Lentiviral-Mediated Gene Therapy (RP-L102) for Fanconi Anemia [Group A] is Associated with Polyclonal Integration Patterns in the Absence of Conditioning

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Disclosures

- Consultant: 48 Bio, GV, Lyrik Therapeutics, Prime Medicine, Spotlight Therapeutics, Stemodontics, Teiko Bio
- Current Equity: 48 Bio, Beam Therapeutics, Decibel Therapeutics, Editas Medicine, GV, Lyrik Therapeutics, Magenta Therapeutics, Prime Medicine, Spotlight Therapeutics, Stemodontics, Teiko Bio, Divested Equity in last 24 months: Forty Seven, Inc., Global Blood Therapeutics
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Introduction

Fanconi anemia (FA) is a rare inherited disorder of defective deoxyribonucleic acid (DNA) repair characterized by:[1-4]

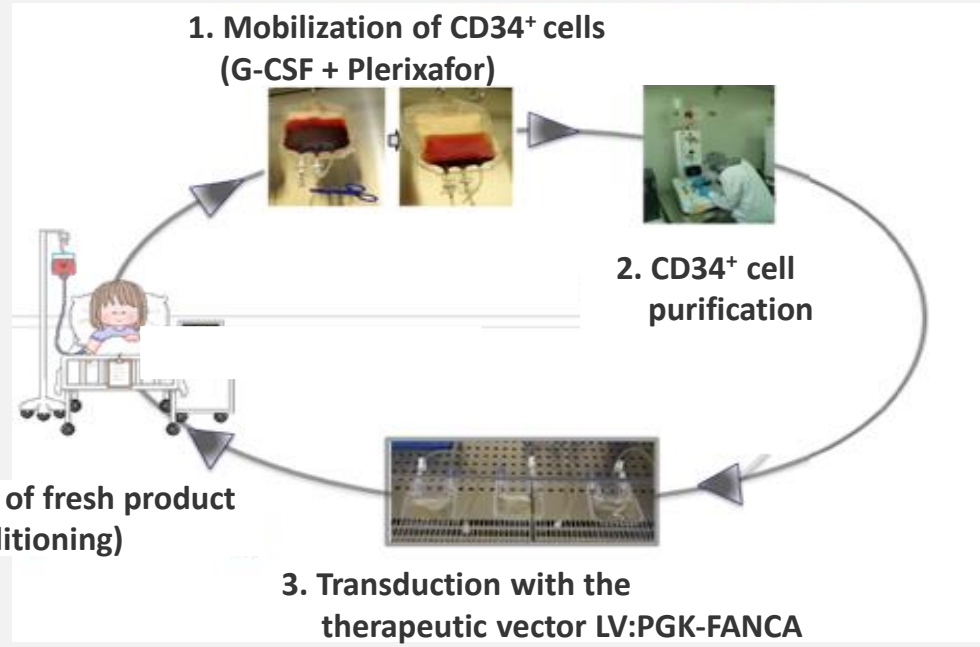
- Progressive bone marrow failure (BMF)
- Cancer Predisposition
- Congenital abnormalities

FA represents a significant unmet medical need[1-4]

- FA-A accounts for ~70% of FA
- Allogeneic HSCT is potentially curative of the hematologic component but confers significant toxicity
  - 100-day transplant-associated mortality
  - GvHD
  - 3-4× increased risk for solid organ malignancies
  - Long-term coronary artery disease, endocrinopathies & neurocognitive dysfunction

RP-L102 Rationale

- Insertion of a functional *FANCA* gene into autologous FA-A CD34+ cells confers resistance to DNA-damage & provides proliferative advantage to modified cells enabling engraftment without cytotoxic conditioning[5]



GvHD=graft versus host disease; LV=lentiviral vector.

1. Anur P et al. *Bone Marrow Transplant.* 2016; 51(7): 938-944; 2. Ebens C et al. *Biol Blood Marrow Transplant.* 2018; 24(4):765-771; 3. Guardiola P et al. *Blood.* 2004;103(1): 73-77; 4. Mehta P et al. *Blood.* 2017; 129(16): 2308-2315; 5. Río P *et al*. Nat Med 2019; 25:1396-1401.

Global RP-L102 Studies

Key Eligibility Criteria

Inclusion:

- FA complementation group A
- Minimum age: 1y
- BM CD34+ cell concentration: ≥30/µL (from aspirate)

Exclusion:

- Available & eligible HLA-identical sibling donor
- MDS or leukemia (including associated cytogenetic abnormalities)
- Mosaicism with stable/improved blood counts

Endpoints

Efficacy:

Phenotypic correction: Increased resistance of BM progenitor cells (CFCs) to DNA-damaging agent mitomycin-C (MMC)

Engraftment: Peripheral blood (PB) and BM vector copy number (VCN)

Clinical response: Hematologic stabilization

Safety: Evaluation of safety and tolerability

BM=bone marrow; BMF=bone marrow failure; FA=Fanconi Anemia; HLA=human leukocyte antigen; MDS=myelodysplastic syndrome; Ph=Phase.

Demographics and Investigational Product Metrics

n=14 patients

Patient #14	Age at Enrollment (years)	Follow Up (months)	CD34+ Cell Dose (cells/kg)	CFCs/kg	Mean VCN: Liquid Culture	Mean VCN: CFCs	Transduction Efficiency in CFCs (%)	CFC Survival MMC 10nM (%)
1 (1001)	5	54	2.0×10^5	5.2×10^4	2.08	0.62*	67	33
2 (1002)	6	18†	3.7×10^5	5.0×10^4	2.21	0.92*	72	47
3 (2004)	3	42	4.8×10^5	1.3×10^5	1.70	0.73	100	63
4 (2008)	2	36	3.2×10^6	5.5×10^5	1.65	1.56	97	63
5 (2009)	3	28‡	1.9×10^6	3.1×10^5	2.16	0.76	61	45
6 (2010)	3	36	4.1×10^6	n/a	0.62	n/a	n/a	n/a
7 (2011)	5	36	2.8×10^6	n/a	1.46	n/a	n/a	n/a
8 (2014)	6	32	5.4×10^5	3.6×10^4	3.68	1.93	87	31
9 (2016)	2	32	3.0×10^5	2.5×10^4	1.96	0.64	88	64
10 (2021)	2	21	2.3×10^6	4.3×10^5	1.55	1.97	78	38
11 (2023)	5	18	2.5×10^5	2.8×10^4	1.70	2.16	87	50
12 (2024)	1	15	1.8×10^6	1.7×10^5	1.69	1.91	88	93
13 (2026)	1	4	1.9×10^6	1.2×10^5	1.30	0.80	75	51
14 (2025)	3	2	3.5×10^5	5.6×10^4	3.45	2.50	92	63

All patients ≤ 6 years old at enrollment

12 patients have ≥ 12 months of follow-up

Trial enrollment is complete

Median Values

VCN (liquid culture): 1.70
VCN (CFC): 1.75
Transduction efficiency: 87.0%
CFC MMC-resistance: 50.5%

*Mean CFC VCN was assessed from a cryopreserved drug product sample.

†Patient withdrawn from RP-L102 study at 18 months post-RP-L102 infusion; received successful allogeneic HSCT for BMF. Safety follow up continues on LTFU study.

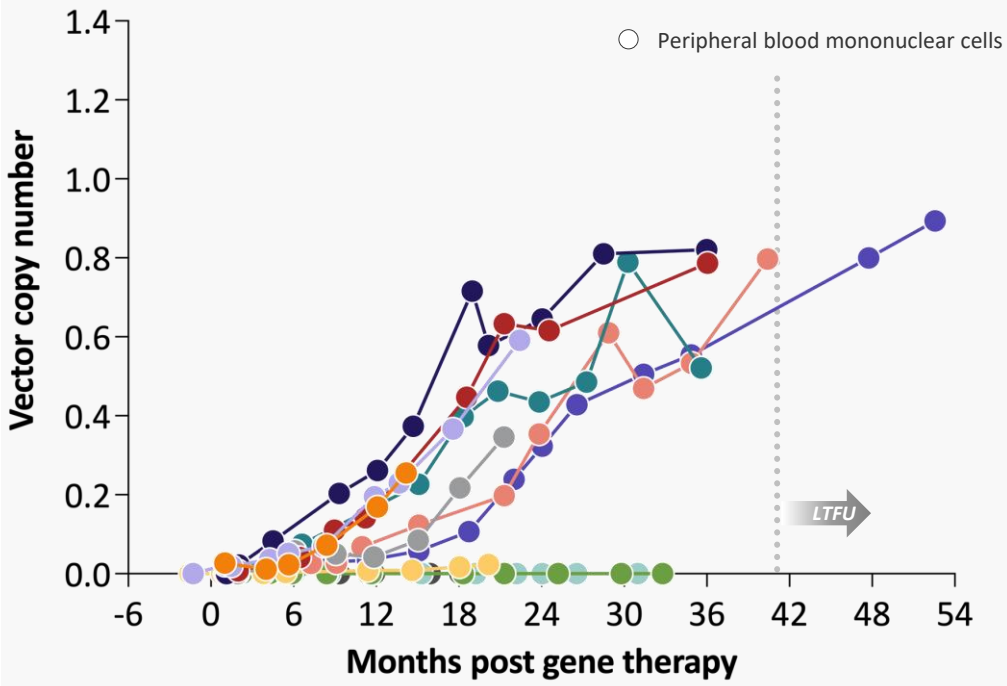
‡Patient withdrawn from RP-L102 study at 28 months post-RP-L102 infusion; received successful allogeneic HSCT for NHL. Safety follow up continues on LTFU study.

BMF=Bone marrow failure; CFC=colony-forming cell; HSCT=Hematopoietic stem cell transplantation; LTFU=long-term follow-up; MMC=Mitomycin-C; n/a: not available; NHL=Non-Hodgkin Lymphoma; VCN=vector copy number.

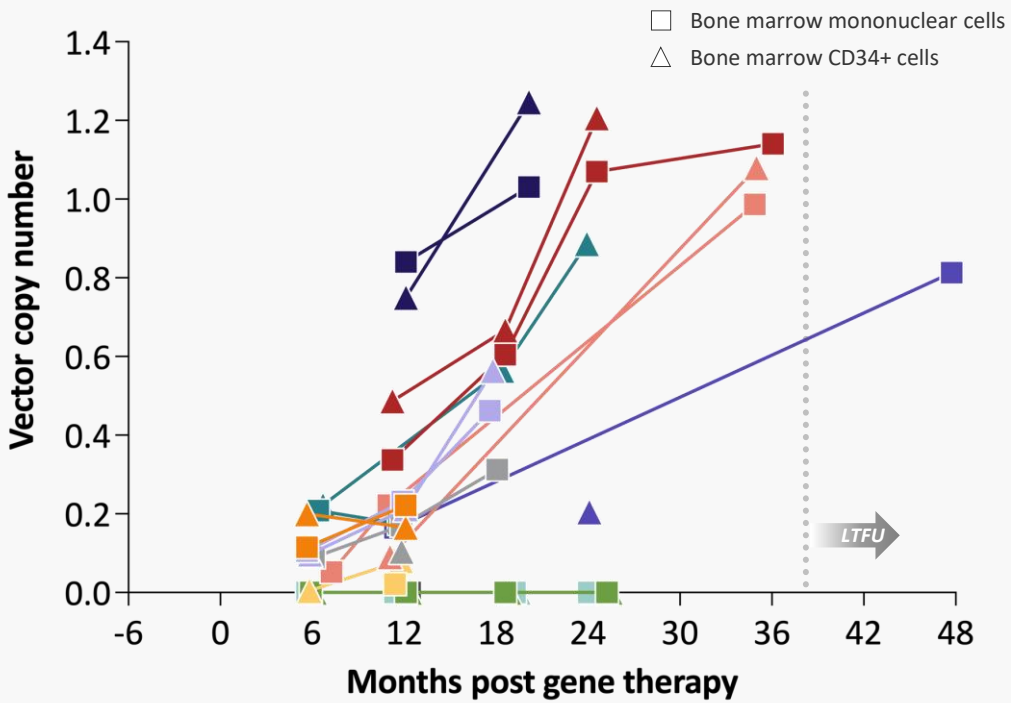
Data cutoff: September 11, 2023

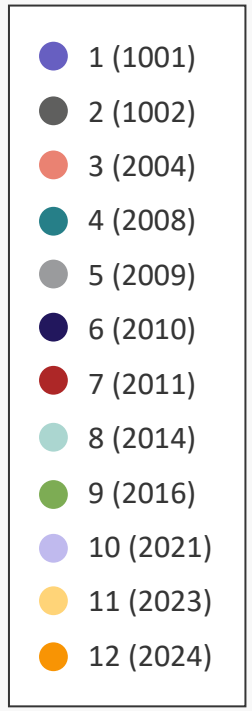
Progressively Increasing and Sustained Genetic Correction in 8 of 12 Patients at ≥1 year post-RP-L102

Peripheral Blood (PB)



Bone Marrow (BM)*





Progressive gene marking increases in PB and BM associated with hematologic stability

*Vector copy number in bone marrow not available at some stipulated time points due to insufficient sample to perform assay.
LTFU=long-term follow-up.

Data cutoff: September 11, 2023

Insertion Site Analysis (ISA) Methodology

- ISA was performed using INSPIIRED (INtegration Site Pipeline for paired-End reaDs)
- Samples‡ analyzed for:
  - abundance of individual integration sites over time
  - presence of common integration sites
  - diversity/polyclonality of samples over time
  - proximity of integrations to proto-oncogenes

Gini index: Describes how equally clones contribute to IS pool.

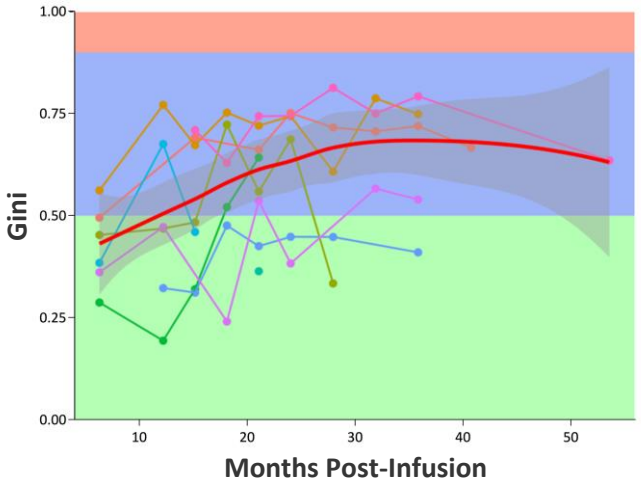
Low value: polyclonal insertion pattern and even sequence distribution

High value: unequal contribution of more dominant sequences to the overall sequence pool

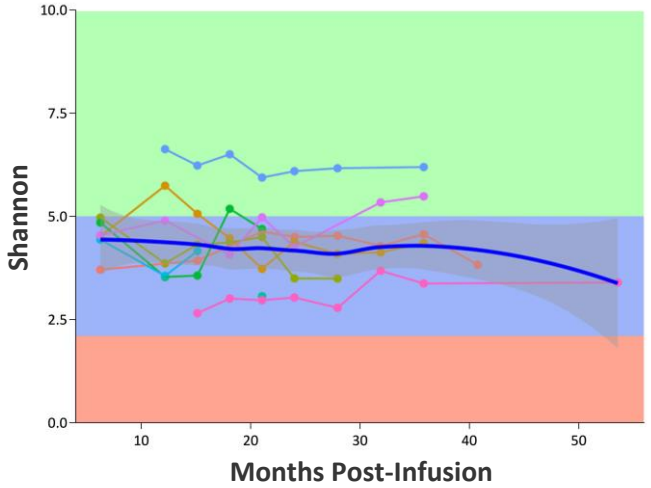
Shannon index: Describes the overall diversity of the IS pool

- ≤2.1: oligoclonal
- >2.1 and ≤5.0: polyclonal
- >5.0: highly polyclonal

Gini Index across Phase 2 Studies*



Shannon Index across Phase 2 Studies*



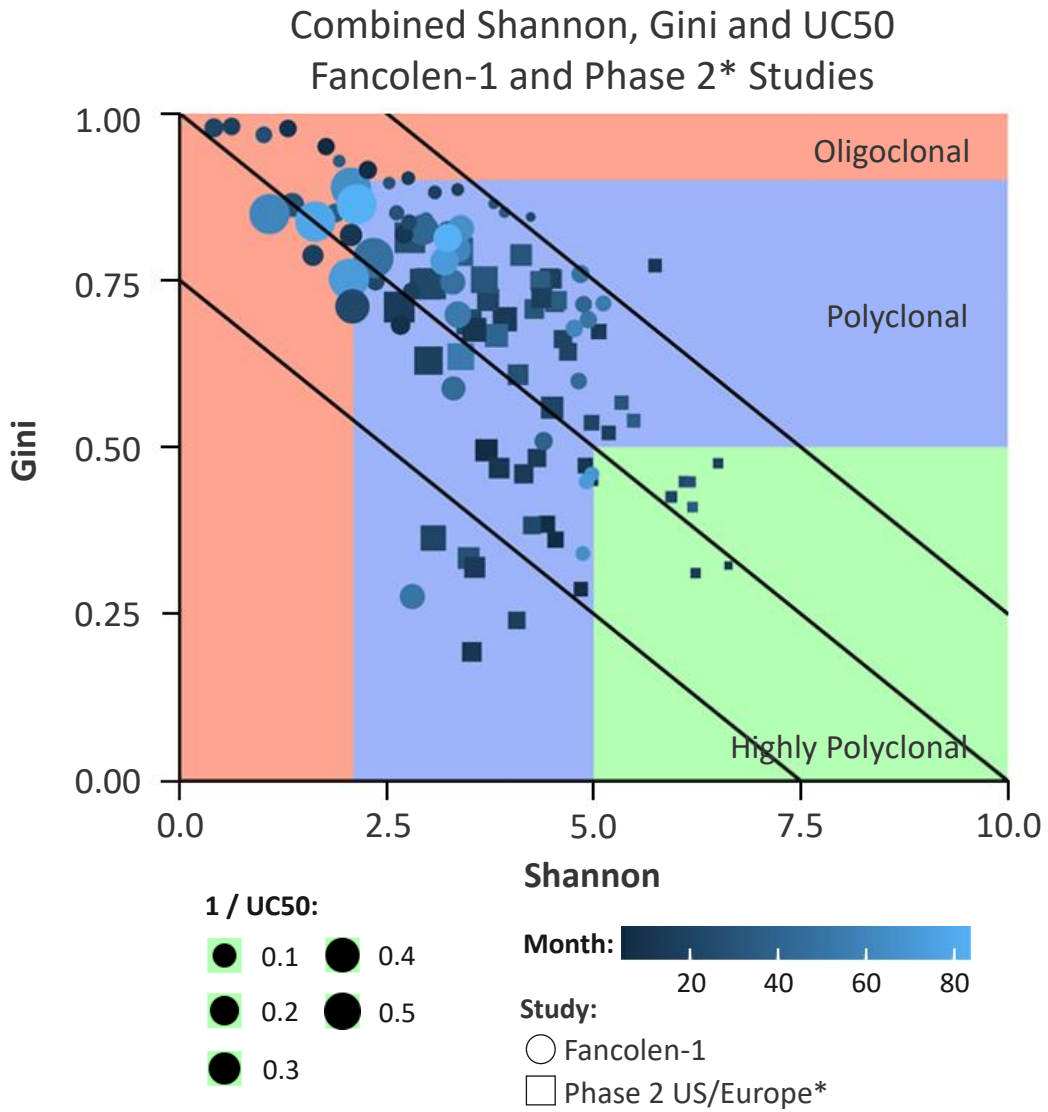
‡ ISA evaluated if VCN ≥0.02

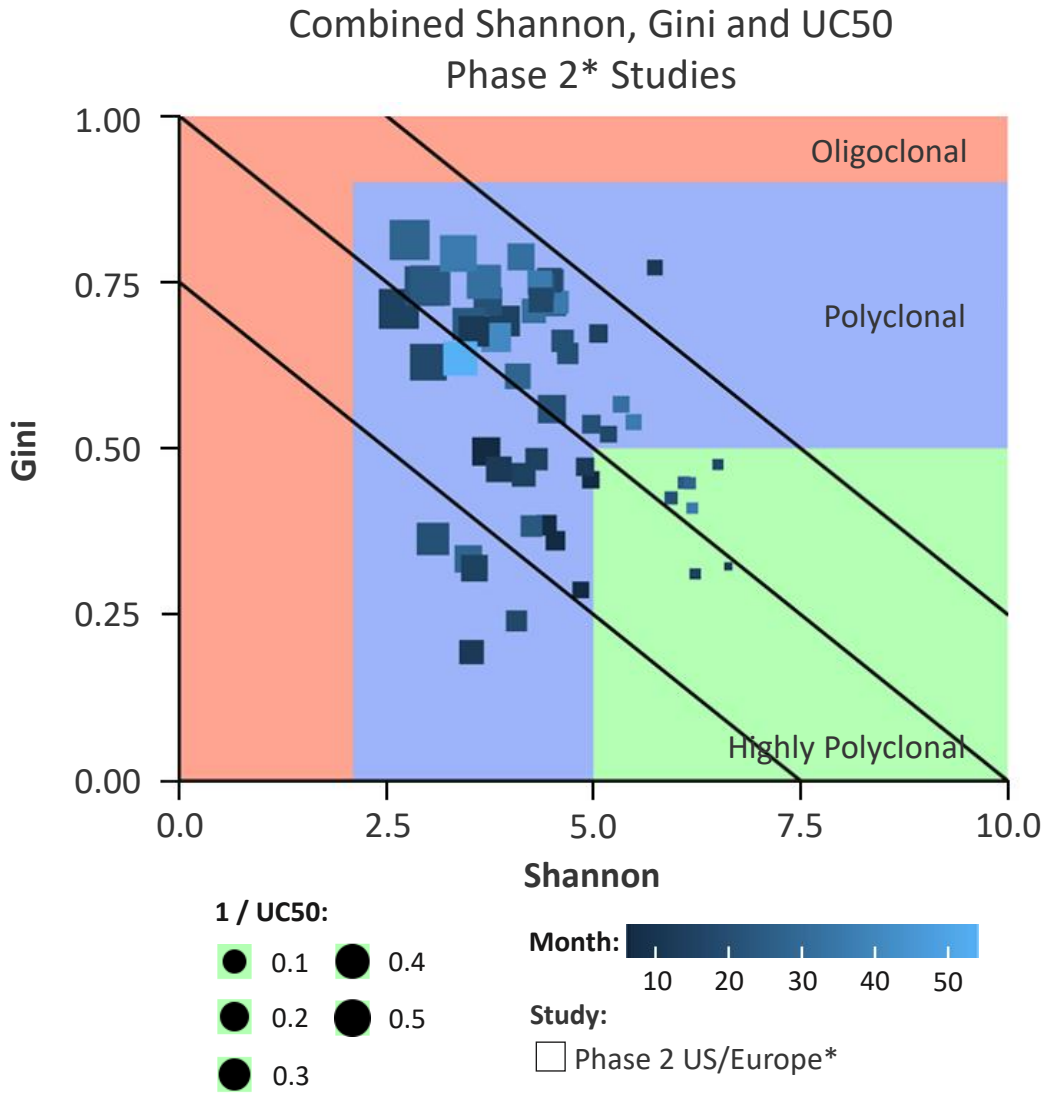
* Phase 2 studies include RP-L102 sponsored US Phase 1 & 2 and EU/UK Phase 2 (Fancolen-2).

IS=integration site.

Data cutoff: September 11, 2023

Aggregate Diversity Indexes for Fancolen-I and Phase 2 Studies

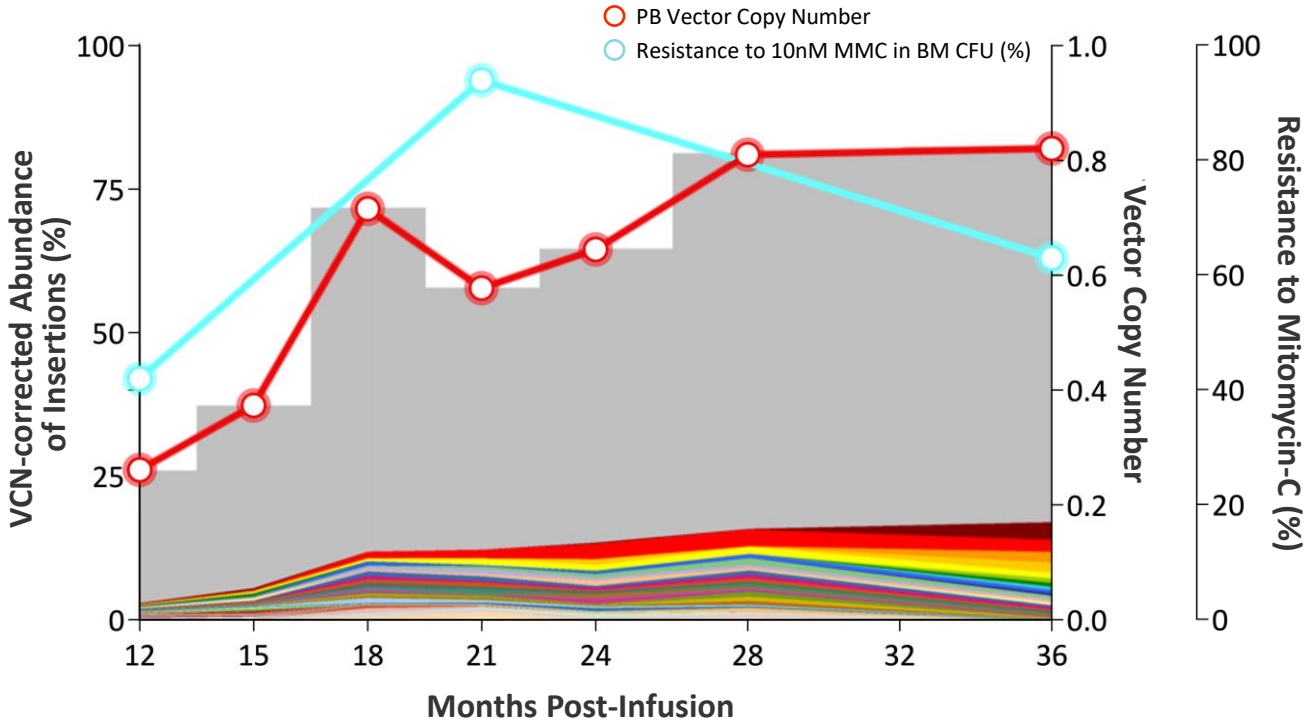




*Phase 2 studies include RP-L102 sponsored US Phase 1 & 2 and EU/UK Phase 2 (Fancolen-2).
UC50= describes how many integrations in a sample contribute to 50% of all sequences

Data cutoff: September 11, 2023

Representative Longitudinal ISA: Patient 6 (2010)



- Colored areas represent all integrations detected in top 10 integrations at any timepoint
- The gray area represents all other identified integrations and reflects the VCN (in %) at each timepoint
- Tables below display the VCN-corrected contributions of top 10 integrations at each timepoint

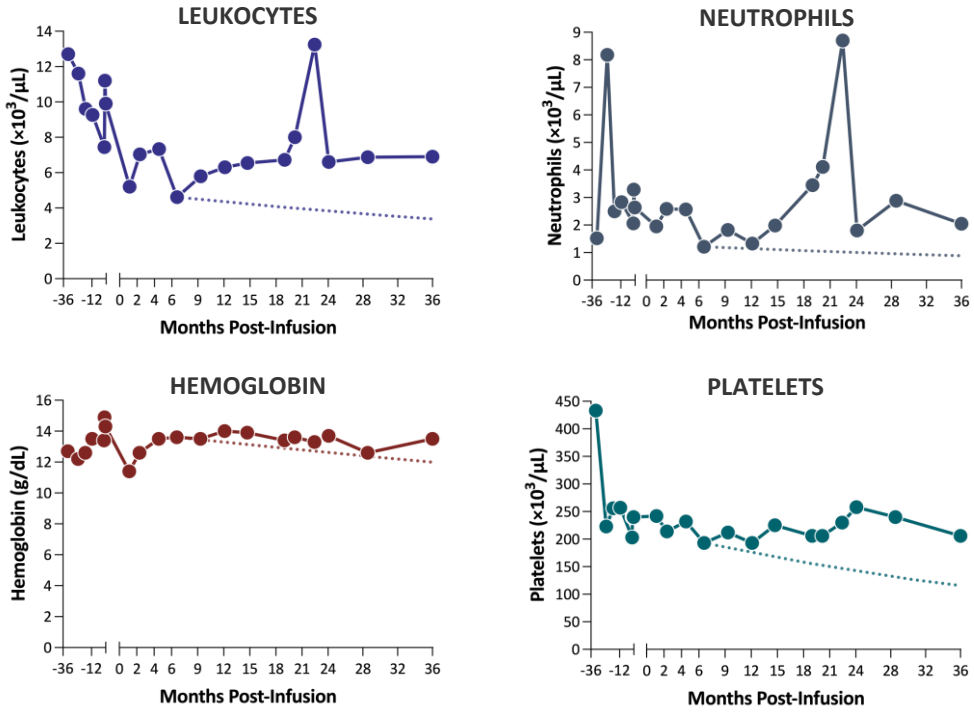
VCN-Corrected Contributions of Top 10 Integrations

Month 12	Month 15	Month 18	Month 21	Month 24	Month 28	Month 36
0.25%: KDM2A*	0.42%: TPX2*	1.11%: NR2C2*	1.49%: NR2C2*	2.66%: NR2C2*	2.89%: NR2C2*	3.10%: CBFB~*
0.21%: LOC105371046*	0.40%: BRD4~*	0.94%: FAT4*	1.18%: TLE3	0.97%: NELFE*	1.24%: TLE3	2.06%: NR2C2*
0.18%: DYRK1A~*	0.40%: ILF3*	0.83%: TCF20*	1.00%: VPS13C*	0.94%: TLE3	1.08%: THCAT158*	1.89%: CNOT6L*
0.17%: DOCK7*	0.33%: CBFB~*	0.73%: CYSLTR1	0.83%: BRD4~*	0.85%: CARD8~*	0.87%: SEC16A*	1.73%: NELFE*
0.15%: CD33*	0.33%: GOLGA2*	0.71%: POLK*	0.83%: TCF20*	0.73%: SEMA3C	0.74%: POLK*	1.01%: TLE3
0.15%: CNNM2*	0.30%: VAV1~*	0.71%: VPS13C*	0.76%: ARHGAP35*	0.70%: PPP6R2*	0.74%: TCF20*	0.78%: SPNS3*
0.14%: MYO9B*	0.30%: VPS13C*	0.69%: PACS1*	0.66%: ZDHHC17*	0.61%: THCAT158*	0.74%: ZNF318~*	0.65%: ILF3*
0.13%: FAT4*	0.26%: ARHGAP35*	0.60%: BRD4~*	0.62%: SEC14L1*	0.59%: SNAP23*	0.67%: ARHGAP35*	0.59%: VSTM5
0.13%: NR2C2*	0.26%: NELFE*	0.56%: ARHGAP35*	0.52%: THCAT158*	0.53%: BRD4~*	0.67%: ARHGAP35*	0.52%: POLK*
0.13%: POLE*	0.23%: DYRK1A~*	0.54%: WASF2~*	0.45%: CYSLTR1	0.53%: GFI1B~*	0.67%: C6orf15	0.46%: DHRS2

* Integration within a transcription unit
~ Integration within 50kb of a proto-oncogene

%VCN-Corrected Abundance of Insertions = [% contribution to all UIS] × [VCN] if PB VCN <1.0 OR
= [% contribution to all UIS] × [1.0] if PB VCN ≥1.0

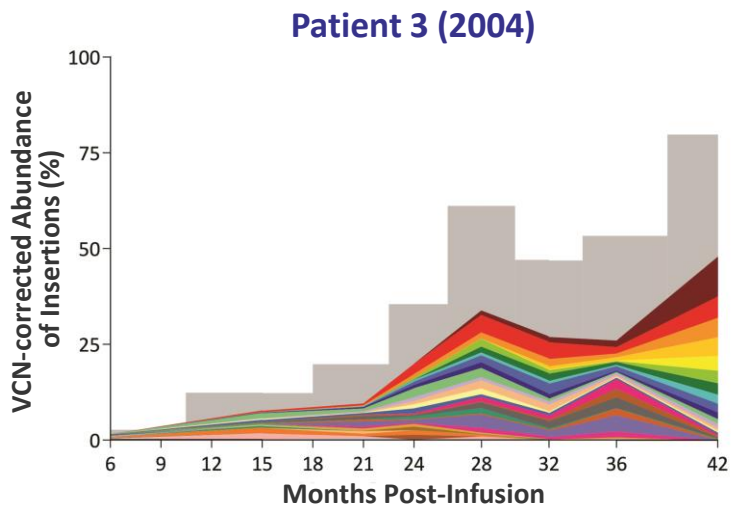
Blood count graphs: *Dotted lines indicate projected blood count decreases based on natural history evaluation from n=139 FA-A patients (IFAR registry, data on file)*

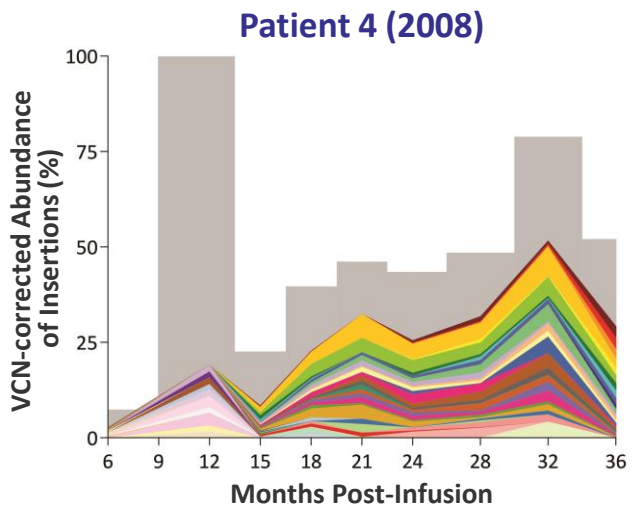


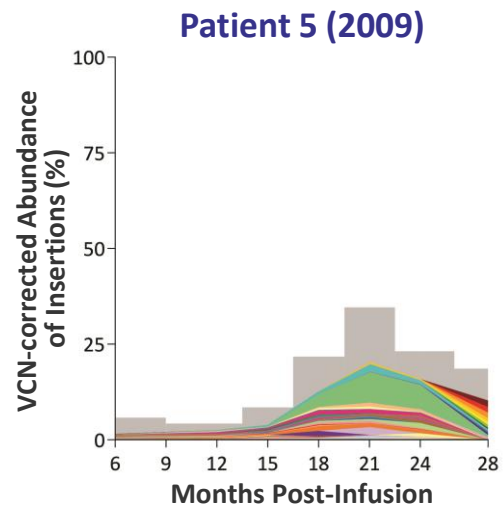
BM=bone marrow; BMF=Bone marrow failure; CFU=Colony-forming unit; MMC=Mitomycin-C; PB=Peripheral blood; RBC=Red blood cell; VCN: Vector copy number; UIS=Unique insertion sites.

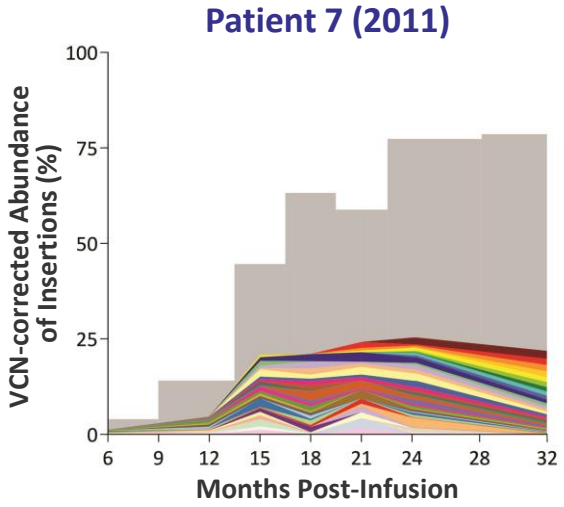
Data cutoff: September 11, 2023

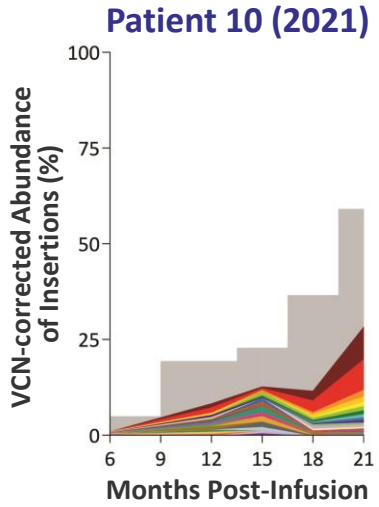
Longitudinal ISA in Additional Patients with Evidence of Comprehensive Engraftment

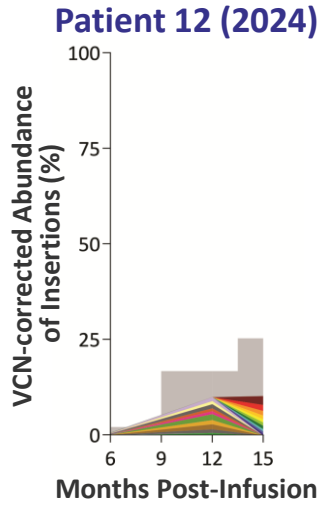










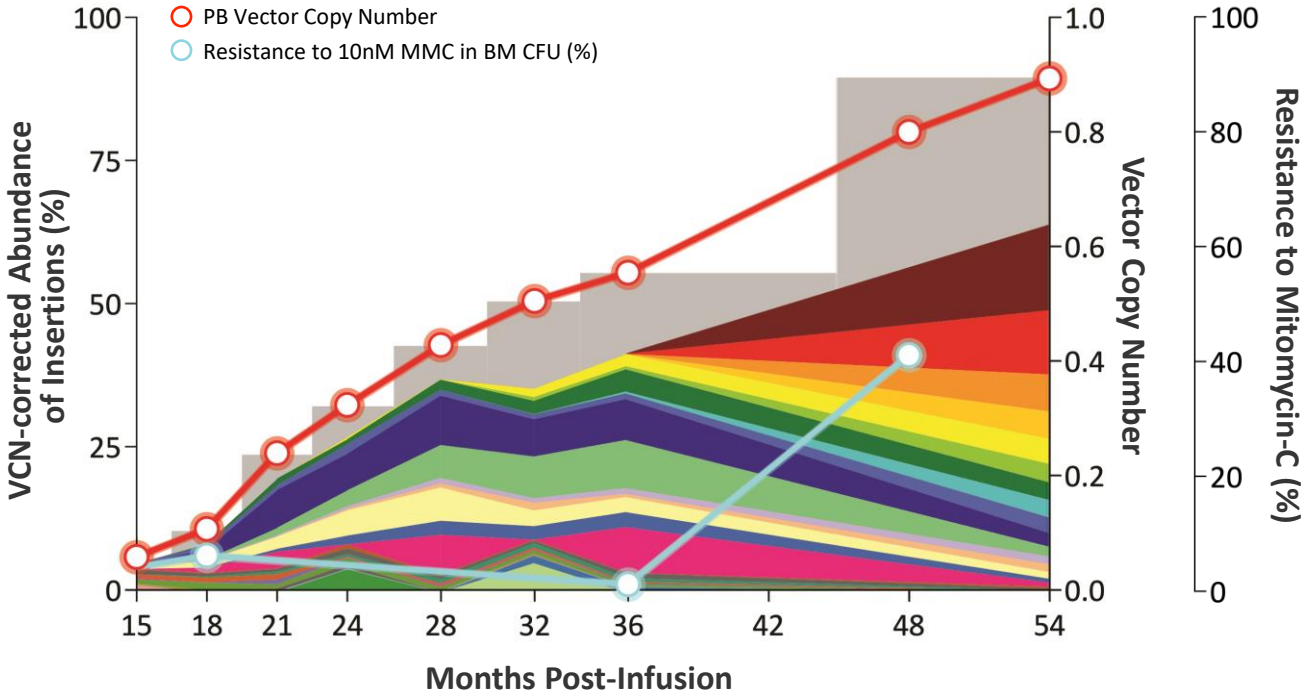


ISA=insertion site analysis; MMC=Mitomycin-C; VCN=vector copy number.

Data cutoff date: September 11, 2023

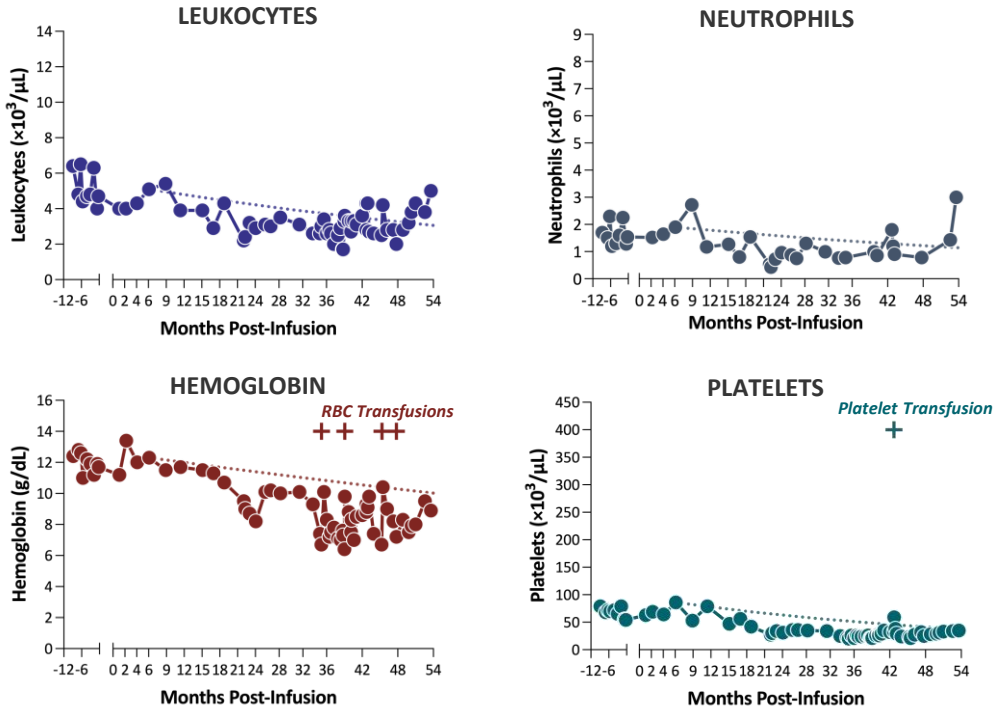
Less Robust Polyclonality Associated with More Limited Engraftment: Patient 1 (1001)

(Including blood count declines during initial 24-months post-infusion)



- Following initial decline requiring transfusion support, blood counts have stabilized, concurrent with the development of BM MMC resistance and sustained genetic correction
- Blood counts remain above thresholds for severe BMF, with no transfusion requirements in most recent 6 months

Blood count graphs: *Dotted lines indicate projected blood count decreases based on natural history evaluation from n=139 FA-A patients (IFAR registry, data on file)*



VCN-Corrected Contributions of Top 10 Integrations

Month 15	Month 18	Month 21	Month 24	Month 28	Month 32	Month 36	Month 54
1.78%: POU2F1*	1.78%: CNOT6L*	6.55%: CNOT6L*	6.36%: CNOT6L*	8.57%: CNOT6L*	7.23%: SLC8A1	8.35%: SLC8A1	14.86%: WDR82*
1.34%: CARD8~*	1.34%: CARD8~*	2.89%: POU2F1*	4.41%: EGR2	6.90%: POU2F1*	6.46%: CNOT6L*	7.89%: POU2F1*	11.15%: SLC1A6*
1.17%: POU5F1P3*	1.17%: EGR2	2.14%: EGR2	4.04%: POU2F1*	5.80%: EGR2	5.08%: POU2F1*	7.09%: CNOT6L*	6.42%: HNRNPU*
0.89%: LINC00571	0.89%: POU2F1*	1.26%: SLC8A1	2.73%: SLC8A1	5.69%: SLC8A1	2.76%: EGR2	3.86%: FCHSD2*	4.73%: TLK1*
0.82%: CNOT6L*	0.82%: LINC00571	1.14%: FCHSD2*	1.68%: SKAP1*	2.42%: LRMDA*	2.32%: LRMDA*	2.63%: EGR2	4.39%: FERMT3*
0.75%: EGR2	0.75%: SKAP1*	0.97%: LINC00571	1.50%: LRMDA*	1.75%: FCHSD2*	2.28%: FCHSD2*	2.60%: LRMDA*	3.21%: RCC2~*
0.45%: ATF1~*	0.45%: POU5F1P3*	0.84%: SKAP1*	1.14%: CARD8~*	1.01%: SKAP1*	1.46%: FERMT3*	2.09%: FERMT3*	3.04%: FCHSD2*
0.24%: CHM*	0.24%: EYA3*	0.81%: ASH1L*	0.95%: POU5F1P3*	0.99%: LY86	1.33%: MSH5*	1.00%: LY86	3.04%: LINC02639
0.21%: INTS6~*	0.21%: CNOT6L*	0.71%: CARD8~*	0.82%: FCHSD2*	0.82%: CARD8~*	1.29%: OAZ1	1.00%: SKAP1*	2.70%: SKAP1*
0.21%: SKAP1*	0.21%: NSRP1*	0.52%: GFI1B~	0.73%: GFI1B~	0.73%: CNOT6L*	0.86%: SKAP1*	0.94%: OAZ1	2.36%: CNOT6L*

* Integration within a transcription unit
~ Integration within 50kb of a proto-oncogene

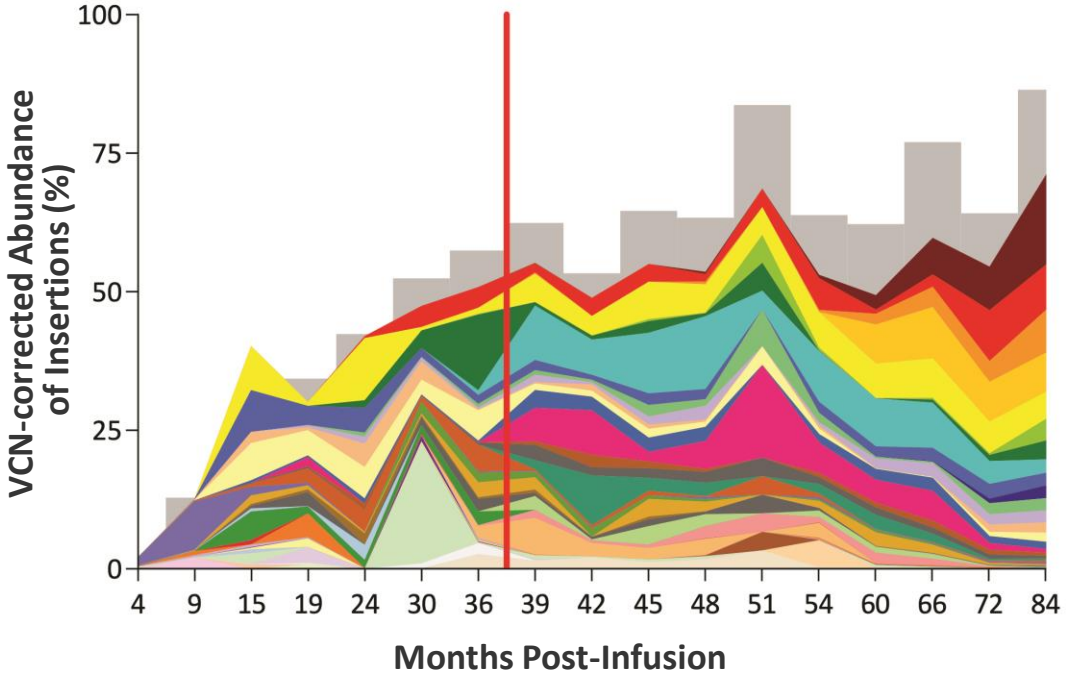
%VCN-Corrected Abundance of Insertions = [% contribution to all UIS] × [VCN] if PB VCN <1.0 OR
= [% contribution to all UIS] × [1.0] if PB VCN ≥1.0

BM=Bone marrow; BMF=Bone marrow failure; CFU=Colony-forming unit; MMC=Mitomycin-C; PB=Peripheral blood; RBC=Red blood cell; VCN: Vector copy number; UIS=Unique insertion sites.

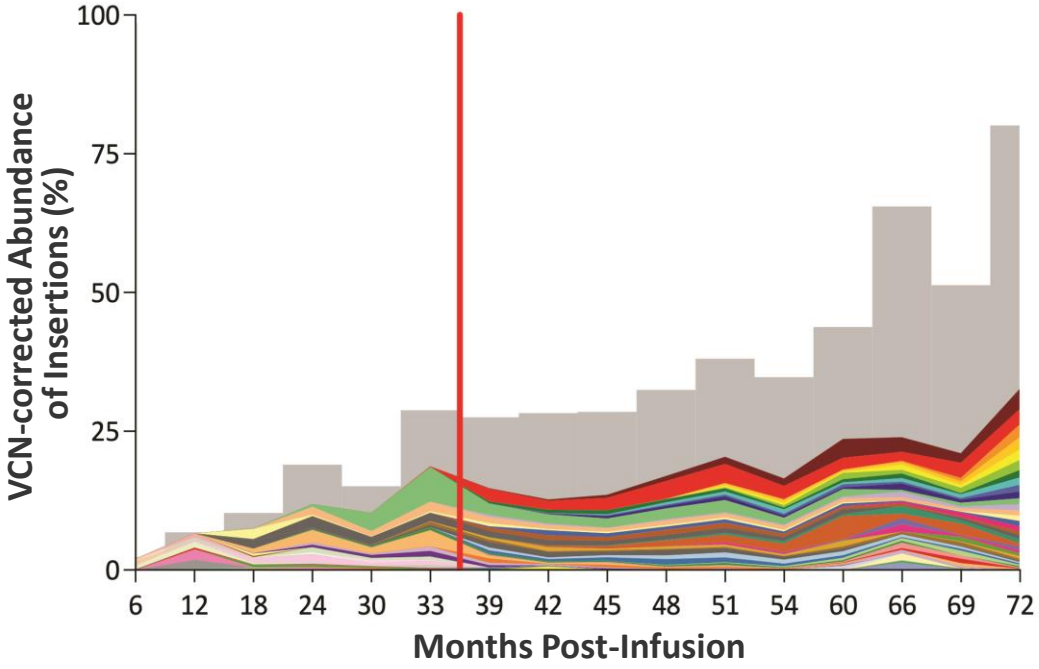
Data cutoff: September 11, 2023

Polyclonal ISA Patterns in Fancolen-1 Patients Receiving Higher Cell Doses and/or VCNs with Hematologic Stability and Increases Over 6-7 Years

Fancolen-1 Patient 002-2002*



Fancolen-1 Patient 002-2006*



*ISA strata to left of red bars indicate retrospective sample analysis at U Hannover; strata to right of bar were performed prospectively.
ISA=insertion site analysis; VCN=vector copy number.

Data cutoff date: September 11, 2023

Conclusions

- RP-L102 is a potentially curative therapy to prevent FA-related BMF, which can be administered without a suitable allogeneic donor or transplant related toxicities
  - 8 of 12 patients with ≥ 1 year of follow up have demonstrated sustained and progressively increasing genetic correction
  - Genetic correction associated with phenotypic correction and hematologic stability

- RP-L102 has conferred polyclonal insertion patterns in Phase 2 studies (utilizing Process B manufacturing optimizations)
  - Relative to Fancolen-1, Phase 2 patients demonstrated higher polyclonality and diversity of gene modified cells
    - Overall 1.3-fold higher mean VCN
    - 1.9-fold higher number of unique integrations
    - 1.4-fold lower Gini index
    - 1.5 fold higher Shannon index

- These diverse integration patterns are indicative of long-term hematopoietic stem cell repopulation of the BM and PB and clonal diversity in the absence of conditioning, without evidence of clonal dominance or potential mutagenesis

BM=bone marrow; BMF=Bone marrow failure; FA=Fanconi Anemia; PB=peripheral blood; VCN=vector copy number.

Data cutoff date: September 11, 2023

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