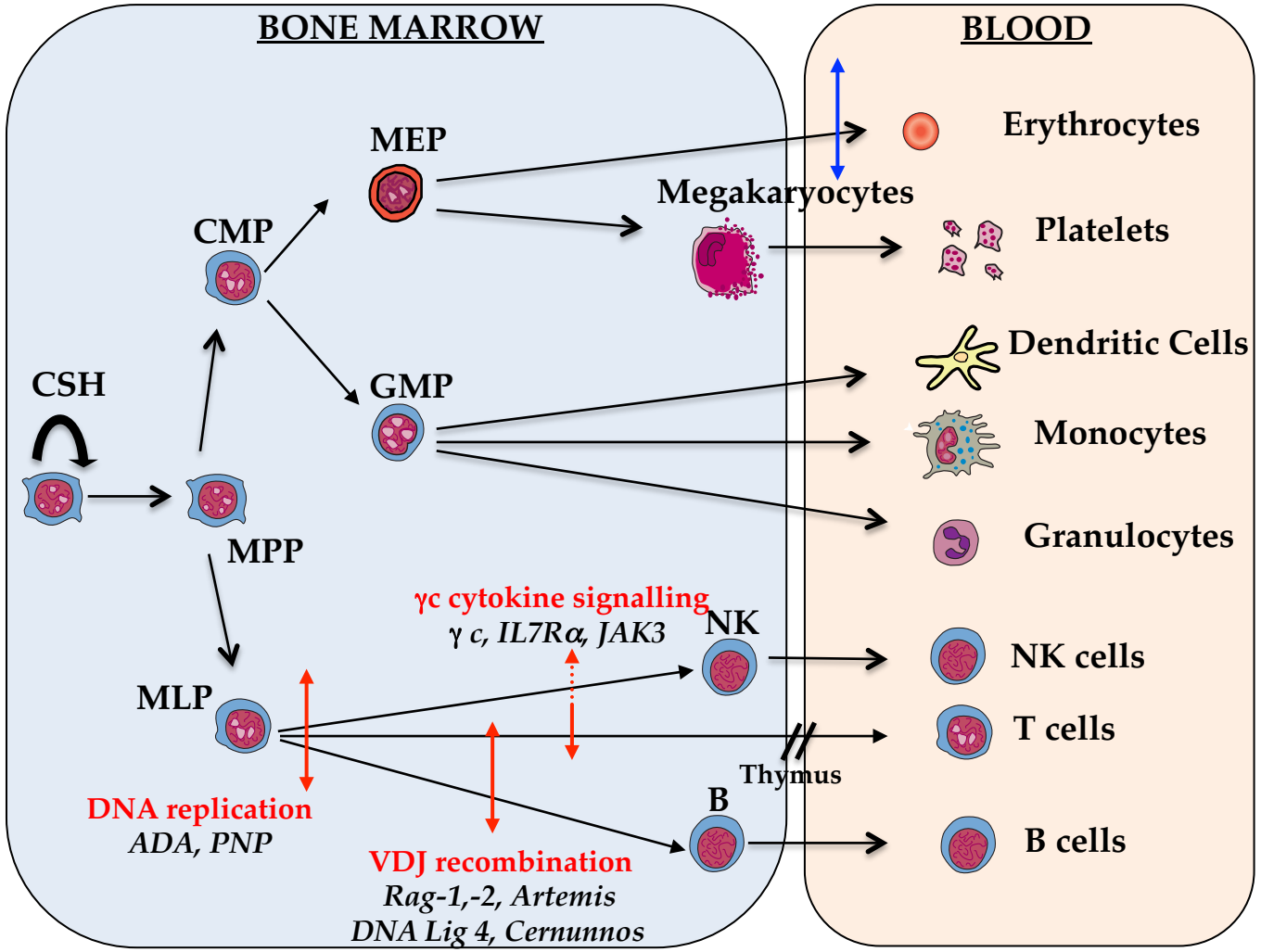
The classical hematopoietic hierarchy



Hematological inherited diseases

β-hemoglobinopathies

β0/βE et βS/βS

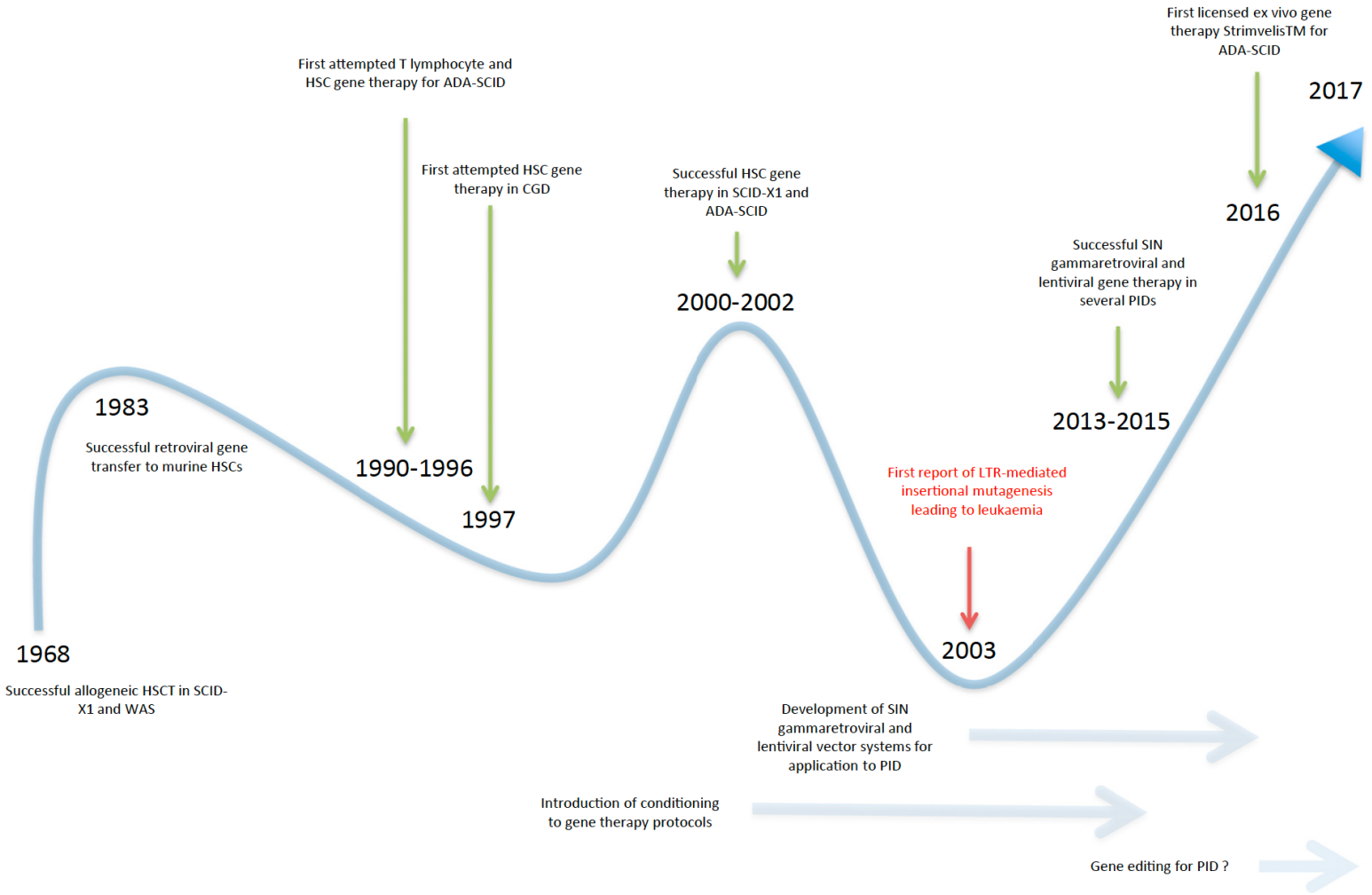


Syndrome Wiskott Aldrich (WAS)
WASp

Granulomatose Septique Chronique (CGD)
NADPH oxydase

Primary Immune Deficiencies (PID)

Milestones in the development of Gene Therapy

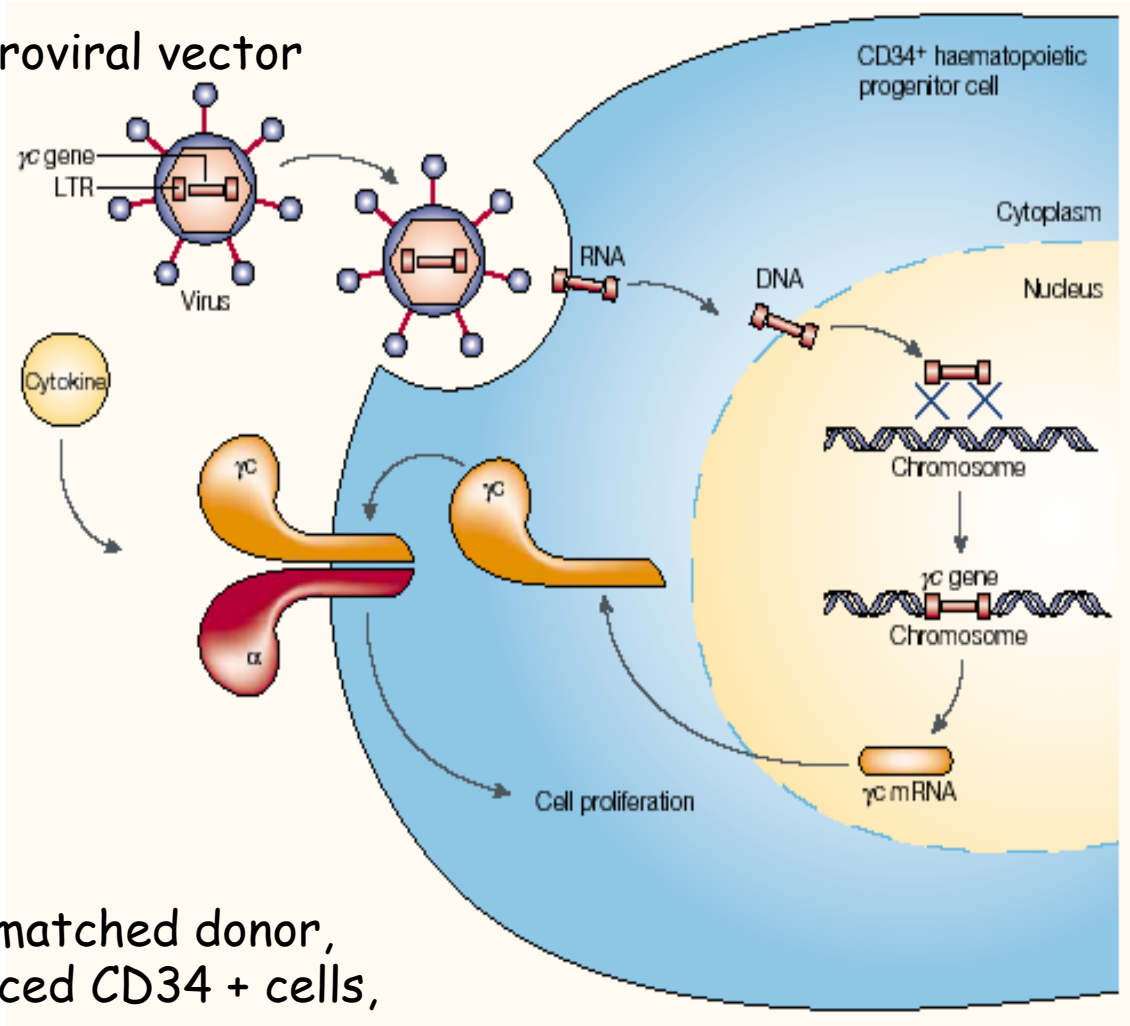


Thrasher et al., Mol Ther 2017

Ex vivo gene therapy of SCID-X1

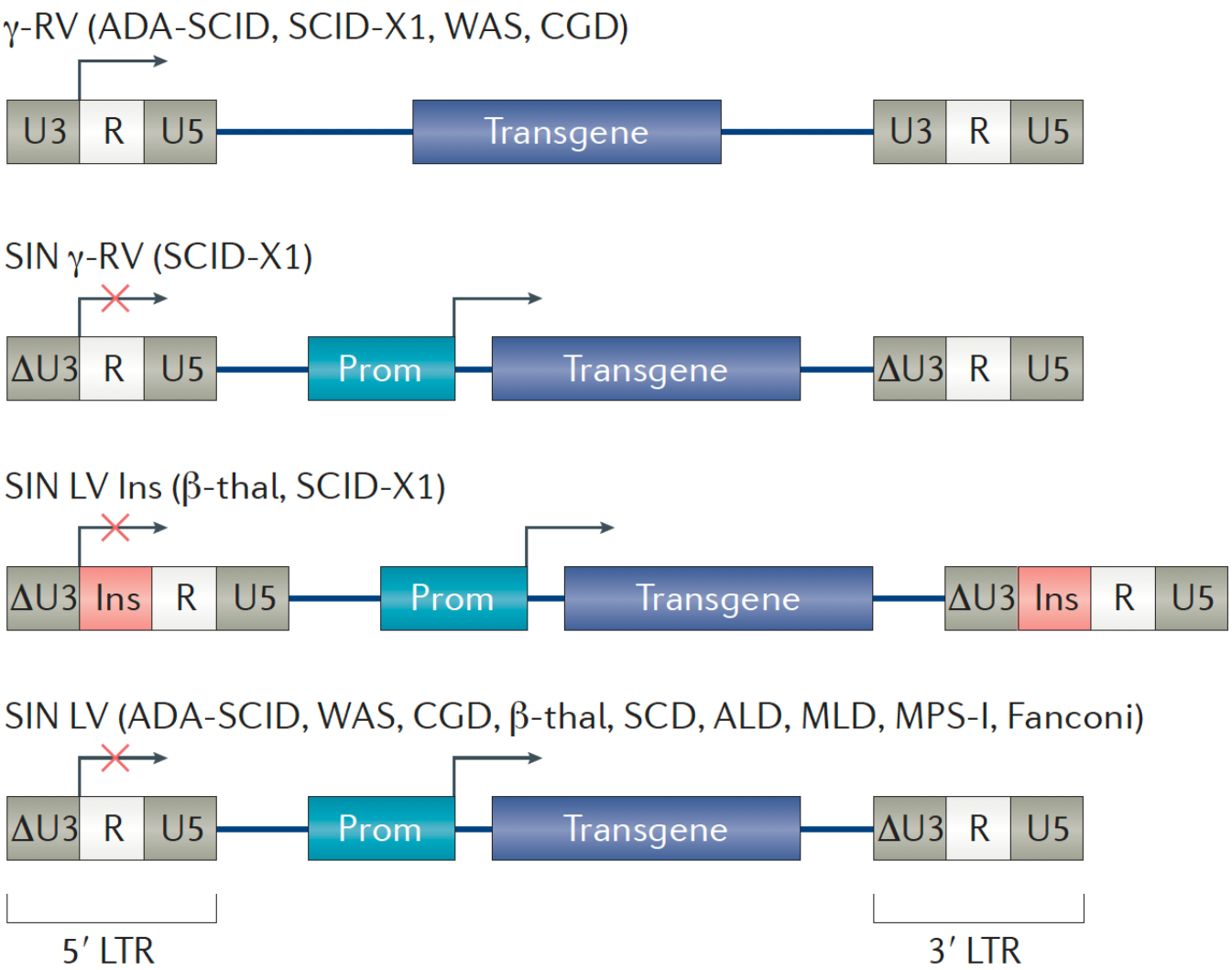
amphotropic gammaretroviral vector

(MFGB2)



patients with no HLA matched donor,
reinjection of transduced CD34 + cells,
no chemotherapy

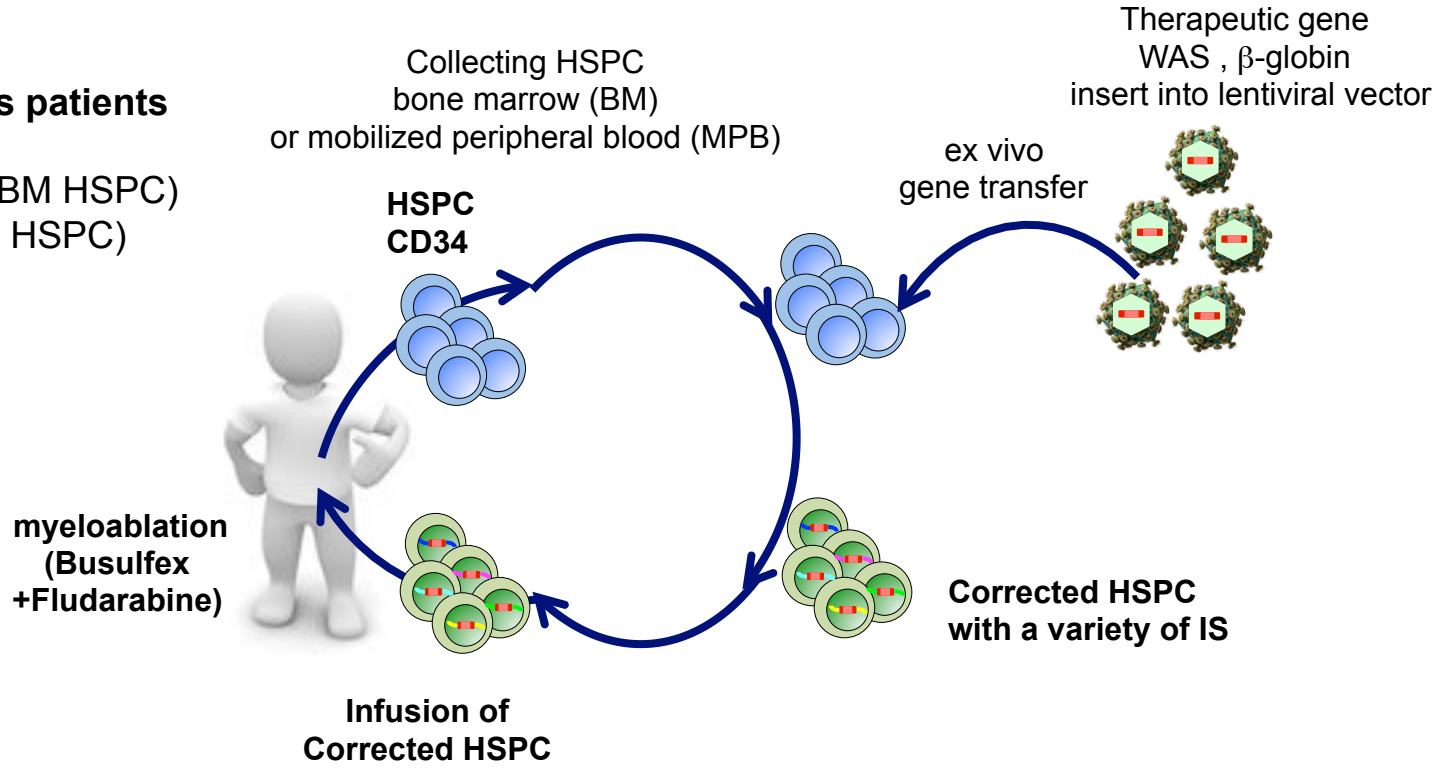
Two generation of retroviral vectors



Wiskott-Aldrich syndrome and β-hemoglobinopathies gene therapy trials

WAS patients
Deficiency in T cell, B cell, NK cells, Platelets
2 patients : BM HSPC

Beta-hemoglobinopathies patients
Deficiency in RBC
Sickle cell disease βS/βS (BM HSPC)
β-thalassemia β0/βE (MPB HSPC)

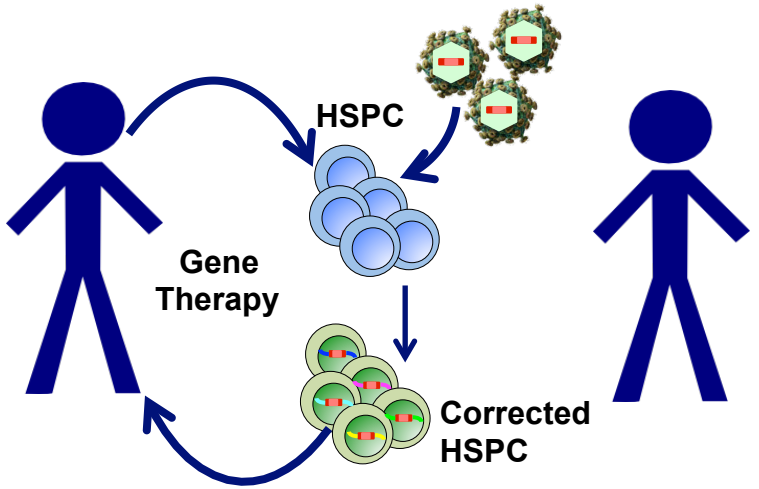


Hacein-Bey-Abina et al., JAMA, 2015
Ribeil et al NEJM 2017 , Ribeil et al NEJM in press

Patients treated

	Pathology	Age at treatment	Source	Mobilizing agent	Conditionning regiment	AUC (Bu)	CD34 10°6/kg	Total corr. CD34 infused	VCN in CD34
WAS4	WAS	10m	BM	-	Bu 3 doses +Flu	NA	7.3	65.7	2.8
WAS5	WAS	3y	BM	-	Bu 3 doses +Flu	17,601	6.8	70.584	0.6
βS/βS	Sickle Cell Disease	13y	BM	-	Bu 4 doses	19,363	5.6	226.8	1
WAS2	WAS	15y	MPB	G-CSF + Plerixafor	Bu 3 doses +Flu	NA	11	660	1.3
WAS7	WAS	3.5y	MPB	G-CSF + Plerixafor	Bu 3 doses +Flu	17,204	15	177.3	0.6
β0/βE	Beta-thalassemia	16y	MPB	G-CSF + Plerixafor	Bu 4 doses		13.6	557.6	1.65

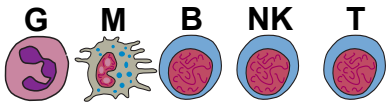
Functional characterization of human HSC
HSC clonal analysis using integration sites tracking



Follow Up

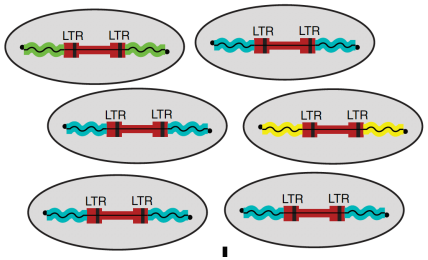
Blood Sample

Facs sorting of blood cell types

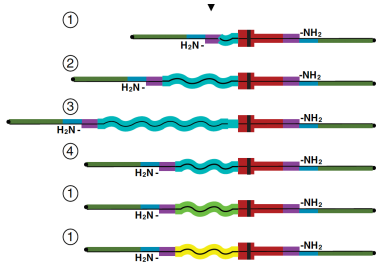


Quantify purity
DNA extraction

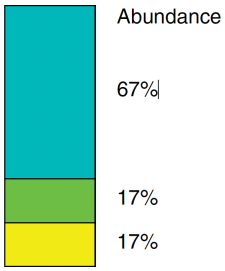
IS library construction
LM-PCR,
Illumina sequencing



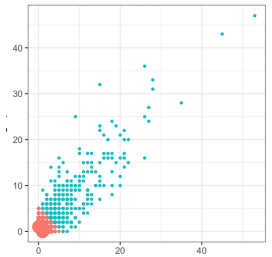
Quantify IS abundance
Sonic Abundance



IS dataset
INSPIIRED pipeline



Extract reliable IS
Abundance threshold



IS contribution to the various lineages

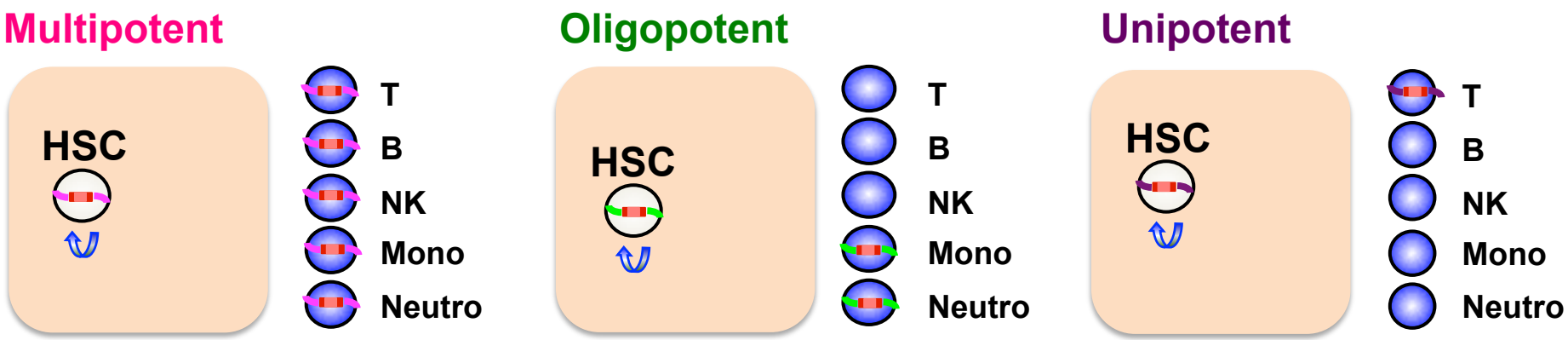
Sherman et al., Mol Ther Meth Clin Dev, 2017
Berry et al., Mol Ther Meth Clin Dev, 2017

Tracking and characterizing Human Hematopoietic Stem Cells

The progeny of the gene corrected HSC

can be tracked by integration sites analysis at long term follow up (> 2years)

in the different lymphoid and myeloid subpopulations



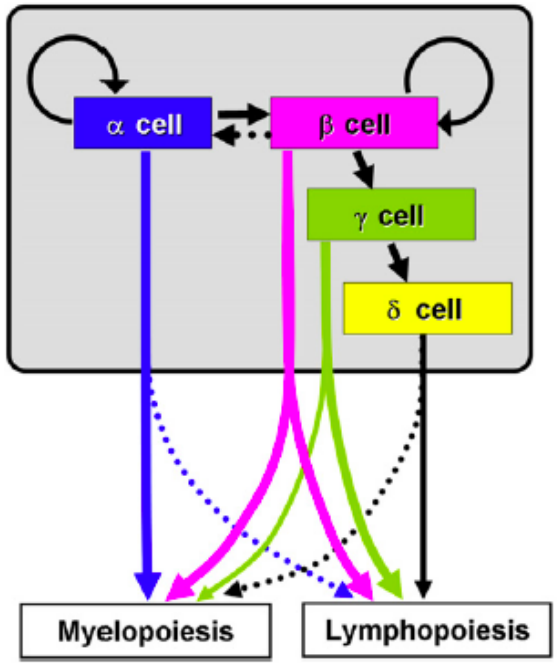
✓ **Defining the various type of HSC**

contributing to long term human hematopoiesis

HSC are heterogenious in their potential

C. Eaves's work

α cell : HSC lymphoid deficient
β cell : HSC balanced
γ cell : HSC myeloid deficient
δ cell : HSC highly myeloid deficient



Dykstra et al Cell Stem Cell 2007
Benz et al Cell Stem Cell 2012

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