Immune Reconstitution after Hematopoietic Stem Cell Transplantation in Immunodeficiency–Centromeric Instability–Facial Anomalies Syndrome Type 1

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Abbreviations
ICF Immunodeficiency, Centromeric instability and Facial anomalies
HSCT Hematopoietic stem cell transplant
RIC reduced intensity conditioning

To the Editor:

Immunodeficiency, centromeric instability and facial anomalies syndrome (ICF) is a rare autosomal recessive condition (OMIM 242860) characterized by pericentromeric chromosome instability and a heterogeneous clinical presentation of recurrent infections, neurologic abnormalities, and facial dysmorphism (hypertelorism, macroglossia, and micrognathia) [1]. In ICF syndrome, defects in DNA methylation at the pericentromeric regions of chromosomes 1, 9, and 16 lead to cytogenetic abnormalities which are prone to breakage [1–3]. Pathogenic variants in four genes have been recognized to date; DNA methyltransferase 3B gene (DNMT3B) causing ICF1, Zinc-finger and BTB domain-containing 24 gene (ZBTB24) causing ICF2, and cell division cycle associated 7 gene (CDCA7) and helicase lymphoid-specific gene (HELLS) causing ICF3 and ICF4, respectively [2]. The most common subtype, affecting approximately 50% of patients, is ICF1 [1]. Immunologic abnormalities in ICF1 result from this epigenetic dysregulation and include defective lymphocyte differentiation, activation, and migration, as evidenced by absent CD19+CD27+ class switched memory (CSM) B cells, hypogammaglobulinemia, and sub-optimal T cell proliferation with antigen stimulation. Subsequently, patients suffer from recurrent pyogenic infections, opportunistic infections, and failure to thrive [3–5].

Treatment for the immunologic manifestations of ICF1 includes early immunoglobulin replacement and prophylaxis for opportunistic organisms; however, recurrent infections frequently lead to shortened lifespan, with affected patients rarely surviving beyond the second decade [4]. The only curative option for the immunodeficiency associated with ICF1 is hematopoietic cell transplant (HCT), which has been reported in less than 10 cases of ICF1 worldwide [3, 6–8]. All previously reported patients seemingly had successful correction of their hypogammaglobulinemia and reported full donor chimerism following either myeloablative or reduced intensity conditioning (RIC); however, data regarding long-term follow up after transplant is limited. Herein, we describe the case of a Caucasian male who presented at age 6 months with recurrent infections, was subsequently diagnosed with ICF1 syndrome, and underwent HCT at 22 months of life with immune

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immunizations (Table 1). Flow cytometry was notable for all mg/dl) and absent vaccine antibody responses to tetanus have panhypogammaglobulinemia (IgG < 30, IgM 7, IgA 7, tures on bronchoalveolar lavage were ultimately negative, superimposed bacterial pneumonia (bacterial and fungal cul-

...for recurrent neutropenic fevers, polyviral respiratory infec-

...2) and thiotepa 10 mg/kg (day −8 to −3) and thiopeta 10 mg/kg (day −1) and received methotrexate and tacrolimus for graft versus host disease (GvHD) prophylaxis. Absolute lymphocyte count (ALC) was 7191 cells/mm² pre-conditioning and 0 following alemtuzumab. Neutrophil engraftment was achieved on day +13. His initial post-transplant course was complicated adenovirus reactivation treated successfully with anti-

...day 130. A CD34+ selected stem cell boost (6.3 × 10⁶/kg) from his brother was given on day +198 with an intent to improve declining chimerism [11]. Chimerism has remained mixed but stable at 2.5 years post-transplant (Table 1). Despite this mixed chimerism, his immunologic studies demonstrated normal-

...at 9 months of age. A targeted next-generation sequencing (NGS) panel for inborn errors of immunity genes showed compound heterozygous variants in trans [c.1957G > A (p.Asp653Asn) and c.2292G > T (p.Arg764Ser)] in the DNMT3B gene, initially both classified as variants of un-

...cates seen in patients with ICF1, confirming the diagnosis at age 12 months (Fig. 2S).

At age 22 months, the patient received 10/10 HLA MRD (matched related donor) bone marrow HCT (total nucleated cells of 6.88 × 10⁸/kg) from his brother, age 4.5 years, who did not carry either of the DNMT3B vari-

...siderable, even in the presence of mixed donor chimerism. There are very few reports of HCT for ICF1 in the liter-

...for ICF in the United States for a patient of ICF1 in the United States, and the first reported case of successful correction of the underlying immune deficiency despite mixed donor chimerism following HCT.

The patient is a Caucasian male born to non-

.....
Immune reconstitution post-HCT

Comparison of baseline immunoglobulins, vaccine titers, immunophenotyping (T/B/NK and CD45 RO/RA), and donor chimerism were collected pre-transplant and monitored for 2.5 years post-transplant (Table 1). Patient had normal phytohemagglutinin-stimulated lymphocyte proliferation, T cell function, and percentages of naïve T cells (CD45 + RA) pre-transplant. Patient received CD34+ stem cell boost on day +197 with no effect on chimerism (between 6-month and 9-month data). At 1 year and 2.5 years following transplant, advanced immunophenotyping of T and B cells was performed (Table 2). At 2.5 years following transplant, additional evaluation for recent thymic emigrants (CD4 + CD45RA + CD31+) was performed which was appropriate for age at 43% of CD4+ T cells (Table 2). Reference ranges are shown in parentheses. Bolded values indicate outside of reference range.

### Flow Cytometry Methodology
Whole EDTA blood was used for multiparametric immunophenotyping. A large panel of T and B cell subset markers and regulatory T cell function, and percentages of naïve T cells (CD45 + RA) pre-transplant. Patient received CD34+ stem cell boost on day +197 with no effect on chimerism (between 6-month and 9-month data). At 1 year and 2.5 years following transplant, advanced immunophenotyping of T and B cells was performed (Table 2). At 2.5 years following transplant, additional evaluation for recent thymic emigrants (CD4 + CD45RA + CD31+) was performed which was appropriate for age at 43% of CD4+ T cells (Table 2). Reference ranges are shown in parentheses. Bolded values indicate outside of reference range.

### Abbreviations
- Ig indicates immunoglobulin; CD4+, T helper cells (CD4+); CD8+, Cytotoxic T cells; CD56+, NK cells; CD19+, B cells, CD45 + RA (naïve T cells).
- * Initial reference range based on established values for our laboratory.
- ** Reference ranges adjusted after changes in laboratory methodology.
- ≠ Data collected for patient and compared with 45 matched control patients, median value for controls is shown in parentheses.
### Table 2  Advanced immunophenotyping post-transplant

| T cells | Naïve T cells % of CD4+ (CD4+ CD45 RA+ CD62L+ + CCR7+) | Central memory % of CD4+ (CD4+ CD45 RO+ CD62L+ + CCR7+) | Effector memory % CD4+ T cells (CD4+ CD45 RA+ CD62L+ CCR7+) | Naïve T cells % of CD8+ (CD8+ CD45 RA+ CD62L+ CCR7+) | Central memory % of CD8+ (CD8+ CD45 RO+ CD62L+ CCR7+) | Effector memory % of CD8+ T cells (CD8+ CD45 RO+ CD62L+ CCR7+) |
|---------|------------------------------------------------|-------------------------------------------------|-------------------------------------------------|------------------------------------------------|------------------------------------------------|-------------------------------------------------|
| 1-year post-transplant | 43.32% | 31.23% | 7.0% | 34.12% | 9.79% | 16.31% |
| 2.5 years post-transplant | 42.2% | 51.1% | 0.1% | 28.3% | 27.7% | 0% |

| B cells | Naïve B cells %CD19+ B cells (CD19+ CD27-+) | Total memory B cells %CD19+ B cells (CD27+ IgM+ IgD+ (8.58%)) | Marginal zone B cells %CD19+ B cells (CD27+ IgM+ IgM- (12.58%)) | Switched memory B cells %CD19+ B cells (CD27+ IgM- IgD- (3.50%)) | IgA+ memory B cells %CD19+ B cells (CD27+ IgA+) | IgG+ memory B cells %CD19+ B cells (CD27+ IgG+) |
|---------|--------------------------------|-------------------------------------------------|-------------------------------------------------|------------------------------------------------|------------------------------------------------|-------------------------------------------------|
| 1-year post-transplant | 92.78% | 7.22% | 2.55% | 2.74% | 0.05% | 2.31% |
| 2.5 years post-transplant | 77.2% | 13.5% | 3.7% | 5.1% | 0.1% | 4.5% |

Comparison of baseline immunoglobulins, vaccine titers, immunophenotyping (T/B/NK and CD45 RO/RA), and donor chimerism were collected pre-transplant and monitored for 2.5 years post-transplant (Table 1). Patient had normal phytohemagglutinin-stimulated lymphocyte proliferation, T cell function, and percentages of naïve T cells (CD45 + RA) pre-transplant. Patient received CD34+ stem cell boost on day +197 with no effect on chimerism (between 6-month and 9-month data). At 1 year and 2.5 years following transplant, advanced immunophenotyping of T and B cells was performed (Table 2). At 2.5 years following transplant, additional evaluation for recent thymic emigrants (CD44 + CD45RA + CD31+) was performed which was appropriate for age at 43% of CD4+ T cells (Table 2). Reference ranges are shown in parentheses. Bolded values indicate outside of reference range.

Flow Cytometry Methodology: Whole EDTA blood was used for multiparametric immunophenotyping. A large panel of T and B cell subset markers and regulatory T cells were assessed by flow cytometry at different time points. Clinical T cell panel analysis prior to transplant was performed on a BD Canto II instrument and data was analyzed with FCS Express v. 7 (De Novo software, Pasadena, CA). Advanced immunophenotyping after transplant was performed as follows: Briefly, one million cells, either from whole blood or PBMCs, were stained with either markers for T cell subsets, B cell subsets to identify naïve and memory T cells and recent thymic emigrants (CD4 + CD45RA + CD31+), memory B cell subsets (CD27, IgD, IgM, IgG, and IgA). For analysis, 10,000 CD45+ lymphocytes for TBNK subset quantitation and 10,000 CD45+ for expanded T and B cell phenotyping were collected on a CytoFlex® flow cytometer (Beckman Coulter, La Brea, CA) with data acquisition using Kaluza C v. 1.1® software (Beckman Coulter).

Abbreviations: Ig indicates immunoglobulin; CD4+, T helper cells (CD4+); CD8+, Cytotoxic T cells; CD56+, NK cells; CD19+, B cells, CD45 + RA (naïve T cells).

‡ Data collected for patient and compared with 45 matched control patients, median value for controls is shown in parentheses.
boost infusion of marrow from the original donor [6]. In contrast, our patient had no response to a stem cell boost and has had persistent stable mixed chimerism, yet achieved effective immune reconstitution with no further chimerism decline or loss of graft.

This patient’s mixed chimerism is likely related to the RIC preparative regimen for HCT. The use of RIC regimens for allogeneic HCT—particularly those containing alemtuzumab, fludarabine, and melphalan—in patients with nonmalignant disease is common. The use of these regimens is often complicated by the high incidence of mixed donor and recipient chimerism, as high as 80% [14]. In our patient the addition of Thiotepa to the conditioning did not change the outcome. Unlike hematologic malignancies where complete replacement with donor derived hematopoiesis is desirable for a cure, non-malignant disorders can often be controlled in the presence of mixed chimerism. Also, patient with nonmalignant disorders where single lineage abnormalities cause disease, relevant lineage specific engraftment is typically curative. Given that ICF is an immunodeficiency affecting the lymphoid lineage, higher level of engraftment in the CD3 and CD19, as was seen in our patient, should be adequate to provide cure. This maybe the result of selective advantage for these lineages that would allow the use of RIC regimens to limit organ toxicity, late effects, and increase tolerability especially in young recipients. Serial tracking of chimerism and immune monitoring is necessary until stability is ensured. In the event of unstable or mixed chimerism, intervals between testing should be shorter to determine additional interventions such as donor lymphocyte infusions, stem cell boosts, or second transplant. We plan to continue chimerism testing, paired with immune monitoring, for our patient every 6 months until 5 years post-HCT if the values remain stable.

ICF1 is a rare disease, and it is difficult to draw conclusions from examination of a few patients. In our case, as in those reported previously, HCT was curative for the immunodeficiency associated with ICF1. Our patient has had improved quality of life and has been off immunoglobulin replacement, has not had any serious infections, and has not been hospitalized since transplant. It is important to note that HCT is unlikely to improve the extramedullary defects and neurological/developmental manifestations of ICF1. Other manifestations such as autoimmunity, for which HCT has an unclear effect, have been described in ICF1 but were not present in our patient. Ultimately, long-term follow up is crucial to better understand the impact of HCT for ICF1, and we hope our case will provide additional insight into this rare disease.

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Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish All authors have consented to the publication of this manuscript.

Competing Interests None of the authors have any conflicts of interest to disclose.

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