Hematopoietic Innovative Therapies

Preliminary Conclusions Obtained in Fanconi Anemia Patients Treated by Lentiviral-mediated Gene Therapy after more than 2 years of Follow-up

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ASCGT 22nd Annual Meeting, Washigton DC, April 29th 2019

Ciemat

ciberer
Centro de Investigación Biomédica en Red
Enfermedades Raras

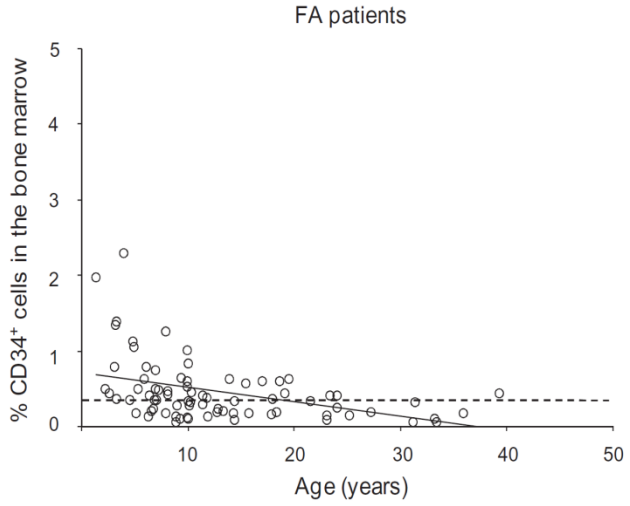
IIS FJD INSTITUTO DE INVESTIGACIÓN SANITARIA FUNDACIÓN JIMÉNEZ DÍAZ

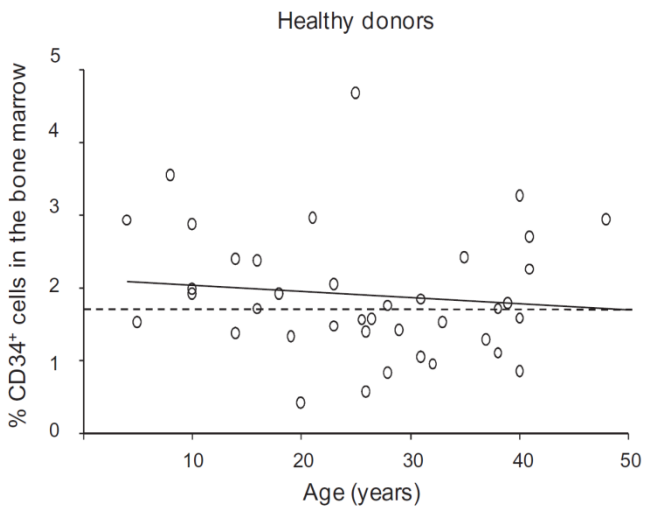
DISCLOSURES:

- *The Innovative Therapies Div. at CIEMAT receives funding for FA gene therapy from Rocket Pharma*

- *PGK-FANCA-Wpre* Lentiviral vector has been licensed to Rocket Pharma*

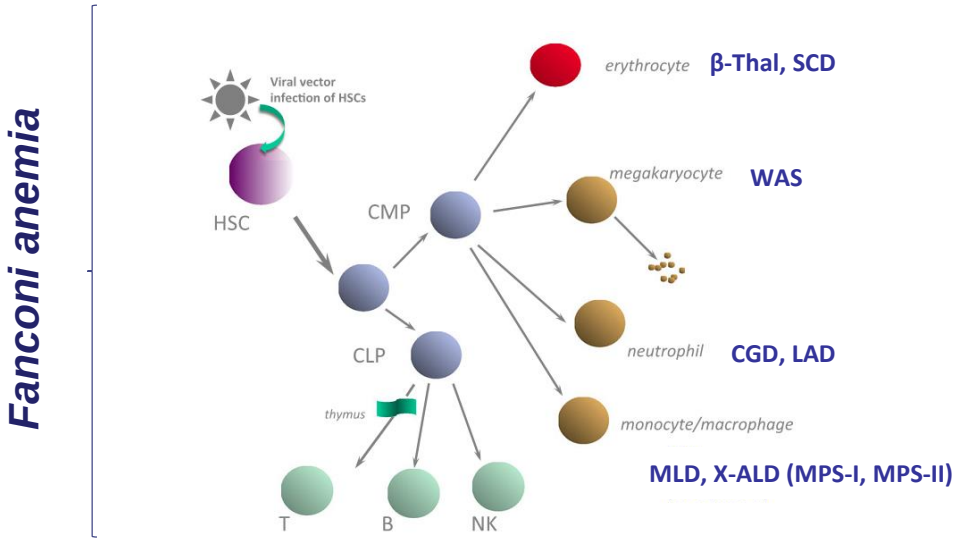
Hematopoietic Stem Cell Defect in Fanconi Anemia: Consequences for Gene Therapy





Ceccaldi et al., 2012

- **The target cells, HSCs are affected:**

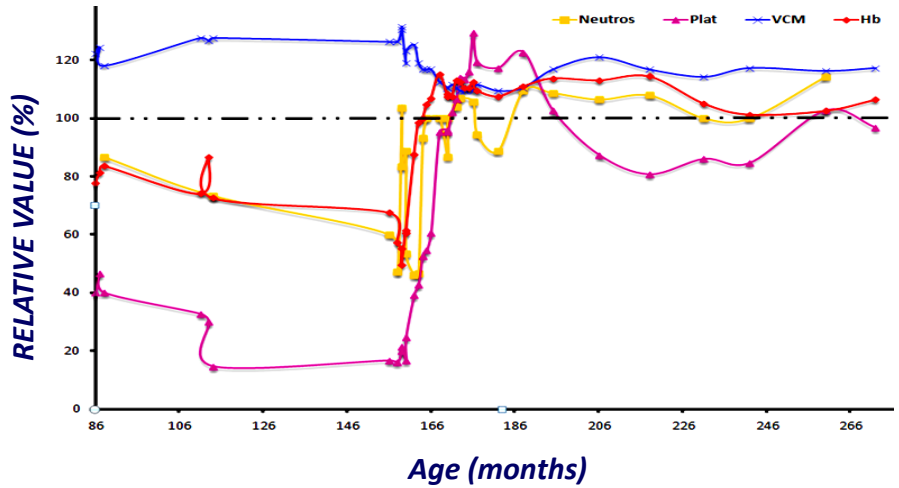


Gosh et al., 2015

- ✓ **Somatic Mosaicism: Natural Gene Therapy**

  (Waisfisz et al. Nat Genet 1999)

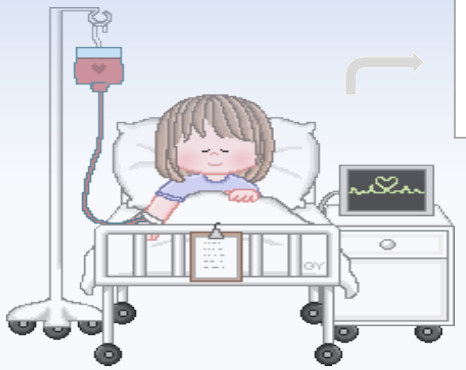
- ✓ **Proliferative advantage of human FA-HSCs corrected by Gene Therapy** (Rio*, Navarro* *et al*. Blood, 2017)



J.Surralles

HSC collection and Gene Therapy Trials of FA-A patients

Fancostem

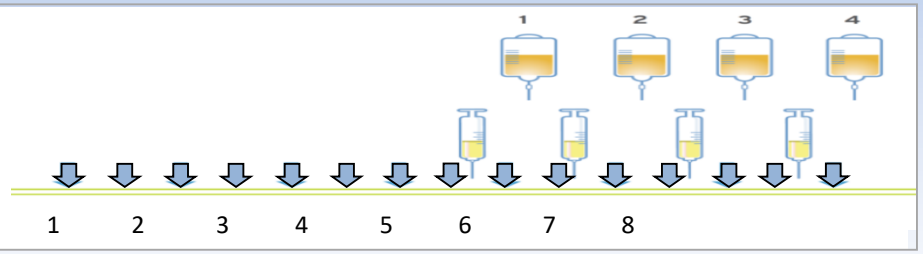


Apheresis

Plerixafor (240 µg/kg/day)

G-CSF (12 µg/kg/12h)

Day



CD34⁺ selection

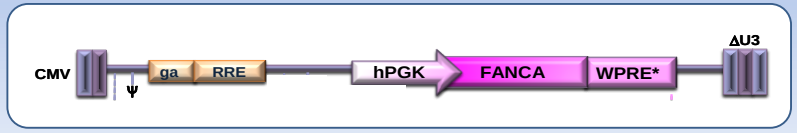
Fresh

Frozen

Inclusion criteria: **One parameter higher than:**

Hb: 8.0 g/dL; Neutrophils: 750/mm³; Platelets:30,000/mm³.

CD34⁺



Pre-stimulation
(8 h)

PGKFANCA:LV (12h)

Final product

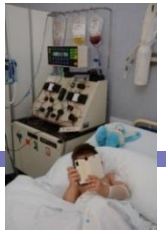
Transduced CD34⁺ cells

NO CONDITIONING

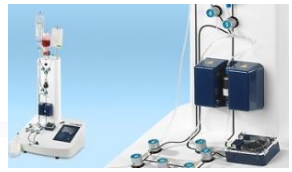
Fancolen

Inclusion criteria: **One parameter lower than:**

Hb: 8.0 g/dL; Neutrophils: 1,000/mm³; Platelets:50,000/mm³.



FANCOSTEM: Purification of mPB CD34⁺ cells



	Apheresis			CD34⁺ immunoselection		
Patient	N^{er} Aph.	**CD34⁺/kg**	**% CD34⁺**	**CD34⁺/kg**	**% CD34⁺**	**Recovery**
01003	3	1.6x10^6	0.05	8.6x10^5	17.4	53.2
02002	2	7.2x10^6	0.16	5.1x10^6	80.0	70.9
02003	3	7.6x10^6	0.17	2.9x10^6	29.3	47.8
02004	3	6.6x10^6	0.15	2.0x10^6	25.6	26.6
02005	2	4.8x10^6	0.11	1.1x10^6	48.7	22.7
01005	2	2.6x10^6	0.18	1.0x10^6	51.8	48.7
02006 *	2	3.1x10^6	0.12	1.3x10^6	8.4*	41.0
02007 *	3	9.9x10^5	0.03	6.0x10^5	1.8*	60.6
02008 $	2	2.8x10^6	0.08	3.4x10^5	1.5$	12.0
MEDIAN *(Range)*	**2** *(2-3)*	**3.1x10^6** *(0.9-7.6x10^6)*	**0.12** *(0.03-0.18)*	**1.1x10^6** *(0.6-5.1x10^6)*	**25.6** *(1.8-80.0)*	**48.0** *(22.7-70.9)*

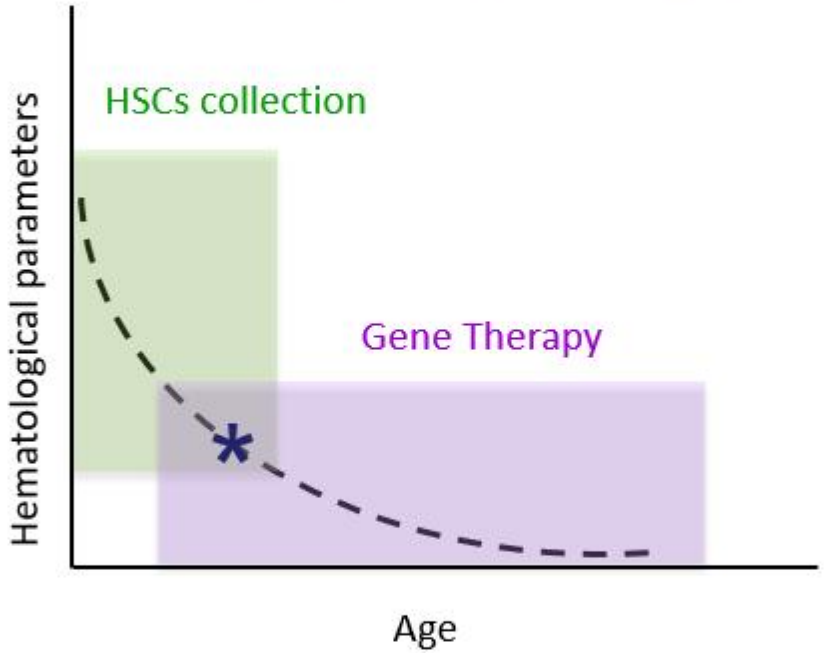
* CD34⁺ cell immunoselection using depletion tubing set

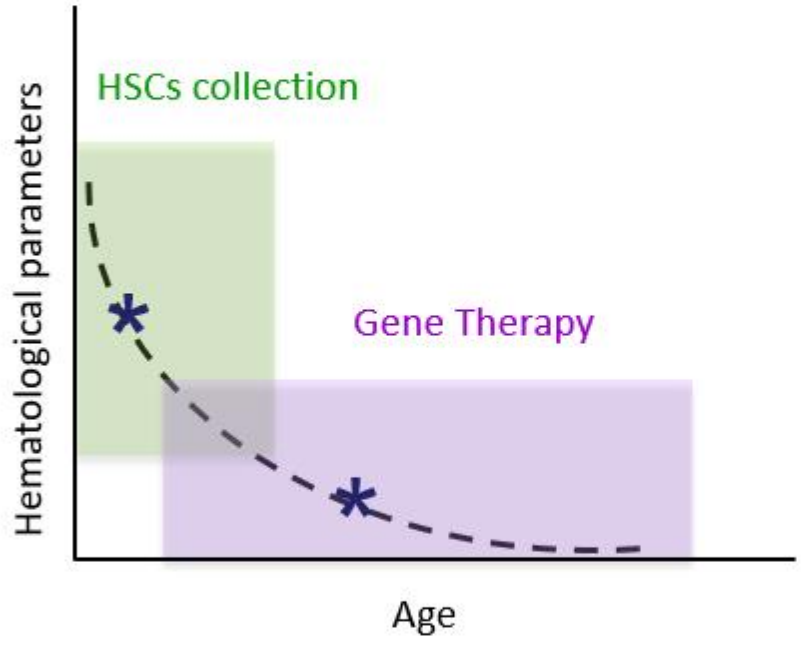
$ CD34⁺ cell immunoselection with gentle washing

Strategy to mobilize and transduce HSCs from FA-A patients included in FANCOLEN

FA-02005 (4 years old): **Hb 12.5 g/dL; NEUTROPHILS 1,680/mm^3; PLATELETS 38,000/mm^3**

FA-02006 (6.6 years old): **Hb 11.3 g/dL; NEUTROPHILS 760/mm^3; PLATELETS 79,000/mm^3**

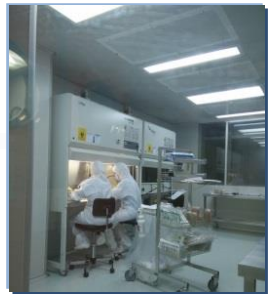




FA-02002 (5 years old): **Hb 10.5 g/dL; NEUTROPHILS 1,600/mm^3; PLATELETS 29,000/mm^3**

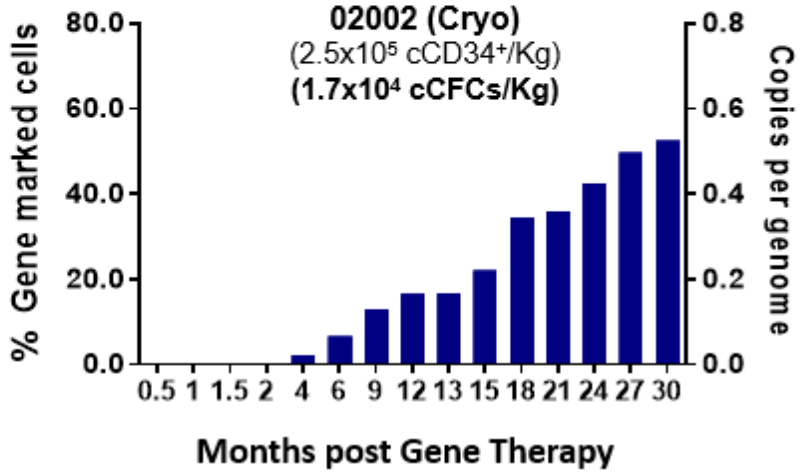
FA-02004 (7.6 years old): **Hb 10.8 g/dL; NEUTROPHILS 900/mm^3; PLATELETS 46,000/mm^3**

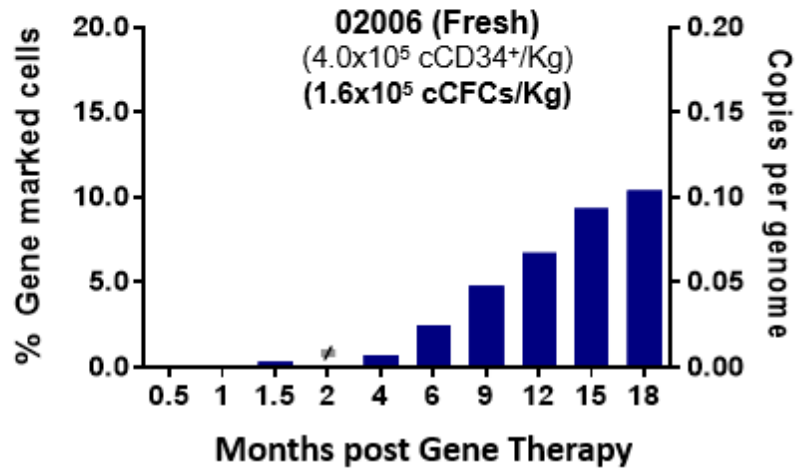
FANCOLEN: Infusion products

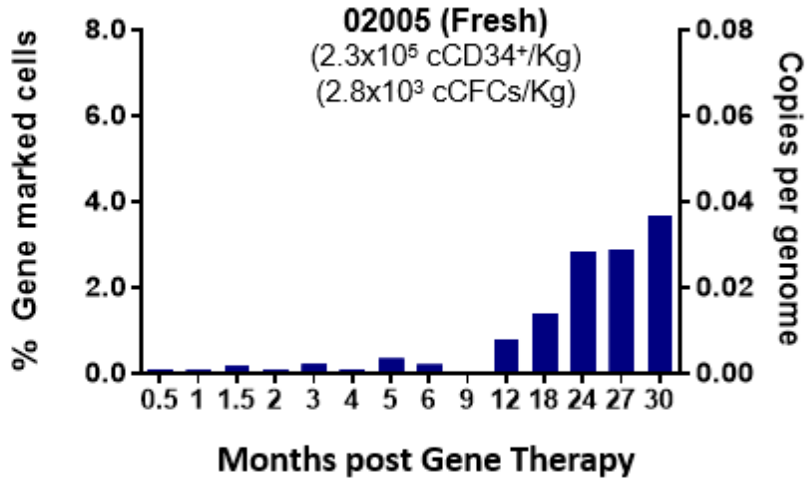


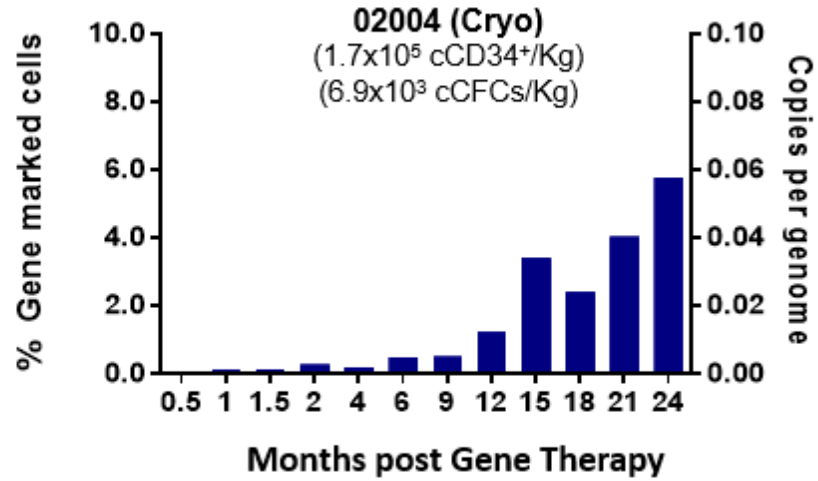
PATIENT	N^{er} of infused cells/kg	CD34^{+} cells/ kg	% CD34^{+}	Mean VCN/cell (CFCs)	Transduced CD34^{+}cells/kg	Transduced CFCs/kg	MMC survival
02005 (Fresh)	1.7x10^{6}	1.40x10^{6}	80.5%	0.17	2.3x10^{5}	2.8x10^{3}	12.1%
02002 (Cryopr)	6.0x10^{5}	0.56x10^{6}	90.6%	0.45	2.5x10^{5}	1.7x10^{4}	30.3%
02004 (Cryopr)	1.2x10^{6}	0.72x10^{6}	57.0%	0.23	1.7x10^{5}	6.9x10^{3}	27.0%
02006* (Fresh)	2.1x10^{7}	0.77x10^{6}	3.5%	0.53	4.1x10^{5}	1.6 x10^{5}	49.2%

Kinetics of Gene-marked Cells in PB of Non-conditioned FA Patients Infused with Transduced CD34⁺ cells





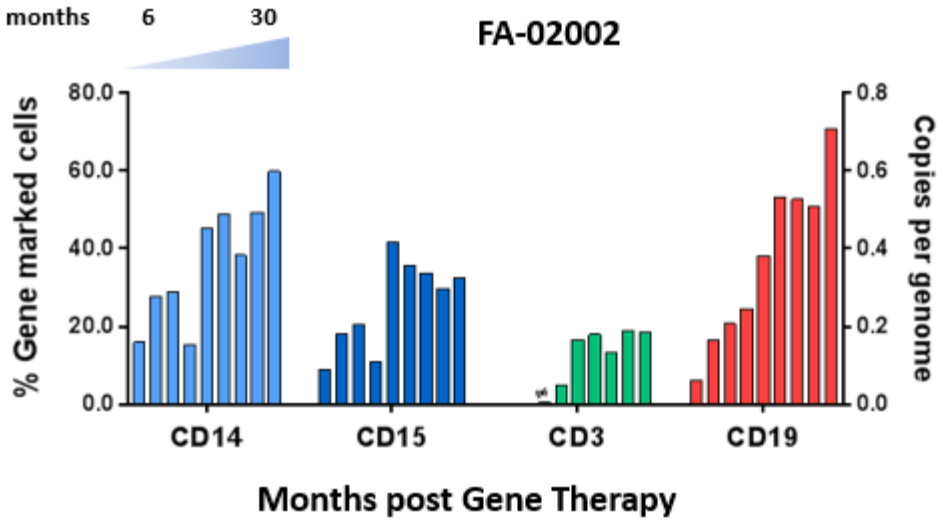


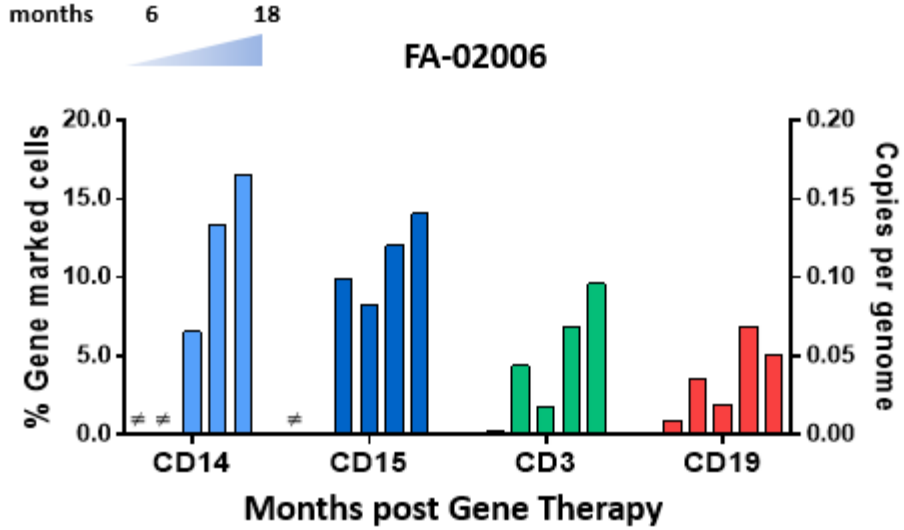


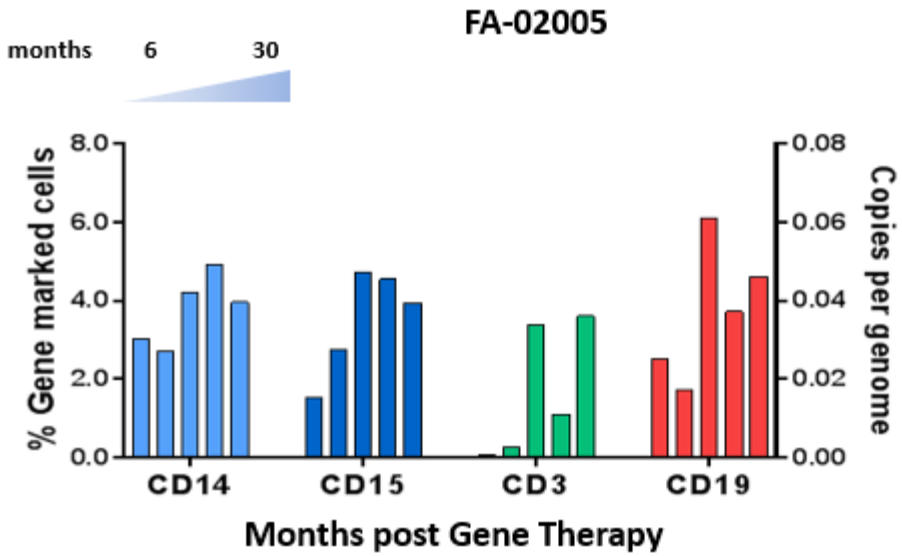
Additional preliminary data from 24-36 month follow-up indicate progressive increases in PB VCNs

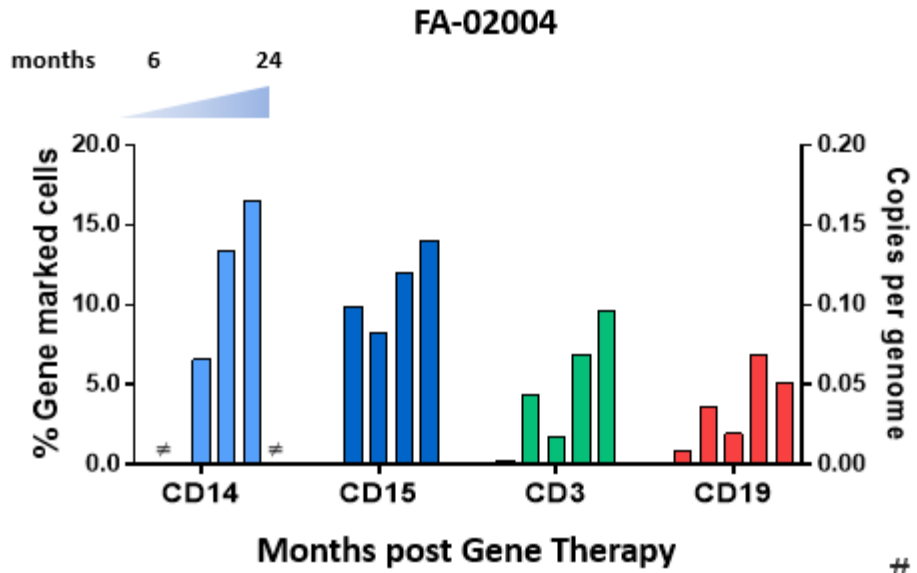
Not analyzed

Progressive increase of gene-marked subpopulations in PB



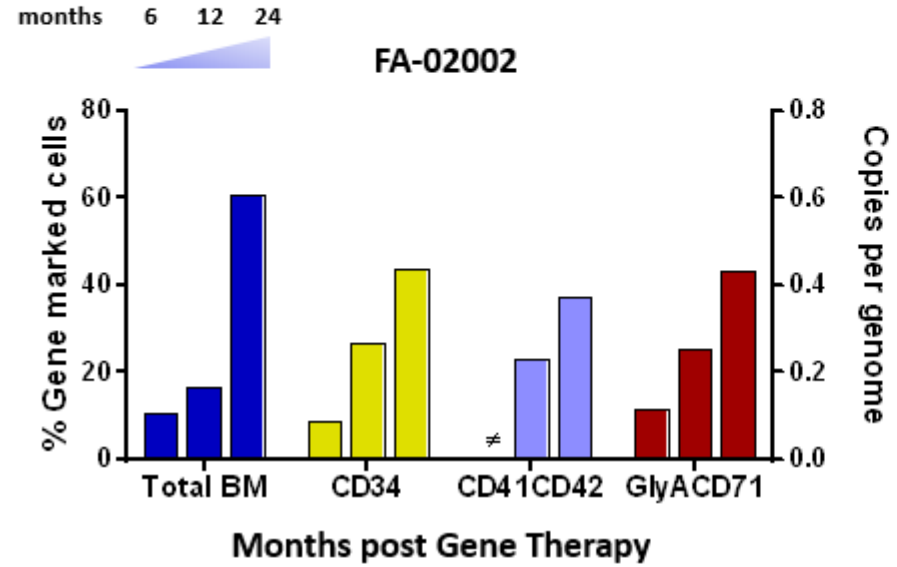


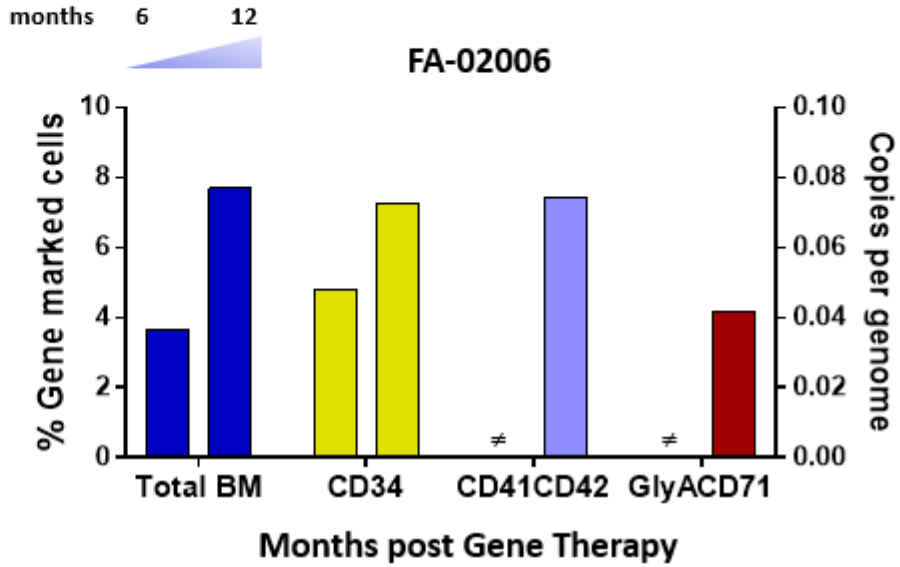


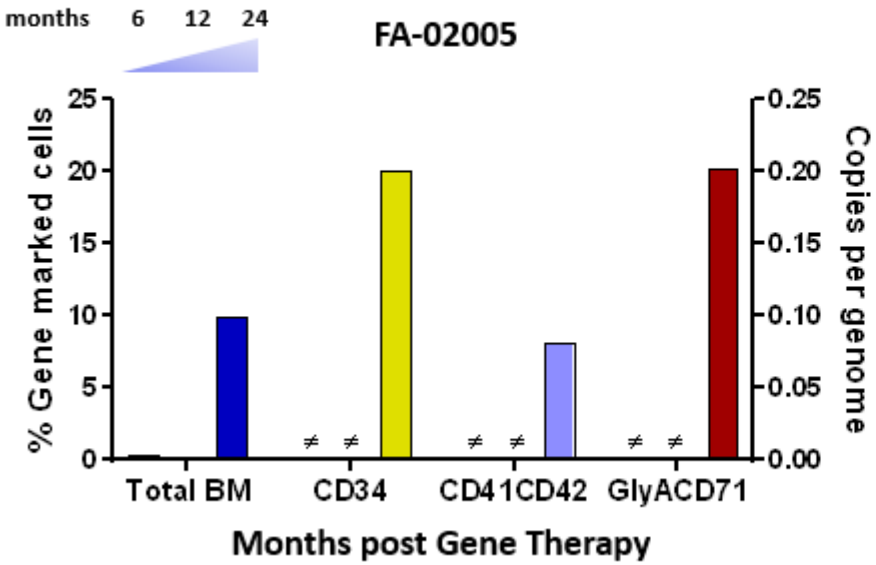


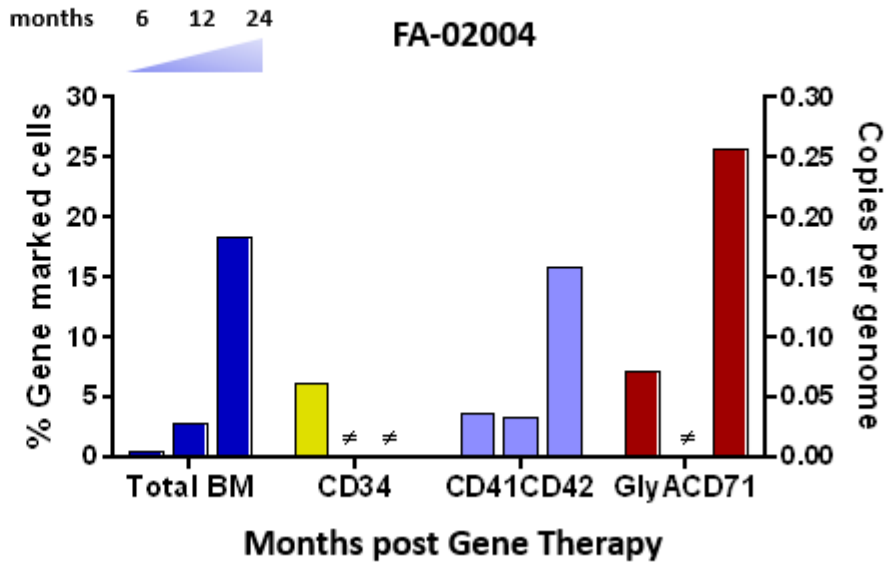
Not analyzed

Progressive increase of gene-marked subpopulations in BM including CD34⁺ cells



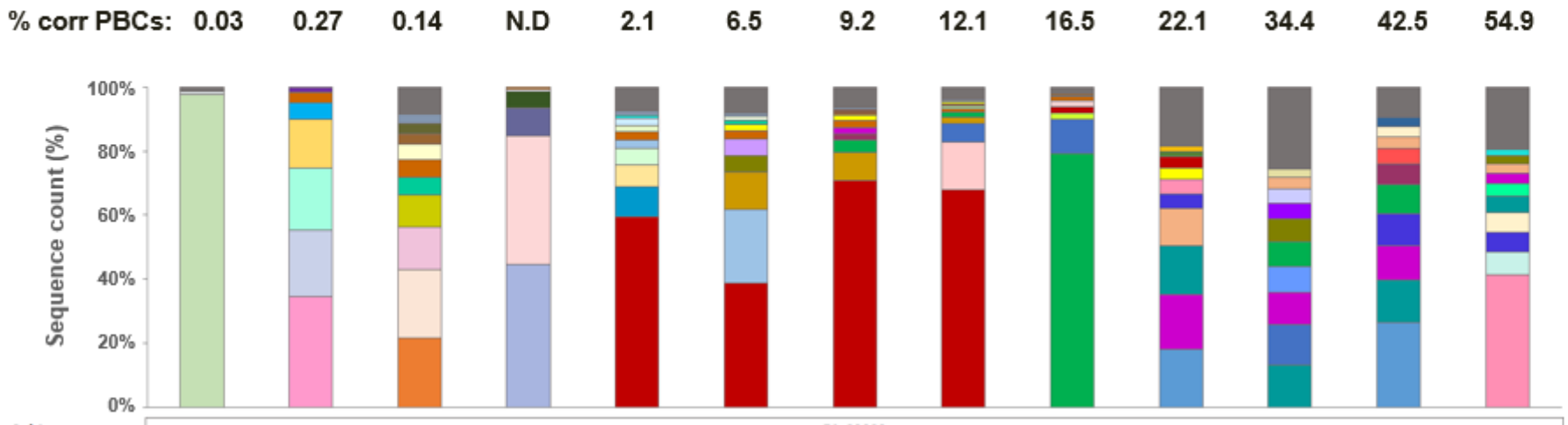




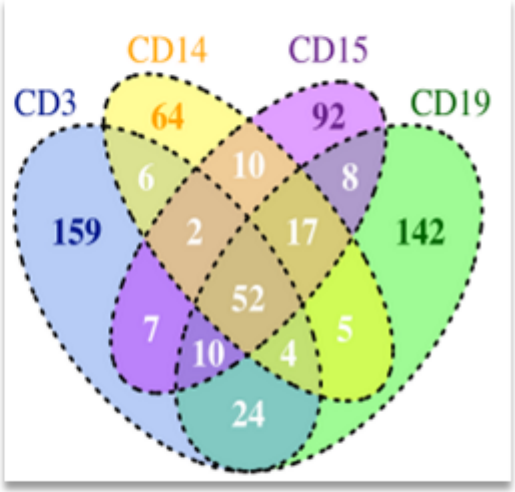


Not analyzed

Integration Site Analysis in Patient 02002



Subject	FA-02002												
Cell Type	PBMCs												
Time Point	2W	4W	6W	2M	4M	6M	7M	9M	12M	15M	18M	24M	30M
Rank													
Top1	SLC37A1	MAP4K3	FCHSD2	FAM129B	FCHSD2	FCHSD2	FCHSD2	FCHSD2	KIAA0232	PPP2R2B	PELP1	PPP2R2B	ADRBK2
Top2	LOC101929555	PDSS2	ALDH6A1	SEPT9	GRB2	PRKACA	RAB40C	FCHSD2	MIR612	CALCRL	MIR612	PELP1	SESN1
Top3	CREM	IRX3	HERC1	FAM129B	RTN3	RAB40C	KIAA0232	MIR612	ARHGAP35	PELP1	CALCRL	CALCRL	VPS8
Top4	LINC00706	STAM	SNX32	STRBP	NKG7	NBEAL1	SCYL1	RAB40C	FCHSD2	PARD3B	HIST2H2AA3	VPS8	HNRNPUL2
Top5	RYR3	MORC3	MECP2	SUZ12	PRKACA	SH3BGRL	CALCRL	KIAA0232	CDCA5	VPS8	KIAA0232	KIAA0232	PELP1
Top6	TSPEAR	UNK	MAPKBP1	ACP5	SPINT2	LINC01467	BPTF	SH3BGRL	RASGRP2	ADRBK2	NBEAL1	SCYL1	NCAPD2
Top7		RGS18	RBM6	MYO1H	RPS6KA1	UBE2F-SCLY	UBE2F-SCLY	ASH1L	LRP1B	UBE2F-SCLY	NR3C1	LRP1B	CALCRL
Top8		ARNT	C16orf62	SUZ12P1	CLIC4	HOOK1	LOC100996291	CALCRL	RAB40C	FCHSD2	DIP2B	PARD3B	PARD3B
Top9		TRAPPC10	UBP1	MIR4472-2	TRAPPC9	CCDC179	PPP2R2B	UBE2F-SCLY	DHX34	NUDT3	PARD3B	HNRNPUL2	NBEAL1
Top10		SS18	PEX14	RPTOR	NSD1	SCYL1	NUDT3	PELP1	ASH1L	MAPRE1	RTN3	RANBP9	TRAPPC9
UIS	6	26	45	20	107	63	98	139	56	126	131	29	104

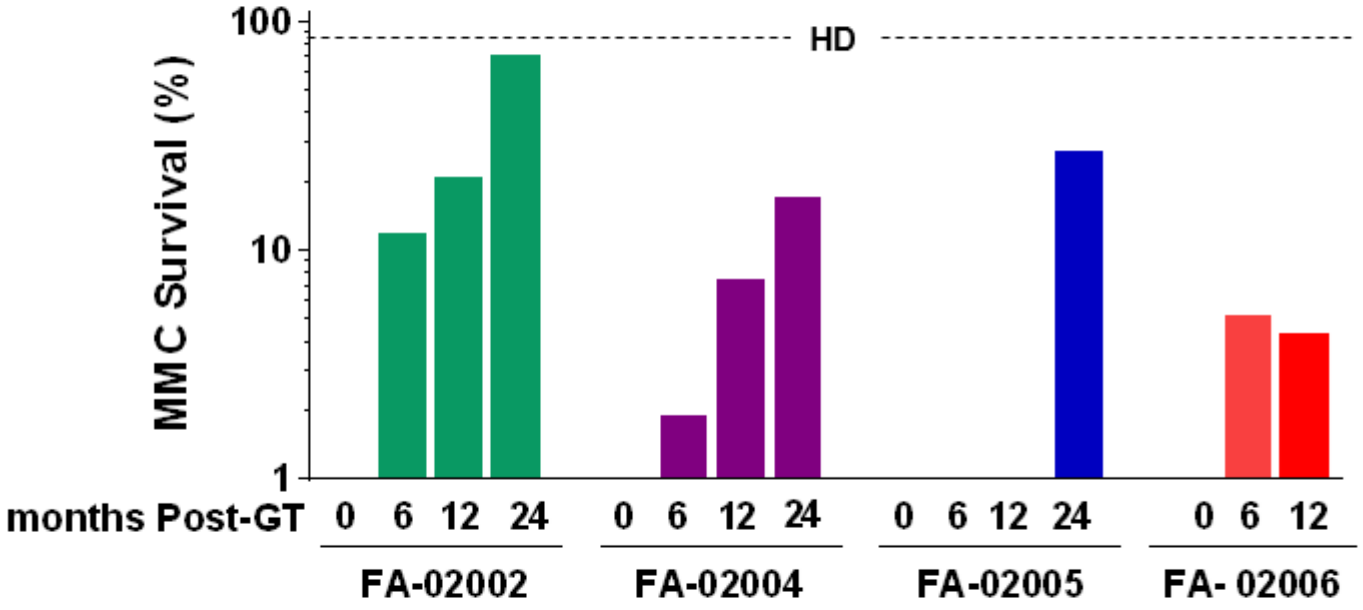


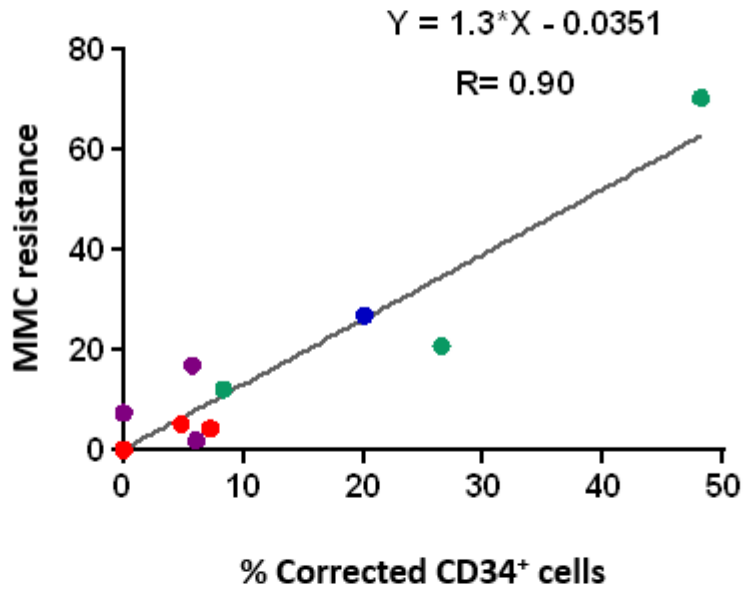
Wang, Wei (GeneWerk GmbH)

Session: Clinical Gene Therapies for Blood Diseases

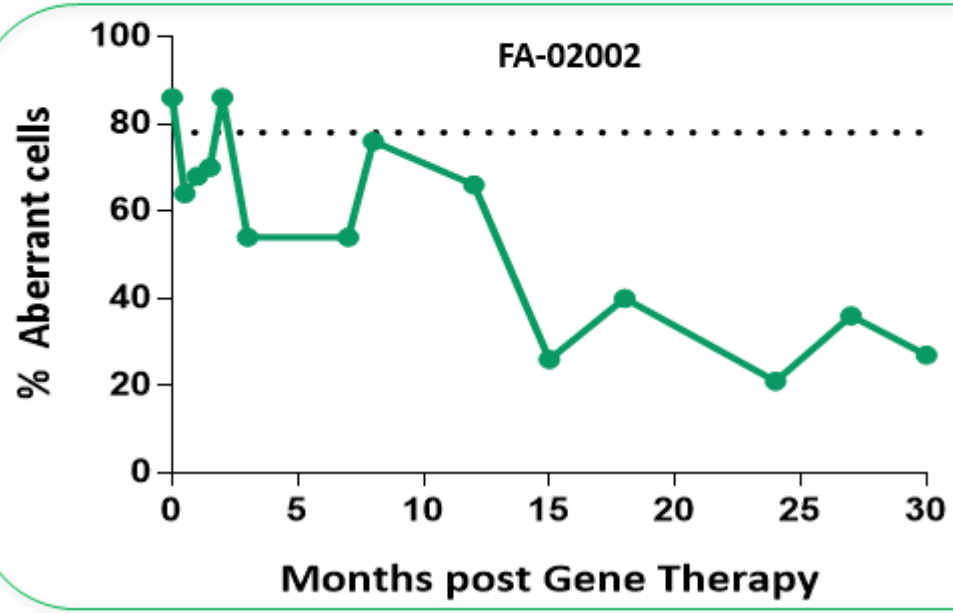
April 29th, 11:15-11:30 (Location: Jefferson)

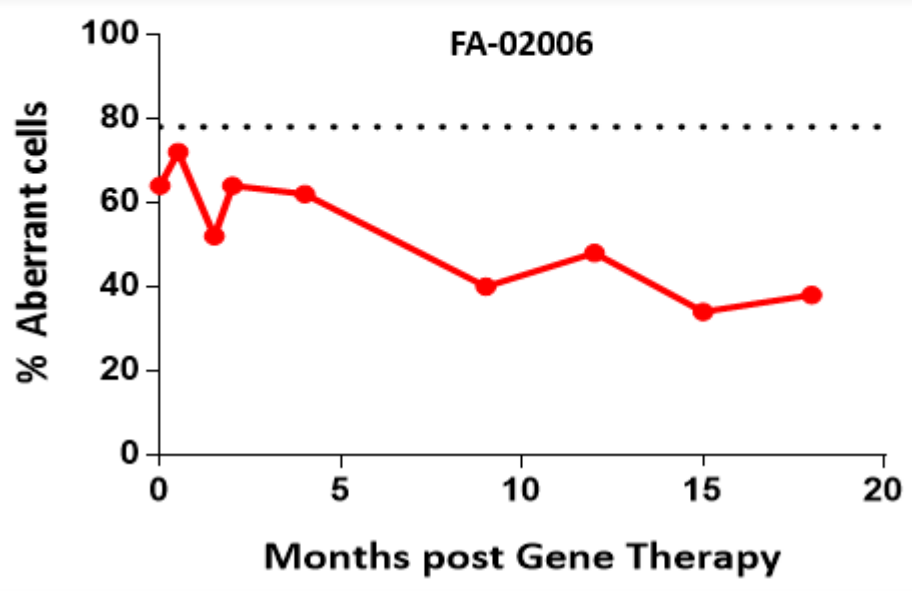
Progressive Phenotypic correction of BM CFCs (MMC-Resistance)

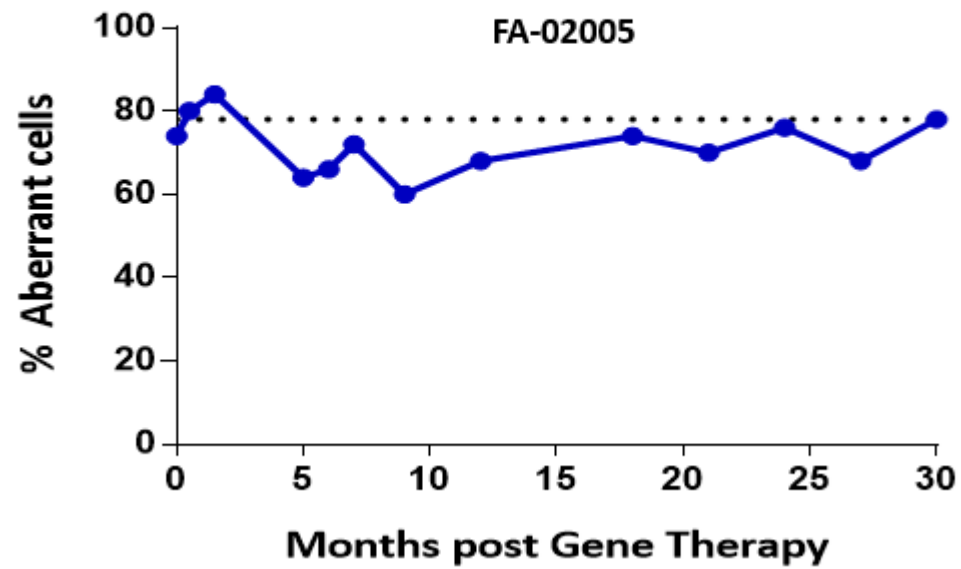


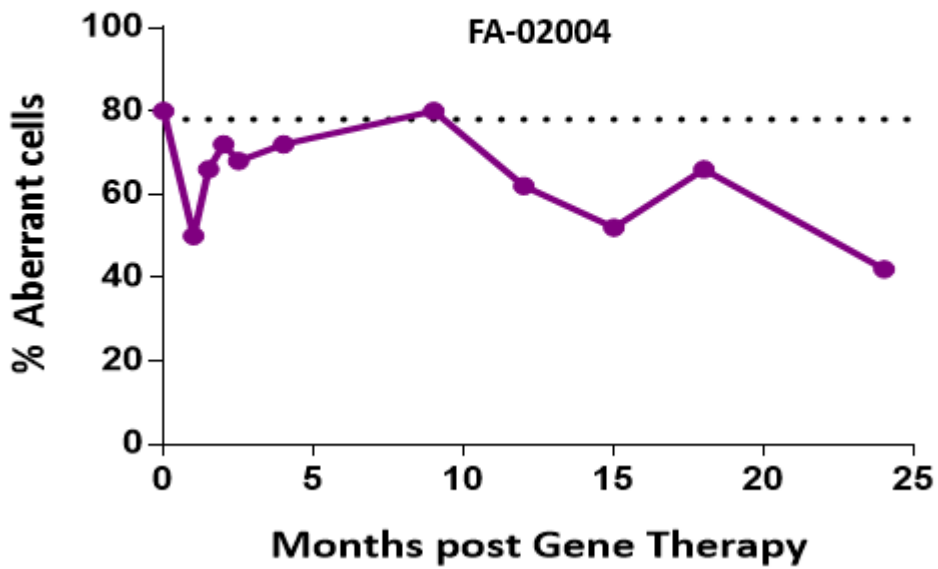


Progressive Phenotypic correction of PB T Cells (DEB-Induced Chromosomal Instability)

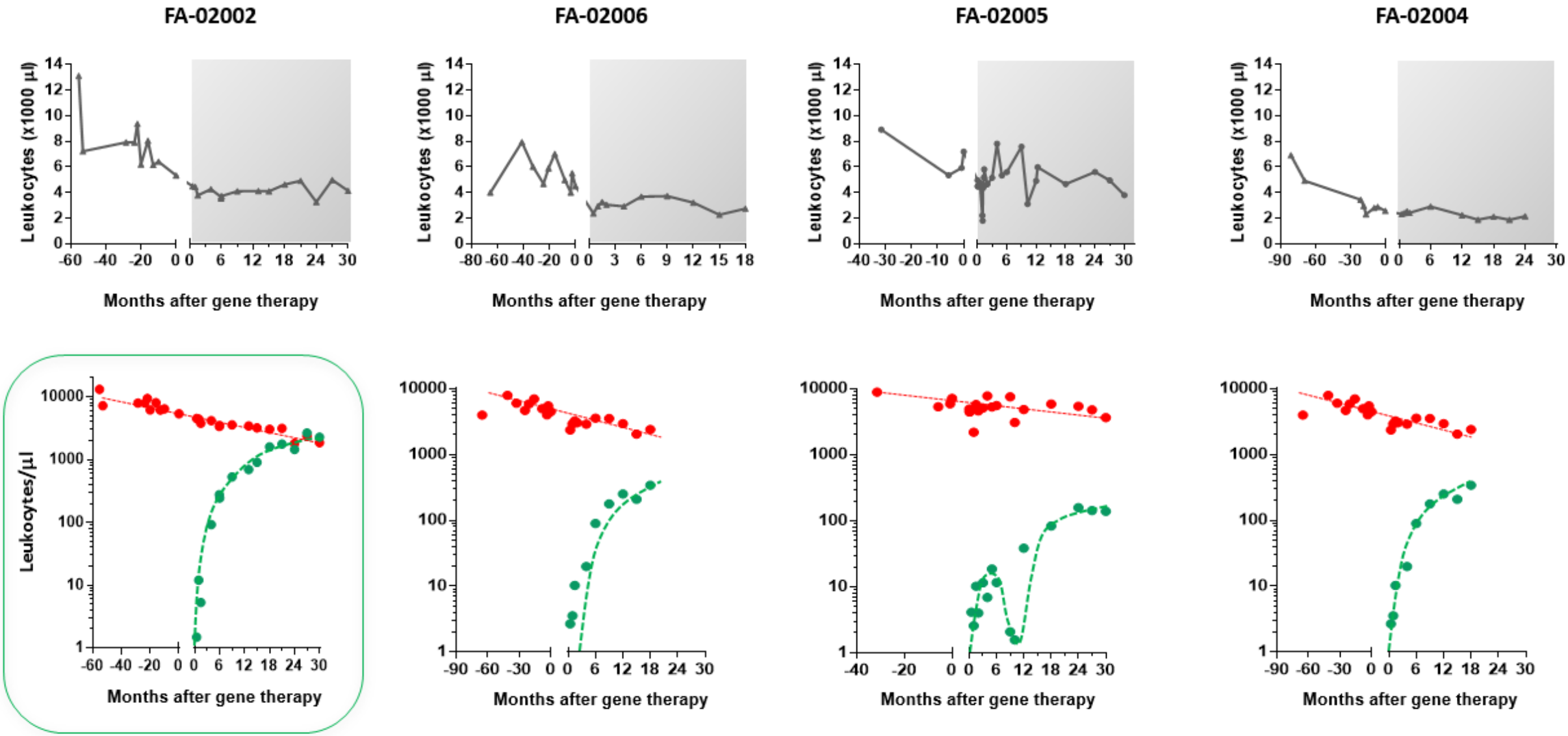






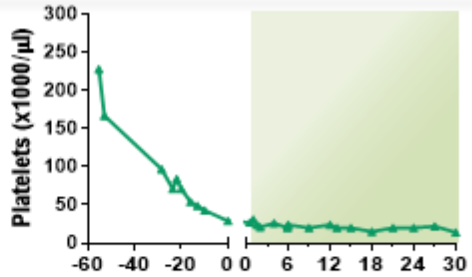


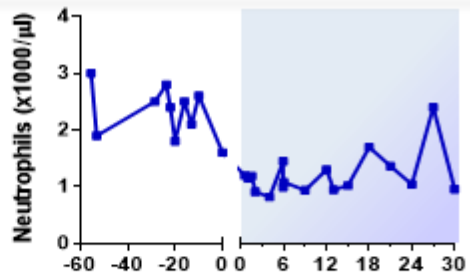
Kinetics of corrected and uncorrected PB leukocytes prior to and after Gene Therapy

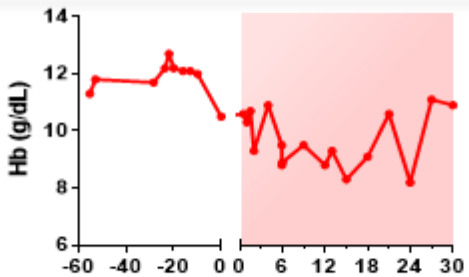


PB Cell Counts in FA Patients prior to and after Gene Therapy

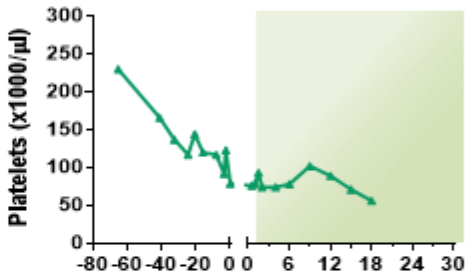
02002 (Cryo)
2.5x10⁵ cCD34⁺/Kg
1.7x10⁴ cCFU/Kg

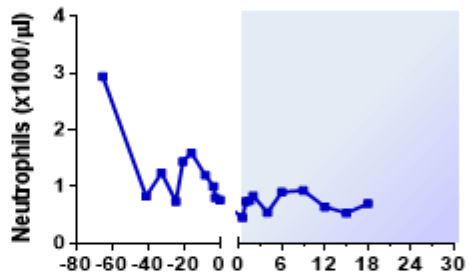


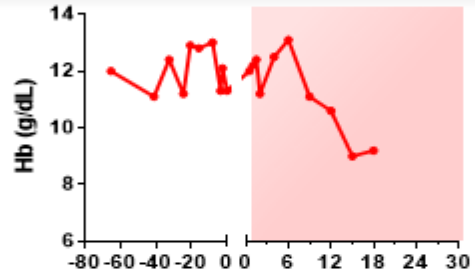




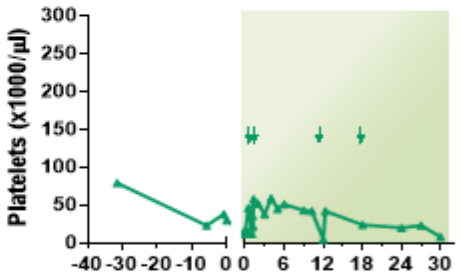
02006 (Fresh)
4.0x10⁵ cCD34⁺/Kg
1.6x10⁵ cCFU/Kg

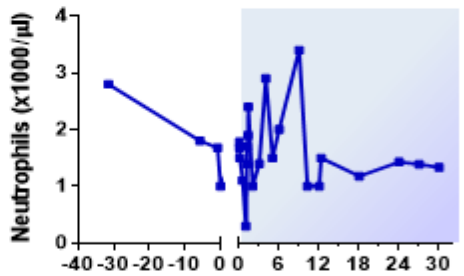


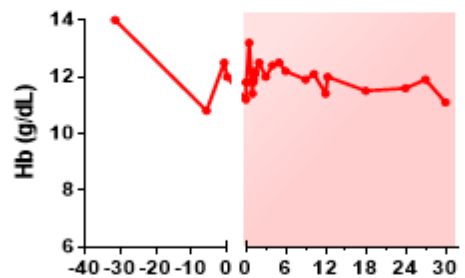




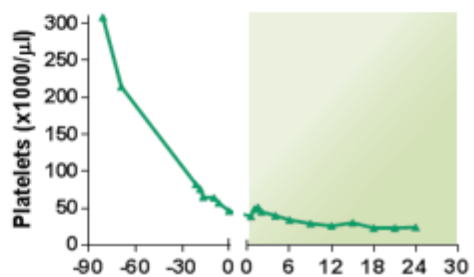
02005 (Fresh)
2.3x10⁵ cCD34⁺/Kg
2.8x10³ cCFU/Kg

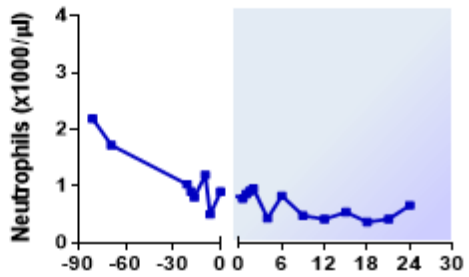


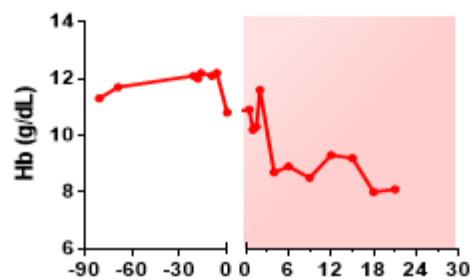




02004 (Cryo)
1.7x10⁵ cCD34⁺/Kg
6.9x10³ cCFU/Kg







Months after gene therapy

Months after gene therapy

Months after gene therapy

Conclusions

- ❑ **No Severe Adverse Events**
- ❑ **Collection of clinically relevant numbers of mPB CD34^{+} cells**
- ❑ **Infusion of corrected CD34^{+} cells in <u>non-conditioned FA patients</u>:**
  - ✓ **Engraftment and repopulation advantage of gene-corrected HSPCs**
  - ✓ **Oligoclonal reconstitution and gene correction of multipotent HSCs**
  - ✓ **Phenotypic correction of BM progenitors and PB T cells**
  - ✓ **Stabilization of the BMF**
  - ✓ **Additional preliminary data from 24-36 month follow-up indicate progressive genetic and phenotypic reversal**

Next Steps

- ***FANCOLEN I: Long term follow up protocol (15 years)***
- ***FANCOLEN II: Hospital Niño Jesús (Madrid)***
  - **Infusion of higher numbers of corrected HSCs:**
    - ***Collection / infusion of HSCs prior to BMF (Fresh/Fresh)***
    - ***Improved transduction of HSCs (transduction enhancers)***
  - **Commercial vector**
- ***Stanford University: Phase 1 Clinical Trial (n=2)***