Evaluation of Bone Marrow in Fanconi Anemia Patients Treated with Briquilimab Antibody-Based Conditioning and TCRαβ+ T-cell/CD19+ B-cell Depleted Haploidentical Grafts

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BACKGROUND

Fanconi Anemia (FA) is the most common cause of inherited bone marrow failure (BMF), with over 80% of patients developing symptoms by age 12. The hematologic aspects of the disease can be cured with allogeneic hematopoietic stem cell transplantation (allo-HSCT), though this has historically required the use of non-specific chemotherapy and/or total body irradiation (TBI) to prepare the host bone marrow (BM), which causes systemic toxicities. We have recently launched a novel Phase 1b/2a clinical trial (NCT04784052) for FA patients in BMF using a targeted anti-CD117 monoclonal antibody, briquilimab, as a non-genotoxic method to facilitate the clearance of recipient hematopoietic stem and progenitor cells (HSPCs) from BM and thereby create BM niche space for donor HSPCs. This method replaces the conventional busulfan chemotherapy or TBI that is often used in FA patients with non-sibling donors and is combined with standard immunosuppression followed by transplantation of TCRαβ+ T-cell and CD19+ B-cell depleted haploidentical grafts from healthy familial donors.

AIMS & METHODS

We aimed to understand the impact of this novel transplant protocol on the BM and investigate the pathways involved in the survival and repopulation of HSPCs. We evaluated treatment efficacy through comprehensive analysis of BM recovery post-HSCT, focusing on HSPC reconstitution and donor cell functionality. Analyses included flow cytometry, MMC-challenged colony assays, single-cell RNA sequencing, and NULISA® (Alamar Biosci). Statistical significance was determined using Mann-Whitney U tests, pairwise Wilcoxon tests, and Kruskal-Wallis chi-squared tests as appropriate.

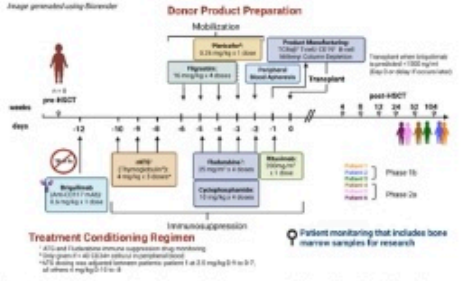

Figure 1. Treatment Regimen & Assessment Timeline. Study schema showing 6 patients with ≥ 24-week follow-up with BM samples (circles) that were analyzed and compared to healthy donor controls (n=3-11) by flow cytometry, colony assays, scRNA-seq, and NULISA proteomics.

Engraftment and Multi-Lineage Donor Chimerism Post-HSCT

Table 1. Patient Characteristics and Post-Transplant Outcomes for Those with ≥ 24 Weeks Follow-up.

Patients	Age, Sex, Race	Mutations associated w/ Fanconi Anemia	Day of Donor Engraftment	Major Acute Toxicities	Donor Chimerism (%) at last follow-up
Patient 1 (BMT373-001)	7 y/o, M, White	FANCA heterozygous for c.4261-2A>G*; c.1433C>G*; C.1638C>T	Neutrophil: Day +11; Platelet: Day +14; Last PBSC infusion: Day +7	No VOD; Grade 3 mucositis, Fevers; No acute or chronic GvHD	All 24 mo: CD34: 100%; CD3: 1.96%/CD15: 100%; CD19 100%; CD56: 100%
Patient 2 (BMT373-002)	10 y/o, F, Asian	FANCA homozygous for c.41-6dupT	Neutrophil: Day +11; Platelet: Day +13; Last PBSC infusion: Day +8	No VOD; Grade 3 mucositis, Fevers; resolved viral infections; No acute or chronic GvHD	All 24 mo: CD34: 100%; CD3: 1.96%/CD15: 100%; CD19 100%; CD56: 100%
Patient 3 (BMT373-003)	8 y/o, F, Black	FANCA heterozygous for c.1A>G*; c.2222+1G>A*	Neutrophil: Day +10; Platelet: Day +11; Last PBSC infusion: Day +5	No VOD; Grade 2 mucositis, Fevers; resolved viral infections; chronic GvHD (moderate, skin GI)	All 18 mo: CD34: 100%; CD3: 1.96%/CD15: 100%; CD19 100%; CD56: 100%
Patient 4 (BMT373-004)	4 y/o, F, White	FANCD1 heterozygous for c.3130+1T>; c.1795_1804del**	Neutrophil: Day +9; Platelet: Day +12; Last PBSC infusion: Day +5	No VOD; Grade 1 mucositis; GIRV resolved; No acute or chronic GvHD	All 12 mo: CD34: 100%; CD3: 99%/CD15: 100%; CD19 99%; CD56: 99%
Patient 5 (BMT373-006)	6 y/o, F, White	FANCC heterozygous for c.67delT*; c.1417G>T*	Neutrophil: Day +10; Platelet: Day +11; Last PBSC infusion: Day +7	No VOD; Grade 2 mucositis; acute GVHD (skin, stage 2, grade1)	At 6 mo: CD34: 100%; CD3: 94%/CD15: 99%; CD19 100%; CD56: 100%
Patient 6 (BMT373-005/007)	10 y/o, F, not reported	FANCA heterozygous for c.1366+5G>C; FANCA heterozygous for a deletion c.(?_-1)_(3552+1_3553-1)del**	Neutrophil: Day +13; Platelet: Day +13; Last PBSC infusion: Day +3	No VOD; Grade 2 mucositis; No acute or chronic GvHD	At 6 mo: CD34: 100%; CD3: 95%/CD15: 100%; CD19 100%; CD56: 100%

* = likely pathogenic mutation
** = classified as a pathogenic mutation
donor chimerism from bone marrow or peripheral blood cells

Repopulation and Clonogenic Potential of HSPCs

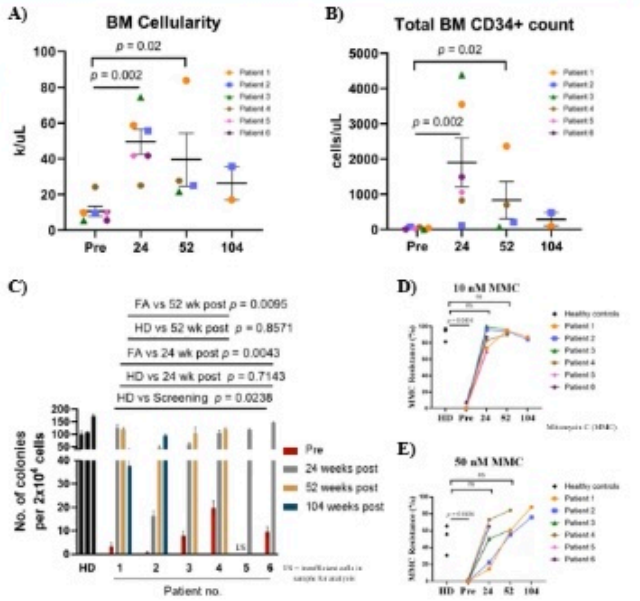

Figure 2. Correction of BMF and DNA-damage Resistance in FA Patients Post-HSCT. A) BM cellularity and B) BM CD34+ cell counts in patients pre-HSCT, and at 24-, 52- and 104 weeks post-HSCT compared to pediatric healthy donor (HD) controls (n=3). C) Increased colony number and D) resistance to 10 nM MMC and E) 50 nM MMC in BM HSPCs in these samples as assessed by colony forming count (CFC) assays. ns = not significant.

Improvement of HSPCs and BM Niche Signaling at ≥ 24 Weeks Post-HSCT

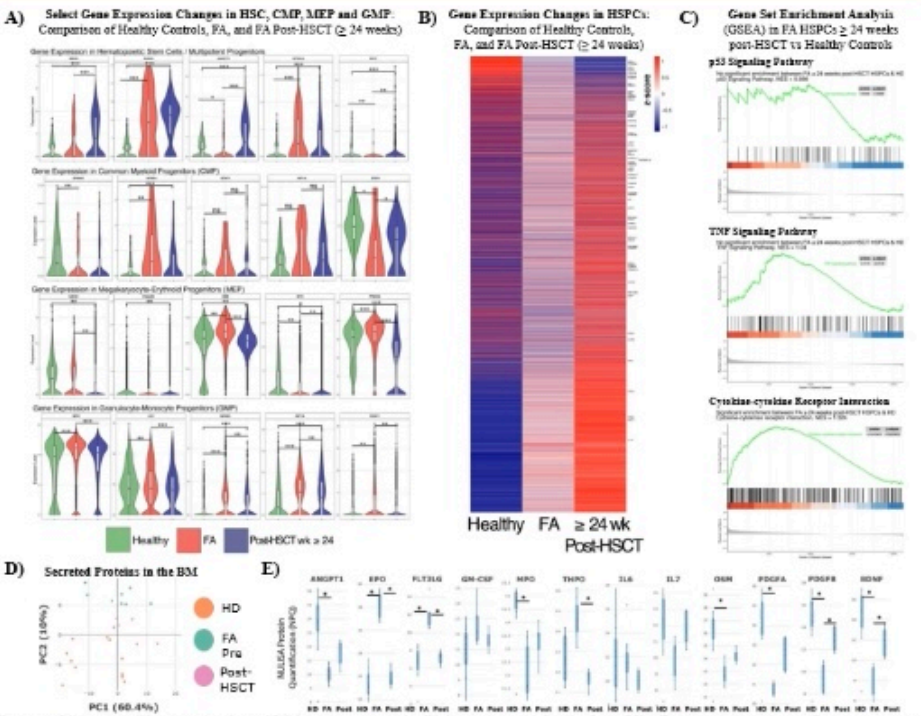

Figure 3. Molecular Characterization of BM Improvement in FA Patients ≥ 24 Weeks Post-HSCT through Integrated Transcriptomic and Proteomic Analyses. A) Violin plots demonstrating restoration of select FA disease-associated genes in HSCs and progenitors post-HSCT by scRNA-seq. B) scRNA-seq heatmap showing restoration of select FA-dysregulated pathways (inflammation, DNA repair, oxidative stress) and elevated hematopoiesis gene expression in donor HSPCs post-HSCT (n=6) compared to both untreated FA patients at screening (n=6) and healthy controls (n=3), suggesting active bone marrow reconstitution. C) Normalization of FA-associated stress pathways (p53 & TNF) and enrichment in cytokine signaling post-HSCT. D) PCA plot of proteomic profiles demonstrating distinct clustering of BM plasma proteins with E) improvement of select hematopoiesis and BM niche-related proteins in FA patients post-HSCT (n=6) compared to healthy donor (HD) controls (n=11). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns = not significant.

SUMMARY & FUTURE DIRECTIONS

Our novel anti-CD117 antibody-based HSCT protocol appears to effectively replace the BM of FA patients with BMF, restoring hematopoiesis. Notably, this was accomplished without the use of busulfan or TBI, which is extremely toxic to FA patients. Post-HSCT analysis reveals restored HSPC function, resistance to DNA damage, and molecular signatures approaching healthy controls. However, additional analyses and assessment of more samples with longer follow-up time points are needed and underway to further understand the full effects of this treatment on the BM microenvironment, including comprehensive evaluation of BM niche interactions and signaling networks. Continued enrollment and analysis will enable further characterization of post-HSCT recovery dynamics and enable evaluation of therapeutic efficacy.

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