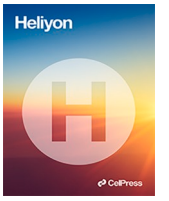
Heliyon 10 (2024) e24026

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Allogeneic bone marrow transplantation for patients with treatment-refractory Crohn's Disease

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ARTICLE INFO

Keywords:
Mucosal immune chimerism
Polygenic risk score
Outcomes
Reduced-intensity conditioning
Crohn's disease

ABSTRACT

Background & aims: Durable remissions of Crohn's Disease (CD) have followed myeloablative conditioning therapy and allogeneic marrow transplantation. For patients with treatment-refractory disease, we used reduced-intensity conditioning to minimize toxicity, marrow from donors with low Polygenic Risk Scores for CD as cell sources, and protracted immune suppression to lower the risk of graft-versus-host disease (GVHD). Our aim was to achieve durable CD remissions while minimizing transplant-related complications.
Methods: DNA from patients and their HLA-matched unrelated donors was genotyped and Polygenic Risk Scores calculated. Donor marrow was infused following non-myeloablative conditioning. Patient symptoms and endoscopic findings were documented at intervals after transplant.
Results: We screened 807 patients, 143 of whom met eligibility criteria; 2 patients received allografts. Patient 1 had multiple complications and died at day 332 from respiratory failure. Patient 2 had resolution of CD symptoms until day 178 when CD recurred, associated with persistent host chimerism in both peripheral blood and intestinal mucosa. Withdrawal of immune suppression was followed by dominant donor immune chimerism in peripheral blood and resolution of CD findings. Over time, mucosal T-cells became donor-dominant. At 5 years after allografting, Patient 2 remained off all medications but had mild symptoms related to a jejunal stricture that required stricturoplasty at 6 years. At 8 years, she remains stable off medications.
Conclusions: The kinetics of immunologic chimerism after allogeneic marrow transplantation for CD patients depends on the intensity of the conditioning regimen and the magnitude of immune suppression. One patient achieved durable improvement of her previously refractory CD only after establishing donor immunologic chimerism in intestinal mucosa. Her course provides proof-

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https://doi.org/10.1016/j.heliyon.2024.e24026
Received 31 October 2022; Received in revised form 16 December 2023; Accepted 2 January 2024
Available online 3 January 2024

of-principal for allografting as a potential treatment for refractory CD, but an immunoablative conditioning regimen should be considered for future studies.
(ClinicalTrials.gov, NCT01570348)

Abbreviations

CD	Crohn's Disease
GVHD	graft-versus-host disease
G-CSF	granulocyte colony-stimulating factor
HCT-CI	Hematopoietic Cell Transplantation-Comorbidity Index
CDAI	Crohn's Disease Activity Index
HBI	Harvey-Bradshaw Index
SIBDQ	Short Inflammatory Bowel Disease Questionnaire
SNPs	Single Nucleotide Polymorphisms

1. Introduction

Recent studies of large patient cohorts have shown Crohn's Disease (CD) to associated with polygenic mutations involving immunity, antigen processing, and epithelial barrier function [1–5]. There are also monogenic disorders with CD-like presentations such as loss of function mutations in interleukin-10 and its receptor [6–9].

Two case series of patients with leukemia and CD suggested that myeloablative conditioning therapy and allogeneic hematopoietic cell transplantation could lead to durable complete remissions of CD [10,11]. One patient's CD recurred following rejection of donor cells and return of host immunity [10]. CD has also developed after allogeneic transplantation from CD-susceptible donor into a naïve host [12]. High transplant-related mortality has precluded myeloablative conditioning and allogeneic transplantation as a treatment for severe CD [13]. There are, however, a minority of patients with CD refractory to all medical and surgical treatment approaches including biological pharmaceuticals [14].

In 2014, we opened a protocol for allogeneic hematopoietic cell transplantation as a cure for refractory, severe CD, based on two recent developments; 1) transplant-related mortality has substantially declined [13,15] and 2) good outcomes were seen in patients transplanted for genetic immune deficiency syndromes associated with gastrointestinal inflammation (for example, IPEX syndrome, chronic granulomatous disease, CTLA4 deficiency) [6,7,16,17]. Our protocol used a reduced-intensity conditioning regimen to minimize regimen-related toxicity; bone marrow from donors with low Polygenic Risk Scores for CD as a cell source to lower the risk of GVHD; post-transplant intravenous cyclophosphamide, oral mycophenolic acid, and protracted tacrolimus dosing to prevent severe GVHD; and prophylactic and pre-emptive antimicrobial drugs to minimize the risk of serious infection [18,19]. The primary aim was to achieve durable remissions of CD in patients with severe disease, as was seen in past patients transplanted for leukemia and less severe CD [10,11]. Secondary aims included assessment of donor chimerism in mucosal immune cells and recording of adverse events (regimen-related toxicity, infection, and graft-vs.-host disease).

2. Methods

2.1. Patient screening and eligibility criteria

Under the aegis of a protocol approved by the Institutional Review Organization of the Fred Hutchinson Cancer Center (*ClinicalTrials.gov*, NCT01570348) and an Investigational New Drug application approved by the U.S. Food and Drug Administration (#015081), we screened patients using a web-based questionnaire, followed by review of detailed medical records. Potentially eligible patients were invited to Seattle for review of eligibility (Supplementary Materials, Table 1). Eligible patients with an HLA-matched donor were given detailed informed consent documents and prepared for transplant.

2.2. Allogeneic bone marrow transplantation

- ***Collection of autologous peripheral blood stem cells.*** We began granulocyte colony-stimulating factor (G-CSF) 16 μg/kg/day for mobilization and storage of autologous hematopoietic cells, to be infused in the event of graft rejection after transplant. To prevent G–CSF–related exacerbations of CD, subjects received prednisone 1 mg/kg/day, starting one day before the start of G-CSF and continued for 10 days. Leukophoresis for $CD34^+$ cells in peripheral blood continued until a minimum of 2.0×10^6 $CD34^+$ cells/kg were cryopreserved.
- ***Supportive care and Infection prevention.*** Supportive care included oral ursodeoxycholic acid (12–15 mg/kg/day), oral N-acetyl cysteine (1200 mg/day) (Twinlab, Hauppauge, New York), and antimicrobial prophylaxis (levofloxacin during neutropenia; oral

fluconazole; valacyclovir for herpes simplex virus- and varicella zoster virus-seropositive patients; trimethoprim-sulfamethoxazole for prevention of *Pneumocystis jiroveci*); and surveillance for cytomegalovirus DNA and Epstein-Barr virus DNA followed by therapy for viremic patients [10,13]. G-CSF was given from day 5 at 5 mcg/kg/day, until the absolute neutrophil count was $>1000/mm^3$ for three consecutive days.

- ***Conditioning therapy.*** A reduced-intensity regimen included cyclophosphamide 25 mg/kg plus mesna 25 mg/kg IV on days −6 and −5; fludarabine 30 mg/M^2 on days −4, −3, and −2; and total body irradiation 200 cGy on day −1.
- ***Donor selection.*** Allogeneic donors were to be an HLA-identical sibling (negative for CD-associated NOD2 alleles) or an HLA-matched unrelated donor. Unrelated donors were matched by high-resolution allele level typing for HLA-A, B, C and DRB1 and intermediate resolution SSOP, identifying alleles in groups of related families historically defined as antigens for DQB1. An unrelated donor was considered matched if patient and donor share HLA-A, B, C alleles with identical sequences at exons 2 and 3, DRB1 alleles with identical sequences at exon 2, and DQB1 results that include the same allele groups.
- ***Infusion of donor hematopoietic cells.*** Patients received HLA-matched, T-cell replete bone marrow on day 0, harvested with a target yield of 4×10^8 nucleated cells/kg recipient weight.
- ***Prevention of graft-versus-host disease.*** Cyclophosphamide 50 mg/kg was given intravenously on days 3 and 4, along with mesna. Tacrolimus was given from day 5 until day 180, with the dose adjusted to maintain trough levels of 5–15 ng/mL, then tapered until discontinuation at day 365. Delayed-release mycophenolate sodium (Myfortic, Novartis Pharmaceutical Corporation, East Hanover, New Jersey) was given through day 35, at 11 mg/kg three times daily.

2.3. Baseline staging and post-transplant monitoring of CD activity after transplant

These baseline studies were collected before the start of conditioning therapy and at intervals after transplant through 5 years of follow-up: Hematopoietic Cell Transplantation-Comorbidity Index (HCT-CI), Crohn's Disease Activity Index (CDAI), Harvey-Bradshaw Index (HBI), CD-related quality of life (SIBDQ), Body Mass Index, esophagogastroduodenoscopy, colonoscopy, and ileoscopy or enteroscopy or enteric imaging.

2.4. Laboratory studies

- ***Donor and host genomic studies.*** Genotyping on an immune-mediated Immunochipv1 array (Illumina, San Diego CA) was performed at Cedars-Sinai Medical Center, Los Angeles, CA. Inflammatory bowel disease-associated markers of interest [1–3] were manually inspected and individual genotype clusters optimized if necessary. Remaining Single Nucleotide Polymorphisms (SNPs) were evaluated based on various SNP statistic parameters. From 196,524 markers present on the array, a total of 175,231 SNPs passed quality control. Polygenic Risk Scores were calculated as a normalized weighted sum of the number of risk alleles carried by each individual (0, 1, or 2) at each known CD locus, with weights proportional to the effect estimates from previously published large-scale association studies [5].
- **Donor and host chimerism in peripheral blood and intestinal mucosa.** Enumeration of donor and host cells in peripheral blood was determined by the Promega PowerPlex16 multiplex STR system that included 15 DNA loci plus amelogenin (Promega Corporation, Madison WI). To assess chimerism in intestinal mucosal biopsies (both patients were female with male donors), sections were stained *in situ* with CEP® X SpectrumOrange™/Y SpectrumGreen™ DNA Probes (Abbott Molecular Inc.) in combination with antibodies targeting CD3 (polyclonal A0452, Dako), CD4 (clone 1F6, Leica Biosystems) or CD8 (Clone 4B11, Novocastra) as previously described [20,21]. Laser scanning confocal microscopy was performed on an Olympus FV1000 (BX61WI) system with 405, 488, 543 and 633 nm laser lines using 20×/0.80 or 60×/1.35 UPlanSApo oil objectives (Olympus). Images were analyzed in ImageJ (NIH) and GraphPad Prism 6 (GraphPad Software, La Jolla California USA).

2.5. Role of the funding source

Study sponsors played no role in the conduct, interpretation, and submission of this work.

3. Results

3.1. Identification of eligible patients

In response to our website questionnaire, we received clinical information from 807 patients, 143 of whom appeared to meet eligibility criteria; 14 were invited to Seattle after review of medical records. Two patients were transplanted, two eligible patients declined to proceed, and ten did not receive approval from medical insurance companies for enrollment.

3.2. Genomic analysis of DNA from patients and their respective donors

CD risk alleles for NOD2 are shown in Supplementary Materials, Table 2 and other previously identified alleles in Supplementary Materials, Table 3 [1,2]. Genotype data for NOD2 showed that Patient 1 was homozygous for the NOD2 SNP rs2066844/SNP8 (R702W). Patient 2 was heterozygous for one NOD2 SNP (rs2066844/SNP8). Donors were negative for all NOD2 SNPs examined. Patient 1 has a higher overall homozygous risk allele count (210) than Patient 2 (198) (Supplementary Materials, Table 3) [1,2].

Polygenic Risk Scores with weights proportional to the effect estimates from large-scale association studies showed that both patients had higher CD risk scores than their respective donors (Fig. 2).

3.3. Medical histories, pre-transplant evaluation, and post-transplant course

Patient 1 was a 40 year old woman with an eleven-year history of recurrent diarrhea and abdominal pain following each of four surgeries (ileal resection, repair of sigmoid-vaginal fistula, resection of ileocolic anastomosis, resection of neo-terminal ileum and adjacent colon). Other medical issues included abscess, pyoderma, anemia, hypertension, IgA nephropathy, and CMV seropositivity. Medical regimens (prednisone, azathioprine, infliximab, adalimumab, methotrexate, cyclosporine, tacrolimus, certolizumab pegol, ustekinumab, natalizumab) either caused side effects or failed to result in durable remissions. At pre-transplant screening, ulcerations were noted throughout the neo-terminal ileum, transverse colon, and distal rectum. CT and MRE imaging showed diffuse mural thickening of the colon and mural hyper-enhancement of the neo-terminal ileum. Disease activity was documented by CDAI 497, HBI 16, and Short IBD Questionnaire score 29/70 (Fig. 1, three panels on the left, red lines). Her donor was an unrelated HLA-matched male, age 28, CMV seronegative, without a history of bowel disease and at low risk for CD based on his Polygenic Risk Score

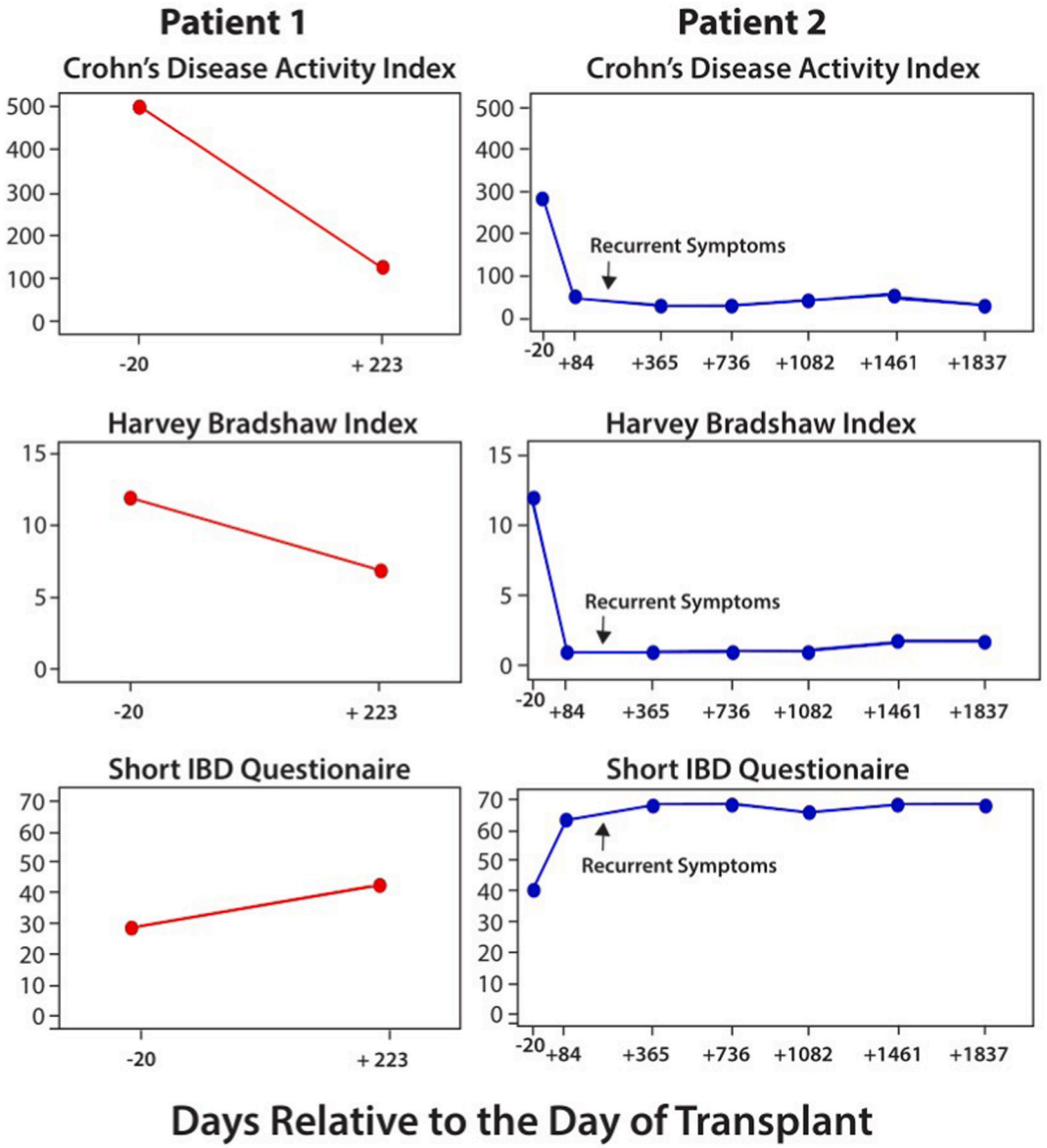

Fig. 1. Display of data for Crohn's Disease Activity Index, Harvey Bradshaw Index, and the Short Inflammatory Bowel Disease Questionnaire at baseline and at time-points to 5 years after allogeneic transplantation.

(Fig. 2, donor data, red circle).

Her post-transplant course was complicated by pulmonary embolus (day 15), CMV reactivation (day 37), spontaneous stress fracture of the femoral neck (day 100), metapneumovirus pneumonia requiring mechanical ventilation (day 108), renal failure (day 118), glucocorticoid-induced myopathy, cardiac arrest (day 318), rib fractures during resuscitation, aspiration pneumonia (day 325), flail chest, and fatal hypoxic respiratory failure (she refused intubation). Peripheral blood chimerism studies on Day 31 showed that $CD3^+$ cells were 99 % donor, 1 % host; $CD33^+$ cells were 92 % donor. On day 84, $CD3^+$ cells were 99 % donor, 1 % host; $CD33^+$ cells were 72 % donor, 28 % host. On Day 324, $CD3^+$ cells were 99 % donor, 1 % host; $CD33^+$ cells were 38 % donor. Restaging of her CD on day 223 revealed no ulcerative lesions in the neoterminal ileum and a solitary 8 mm. Ulcer in the sigmoid colon consistent with CD. At this time, CDAI was 121, HBI 7, and the Short IBD Questionnaire score was 42/70 (Fig. 1, three panels on the left, red lines). Repeat sigmoidoscopy on day 332 showed no lesions or histologic changes of CD. There was histologic evidence of mild gastrointestinal GVHD in endoscopic biopsies on days 28, 53, 126, and 332. Histologic sections of intestinal mucosa, examined *in situ* with fluorescent *in situ* hybridization (FISH) probes targeting X/Y chromosomes and T-cell markers, revealed very few persistent host T-cells at all time points up to day 332.

Patient 2 was a 45 year old woman with onset of CD at age 18. Her CD was confined to the small intestine, leading to multiple surgeries and stricture dilations: Resection of two 30 cm. Ileal segments, dilation of two proximal jejunal strictures; resection of 45 cm. Jejunum; stricturoplasty proximal to ileocecal valve; resection of 50 cm. Jejunum, starting 70 cm. From ligament of Treitz; balloon dilation of two jejunal strictures, complicated by perforation, closed surgically. Multiple medical regimens (sulfasalazine, prednisone, mesalamine, metronidazole, azathioprine, budesonide, infliximab, adalimumab, ustekinumab) failed to result in durable remissions. At pre-transplant screening, wide-spread ulcerations and multiple strictures were noted from 40 to 70 cm. beyond the ligament of Treitz (Fig. 3A–E). MRE imaging showed a 5 cm. Area of strictured jejunum with wall thickening; two strictures in the proximal and mid-jejunum, diffuse mural thickening of the colon and mural hyper-enhancement of the neo-terminal ileum; disease activity was documented by CDAI 279, HBI 12, and Short IBD Questionnaire score 40/70 (Fig. 1, three panels on the right, blue lines). Her symptoms were abdominal pain and distention following meals along with 3–6 diarrheal stools per day. Her physicians were reluctant to repeat balloon stricture dilations because of prior perforation and believed that yet another resection of involved jejunum would lead to short bowel syndrome. Her donor was an unrelated HLA-matched male, age 26, CMV seronegative, without a history of bowel disease and at low risk for CD based on his Polygenic Risk Score (Fig. 2, donor data, blue circle).

She had an almost event-free, uncomplicated course until day 145. Peripheral blood chimerism studies on Day 82 showed that $CD3^+$ T-cells were 93 % donor, 7 % host and $CD33^+$ myeloid cells were 92 % donor, 8 % host. On day 86, CDAI was 44, HBI 1, and the Short IBD Questionnaire score was 64/70. Her gastrointestinal CD activity was restaged on Day 86, revealing gastric mucosal edema, re-epithelialization of the previously ulcerated, strictured area in the jejunum, and normal colonic and ileal mucosa (Fig. 3B–F). Histology of the previously ulcerated jejunal mucosa showed patchy acute inflammation. Tacrolimus was continued per protocol and she returned home to be followed at Oslo University Hospital, only to develop recurrent abdominal pain and diarrhea around Day 145, with a fecal calprotectin value of 510. Endoscopic findings (Fig. 3C–G) on Day 178 were consistent with recurrent CD: two superficial ulcerations at 30 cm. beyond the ligament of Treitz, then prestenotic dilation, then more extensive ulceration in a strictured area of ~2–3 cm. length. The stricture was balloon dilated, and mucosa was then normal in appearance to an anastomosis at ~70 cm. beyond Treitz. Biopsy histology from strictured area showed ulcerations, edema, and acute inflammation. Colon mucosa was normal. Treatment with prednisone 1 mg/kg and oral budesonide was started and tacrolimus was continued. To determine whether host T-cells persisted in the intestine, we stained sections from endoscopic biopsies with a combination of FISH probes targeting X and Y chromosomes, and antibodies to the T-cell antigen CD3 [22,23]. At day 178 post transplantation only 21 % of $CD3^+$ T cells in non-inflamed jejunum were derived from donor, whereas in colon the donor contribution was lower at 16 % donor (Fig. 4A–F). Both host $CD4^+$ and

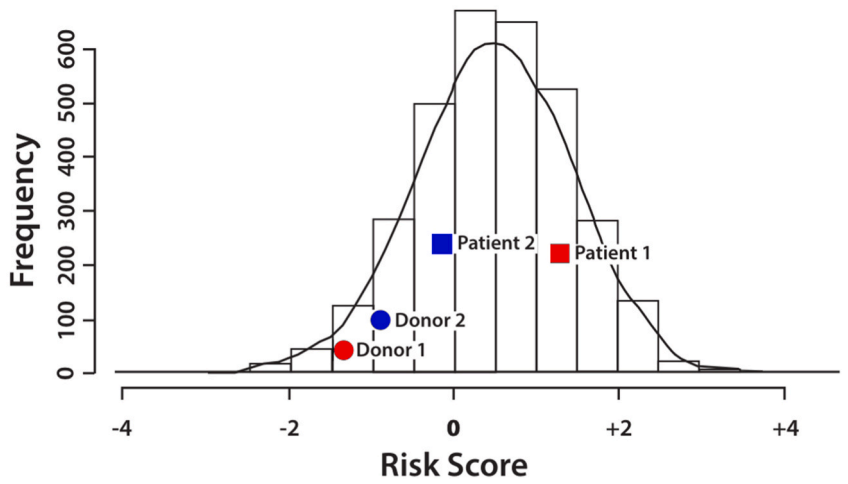

Fig. 2. Histogram of Crohn's Disease Polygenic Risk Score, with patients and their respective donors plotted in relation to a larger Inflammatory Bowel Disease cohort from Cedars-Sinai Medical Center, Los Angeles CA.[2,3].

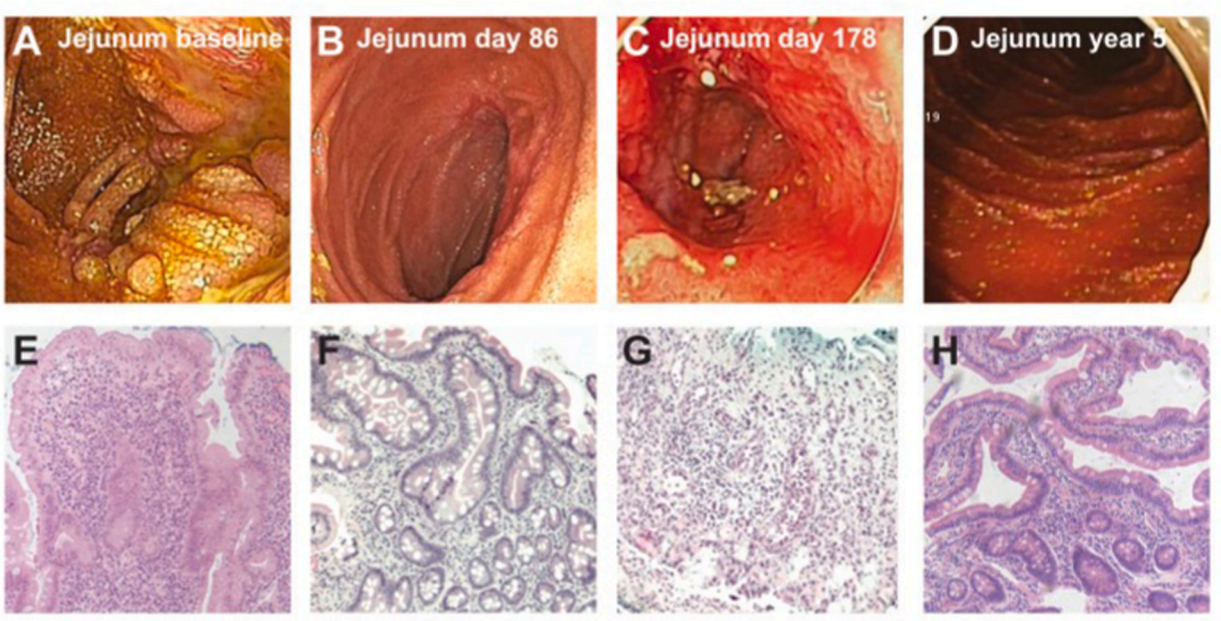

Fig. 3. Endoscopic photographs and histology of the same jejunal site from patient 2. Panels A and E are from before the start of conditioning therapy; panels B and F, from day 86, prior to discharge from Seattle; panels C and G, from day 178, during a flare of Crohn's Disease; and panels D and H, from 5 years after transplant.

$CD8^+$ T cells were present (not shown). Peripheral blood chimerism studies at this time showed that $CD3^+$ T-cells were 86 % donor, 14 % host, and $CD33^+$ myeloid cells were 93 % donor, 7 % host. These findings demonstrated that host T-cell residency was extremely durable in the intestinal mucosa, consistent with recurrent CD, in turn related to failure of allogeneic donor cells to eliminate host immune cells, due to the intense immune suppression that was used to prevent GVHD. On Day 206, all immune suppressive medications were tapered and then discontinued, with the aim of establishing donor hematopoietic cell dominance while minimizing host immunity. Her symptoms improved over the subsequent months, with fecal calprotectin value of 18 on Day 243. At 1 year after transplant, CDAI was 25, HBI 1, the Short IBD Questionnaire score was 69/70 (Fig. 1, three panels on the right, blue lines). And restaging endoscopic and microscopic examination at 1 year showed only a small ulcer in mid-jejunum. Annual examinations up to 5 years were endoscopically and microscopically normal except for focal erosions and inflammatory foci. The jejunal stricture present at baseline has been unchanged throughout the 5-year period, but with clearing of mucosal ulceration and less prestenotic retention. Her symptom and quality of life scores, off all CD medications, were similar at years 2, 3, 4, and 5 post-transplant (Fig. 1, three panels on the right, blue lines). Peripheral blood donor T cell chimerism was 90 % at 1 year and gradually increased to 99 % at 3 years. Combined XY FISH/T-cell *in situ* staining of intestinal biopsies showed a substantial number of host T cells in the intestine more than 2 years after transplant (Fig. 4G-L). Over subsequent years, a gradual increase in donor mucosal T-cells (Fig. 4C–F) was seen. Stable clinical symptoms off medications continued until ~ Day 2252 (6^+ years), when worsening jejunal obstruction led to stricturoplasty and anastomosis revision, followed by relief of obstructive symptoms (CDAI on Day 2542 (7 years) was 84). Peripheral blood chimerism studies on Days 2290 (6.3 years) and 2628 (7.2 years) showed dominant donor immune cells (>99 % T-cells, 93–94 % granulocyte/monocyte). Y-body immunohistology of intestinal mucosal biopsies showed donor T-cells (XY^+) increasing over time (Fig. 4C–F). At 8^+ years post-transplant, she remains stable off medications but with intermittent abdominal pain.

4. Discussion

The 2 cases presented here cannot provide any conclusions on whether reduced-intensity conditioning and allogeneic bone marrow transplantation is feasible for therapy of refractory CD. The lessons learned from both patients, however, serve to inform the design of future studies whose aim would be to achieve durable remissions of CD in patients with severe refractory disease. Patient 2 ultimately met this goal only after a course complicated by recurrent CD some 6 months after transplant, discontinuation of immune suppression, and gradual increase in donor density of intestinal mucosal immune cells. Resolution of CD in this patient and others undergoing allogeneic transplants [10,11] provide proof-of-principle that replacing host immunity with donor cells can be an approach to treatment of patients with unrelenting CD. However, too few CD patients have been studied to recommend allogeneic transplants outside of research protocols. We learned that reduced intensity (non-myeloablative) conditioning therapy with cyclophosphamide, fludarabine and TBI 200 cGy was successful in avoiding organ toxicity in both patients but was a potential contributor to persistence of host immunity in intestinal mucosa in patient 2. Whether newer reduced-intensity conditioning regimens (for example, fludarabine with busulfan or thioTEPA or treosulfan) would lead to a lower frequency of host immunity in intestinal mucosa and durable CD

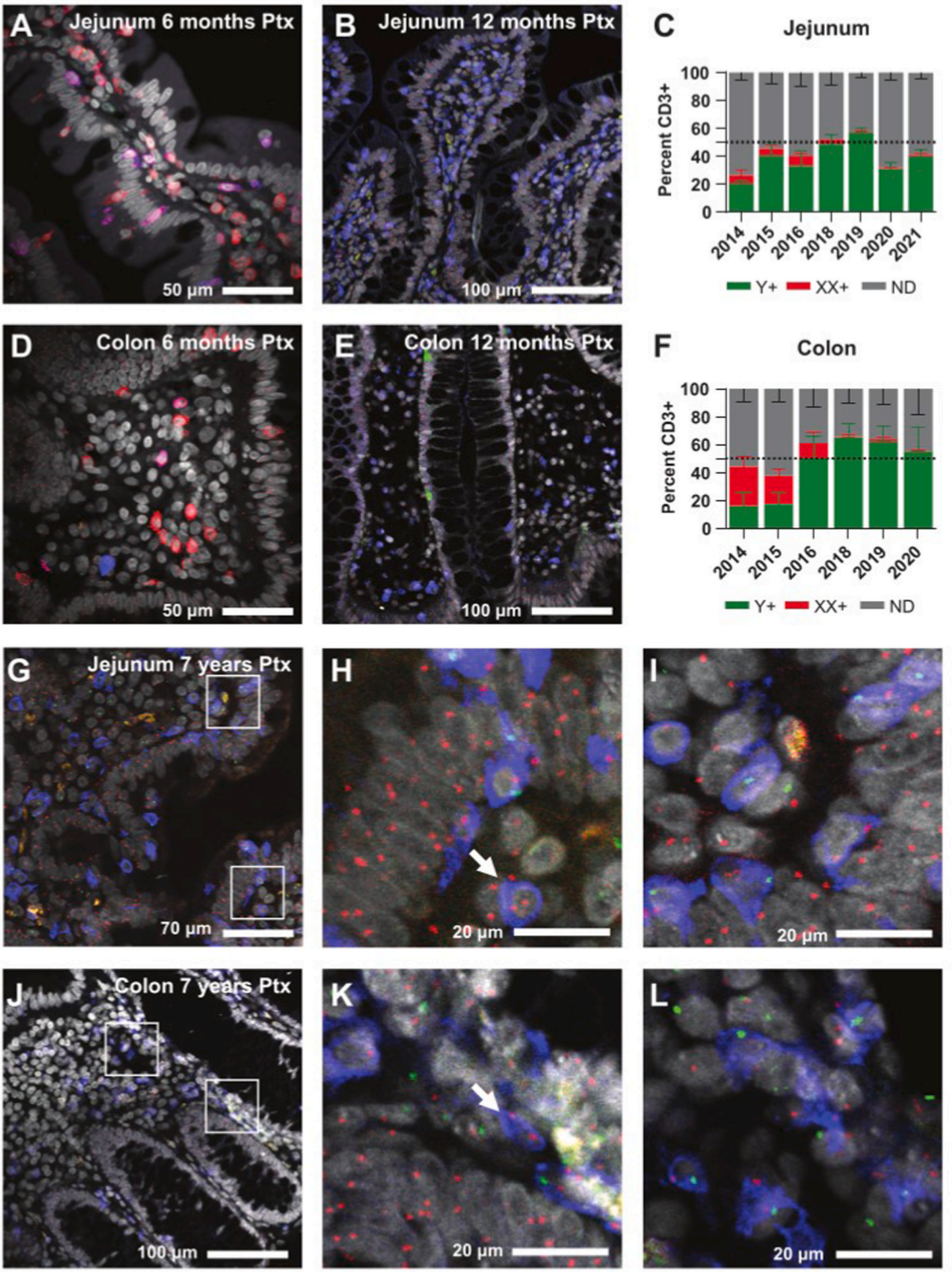

Fig. 4. Patient 2, representative micrographs with T cell counts and origin in jejunum and colon after transplantation. Micrographs were taken by confocal microscopy of tissue sections from biopsies obtained by endoscopy (A, B and H–I) or colonoscopy (D, E and J-L) at indicated time-points post transplantation (Ptx). Panels A and D present CD3 and X-chromosomes (both in red), Y-chromosomes (green), CD8 (blue) and nuclei (grey). Panels B, E and G-L show X- (red) and Y-chromosomes (green), CD3 (blue) and nuclei (grey) without CD8 staining to identify XX positive patient derived T cells. Panels H–I and K-L are magnifications from G and J, respectively, and arrows indicate persisting host T cells at 7 years post transplantation. Graphs in panel C and F present mean counts of CD3 cells containing one Y-chromosome (green), two X-chromosomes (red) or cells

that could not be confidently identified as either Y-, or XX-chromosome positive (not determined (ND) in grey). Percent cells and standard deviation were generated by manual counting $CD3^+$ cells in 3–7 micrographs from 1 to 3 biopsies per time-point, in total $>8 \times 10^3$ individual T cells. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

remissions is an open question. Previous reports of durable CD remissions following allogeneic transplantation have followed myeloablative conditioning regimens [10,11]. Measures to prevent more severe GVHD were effective. However, intense immune suppression to prevent GVHD contributed to high levels of persistent host immunity in both peripheral blood and especially intestinal mucosa. Recurrence of Patient 2's CD after transplant was treated by withdrawal of immune suppression resulting in >98 % donor immune chimerism in peripheral blood and resolution of CD findings. As time passed, her host mucosal T-cells decreased in numbers while donor T-cells became dominant (Fig. 4C–F), similar to the findings in peripheral blood. The obvious limitations of this study are the enrollment of only 2 of 12 patients and the untimely death of Patient 1, before her post-transplant CD course could be evaluated. The strengths include avoidance of regimen-related toxicity and more severe GVHD, genomic analysis of patients and donors, serial intestinal biopsies for evaluation of mucosal immune chimerism, and long-follow-up of Patient 2.

A small percentage of persistent host hematopoietic chimerism in peripheral blood is the expected result of an allograft after non-myeloablative conditioning. Host lymphoid cells can persist in tissues far longer than in marrow and peripheral blood. Similar observations related to persistent host immune cells have been made in organ transplant recipients [20,21]. We halted enrollment after the post-transplant flare of CD in Patient 2. Even though this patient's flare of CD symptoms and mucosal injury resolved after immune suppressive therapy was discontinued, we could not be sure what her long-term course would be, and thus, were reluctant to offer this protocol to other CD patients.

We speculate that persistent mucosal host T-cells in Patient 2 were directly involved in the recurrence of CD. Because Patient 2 showed mixed chimerism in blood we cannot rule out the possibility that host T cells activated elsewhere were recruited to the intestinal mucosa after transplantation. Non-lymphoid tissues contain high numbers of non-circulating resident memory T cells, and in transplanted intestine both $CD4^+$ and $CD8^+$ T cells can persist for more than a year without replenishment from blood [21,24]. In another study, there was no difference in the survival of host T cells between allografted patients receiving non-myeloablative versus myeloablative conditioning; in the latter case all patients displayed 100 % blood chimerism shortly after transplant [25]. We speculate that more intense post-transplant immune suppression was responsible for failure of donor cells to reject host hematopoietic cells, thus, leading to persistence of host immune cells in peripheral blood. This hypothesis is supported by the absence of symptoms of CD at 1 year in Patient 2 (Fig. 1, three panels on the right, blue lines) and endoscopic and histologic improvement following discontinuation of all immune suppressive medications.

Autologous hematopoietic cell transplantation has been carried out in patients with CD as the indication [26–29]. A meta-analysis describing outcomes of autologous transplantation for CD reported 54.1% treatment-free remissions at one year and 6.4 % treatment-related mortality [28]. While the majority of autologous graft recipients with CD show improvement, the cumulative incidence of recurrent CD increases over time [26]. Clinical experience suggests that CD recurrences after autologous transplant, however, are more responsive to treatment and less severe than therapies during the time period before transplantation [29]. Strictures, however, often persist after autologous transplants and may require resection, as did our Patient 2 [26,29].

Recent genomic studies have shown that polymorphisms in >240 loci are related to susceptibility to developing CD, with different patterns of polymorphisms for small intestinal vs. ileocolic vs. colonic CD as defined by clinical and endoscopic criteria [1–4,30]. A Polygenic Risk Score (Fig. 2) confirms that both patients had a higher genetic burden compared to their respective donors. A recent genomic study suggests that SNPs in four gene regions — unrelated to gene regions associated with CD susceptibility — were related to severity and prognosis of patients with CD [3]. Three of these genetic loci were related to the immune system (FOXO3, a region near the IGFBP1 gene, and the MHC region) and one related to embryonic stem cells (XACT), with high expression in the intestine [22]. CD in Patient 2 was confined to the small intestine and dominated by fibrostenotic lesions, for which a recent genomic association study has identified a variant in the WWOX gene [23].

On the negative side, the death of Patient 1 on Day 337 precluded any meaningful analysis of her CD course after transplant. She engrafted promptly but died after a series of complications. Mucosal erosions in her neoterminal ileum and colon that were present at baseline were absent just before her death from respiratory failure. Unlike Patient 2, her peripheral blood chimerism studies showed the usual pattern of >98 % donor dominance. We speculate that non-compliance with tacrolimus dosing allowed donor cell dominance, resulting in absence of recurrent CD but leading to development of acute GVHD.

Of the mutations in genes and gene regions that have been found to increase susceptibility to CD, most are related to defects in immune recognition and response, but some involve defective antigen processing by epithelial cells. We speculate that while allogeneic hematopoietic cell transplantation will correct defects in immunity, as seen in children with inborn errors [25–27], mutations related to epithelial cell dysfunction would not be corrected by hematopoietic cell transplantation. Myeloablative conditioning regimens should avoid persistent host immune chimerism in intestinal mucosa and result in durable remissions [10,11]. When the risk of mortality after myeloablative allografts has fallen to low levels [29], protocols for patients with refractory CD using myeloablative conditioning and allogeneic transplantation should be considered. At this time, the mortality risk posed by current myeloablative conditioning regimens and their regimen-related toxicity is unacceptably high as a treatment for refractory CD [19]. We foresee future transplant research for CD aimed at reducing the toxicity of myeloablative conditioning regimens by pretransplant screening using morbidity indices and by therapeutic drug monitoring of components of conditioning drugs, along with prevention of more severe GVHD.

Grant support

Funding for Seattle-based research for the Crohn's Allogeneic Transplant Study was provided by a generous grant from the Eli and Edythe Broad Foundation, a donation from an anonymous source, and support from the Seattle Cancer Care Alliance. Genotyping data at Cedars-Sinai Medical Center, Los Angeles CA. was supported by the Helmsley Charitable Trust and the National Institutes of Health NIDDK IBD Genetics Consortium (U01 DK062413). Follow-up evaluation of Patient 2 was carried out in Oslo, Norway, and supported by research funds from South-Eastern Norway Regional Health Authority.

Transcript profiling

Not applicable.

Writing assistance

The manuscript was written entirely by the authors.

Ethics statement

This study was reviewed and approved by the Institutional Review Organization of the Fred Hutchinson Cancer Center, with the approval number Protocol 2551 (NCT01570348). All participants/patients provided informed consent to participate in the study. All participants/patients provided informed consent for the publication of their anonymized case details and images.

Data availability statement

In the Results section, Tables, Figures, and Supplemental Material, we have provided data that are publicly available. The data have not been deposited in a separate repository as data in the manuscript serves this purpose. Further clinical details are available within the constraints of consent documents and patient privacy.

CRediT authorship contribution statement

George B. McDonald: Writing - review & editing, Writing - original draft, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Ole J.B. Landsverk:** Visualization, Methodology, Investigation, Formal analysis, Data curation. **Dermot P.B. McGovern:** Validation, Supervision, Methodology, Formal analysis, Data curation. **Anders Aasebø:** Visualization, Methodology, Investigation, Formal analysis, Data curation. **Vemund Paulsen:** Visualization, Investigation, Data curation. **Talin Haritunians:** Validation, Methodology, Investigation, Formal analysis, Data curation. **Henrik M. Reims:** Resources, Investigation, Funding acquisition. **Bernadette M. McLaughlin:** Validation, Project administration, Investigation, Data curation. **Timothy Zisman:** Visualization, Investigation, Data curation. **Dalin Li:** Validation, Methodology, Formal analysis, Data curation. **Elisabeth T.M.M. Elholm:** Visualization, Investigation. **Frode L. Jahnsen:** Validation, Investigation, Funding acquisition, Formal analysis. **George E. Georges:** Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Tobias Gedde-Dahl:** Validation, Investigation, Funding acquisition, Formal analysis, Data curation.

Declaration of competing interest

None of the authors has a conflict of interest related to the content of this work.

Acknowledgements

Funding for Seattle-based research for the Crohn's Allogeneic Transplant Study was provided by a generous grant from the Eli and Edythe Broad Foundation, a donation from an anonymous source, and support from the Seattle Cancer Care Alliance. Genotyping data at Cedars-Sinai Medical Center, Los Angeles CA. was supported by the Helmsley Charitable Trust and the National Institutes of Health NIDDK IBD Genetics Consortium (U01 DK062413). Follow-up evaluation of Patient 2 was carried out in Oslo, Norway, and supported by research funds from South-Eastern Norway Regional Health Authority.

We are grateful for the care provided for our patients by nurses, physicians, pharmacists, and support staff in both Seattle and Oslo.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e24026.

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