Leukemia (2019) 33:2732–2766
https://doi.org/10.1038/s41375-019-0510-0

LETTER

Stem cell biology

Clonal hematopoiesis by *SLIT1*-mutated hematopoietic stem cells due to a breakdown of the autocrine loop involving Slit1 in acquired aplastic anemia

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Received: 7 February 2019 / Revised: 23 April 2019 / Accepted: 1 May 2019 / Published online: 11 June 2019

To the Editor:

Acquired aplastic anemia (AA) is a hematopoietic disorder caused by an immune attack on hematopoietic stem and progenitor cells (HSPCs) and has been thought to be rarely associated with the acquired genetic defects in HSPCs. However, recent studies have found that clonal hematopoiesis from HSPCs with genetic alterations is not so uncommon in AA [1]. For instance, clonal granulocyte populations of the paroxysmal nocturnal hemoglobinuria (PNH) phenotype and uniparental disomy of chromosome 6p are detected in 50 and 13% patients with AA, respectively [2, 3], and represent the immune pathophysiology of bone marrow (BM) failure. Given that even a chromosomal abnormality like del(13q) is associated with good response to immunosuppressive therapy (IST) [4], hematopoiesis in AA patients in remission after IST is likely supported by a limited number of HSPCs, with acquired genetic changes that survived the attack on the immune system. At present, however, how these mutant HSPCs contribute to hematopoiesis in AA patients remains largely unknown.

Slit1 is a member of the Slit family that binds to members of the Robo receptor family [5]. The interaction between Slit and Robo inhibits leukocyte chemotaxis, migration, and proliferation, and induces apoptosis in normal and tumor cells [6]. Some studies have found that the Slit/Robo pathway regulates cell function in both an autocrine and paracrine manner [7, 8]. However, little is known about the role of this pathway in the regulation of hematopoiesis. We detected clonal hematopoiesis by HSPCs with *SLIT1* mutations in two patients with acquired AA, who were in remission after IST. Since Slit1 negatively regulates various cell types, mutations in Slit1 could represent a novel mechanism underlying "benign" clonal hematopoiesis in patients with immune-mediated BM failure. To test this hypothesis, we investigated the mechanism underlying clonal hematopoiesis, using a mutant Slit1 protein that harbors a mutation identified in one of the AA patients.

Three moderate AA patients with minor populations of GPI(–) granulocytes who readily responded to cyclosporine monotherapy were chosen for the first screening of genetic abnormalities associated with clonal hematopoiesis. DNA extracted from granulocytes of these three patients was subjected to exome sequencing, using the Illumina Genome

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Supplementary information The online version of this article (https://doi.org/10.1038/s41375-019-0510-0) contains supplementary material, which is available to authorized users.

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Bone Marrow Transplantation (2019) 54:1129–1132
https://doi.org/10.1038/s41409-018-0420-1

CORRESPONDENCE

Escape hematopoiesis by donor-derived 6pLOH(+) hematopoietic stem cells in a marrow transplant recipient with late graft failure

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Received: 14 September 2018 / Revised: 12 November 2018 / Accepted: 28 November 2018 / Published online: 10 January 2019

Late graft failure (LGF) is a rare but serious complication in allogeneic hematopoietic stem cell transplant (allo-HSCT) recipients with complete donor chimerism. A second allo-HSCT from the original donor with or without preconditioning may be effective [1, 2]; however, it is often associated with transplant-related mortality. Some patients with LGF can be successfully treated with immunosuppressive therapy to inhibit donor T cells capable of specifically eliminating autologous hematopoietic stem cells [3, 4]. However, it is difficult to confirm the involvement of donor-derived cytotoxic T cells in the development of LGF. Here, we report a case of donor-type LGF, which occurred 17 years after an allogeneic bone marrow transplantation from a 6/8 human leukocyte antigen (HLA)-matched half-sibling donor. Immune-mediated hematopoietic failure was diagnosed based on the presence of HLA-A allele-lacking leukocytes (HLA-LLs) attributed to copy number neutral loss of heterozygosity in the short arm of chromosome 6 (6pLOH).

A 38-year-old female was diagnosed with having severe aplastic anemia in November 1997. She did not respond to treatment with horse anti-thymocyte globulin (ATG), cyclosporine (CsA), and danazol: thus, there was a risk of death due to severe pancytopenia. As no HLA-matched donors were available from her family and the Japan Marrow Donor Program, she underwent bone marrow transplantation from an HLA-haploidentical donor at the age of 40 after a myeloablative conditioning regimen with cyclophosphamide (60 mg/kg/day on days −4 and −3), TBI (3 Gy × 2/day on days −9 and −8), and cytarabine (3 g/m^2 × 2/day on days −6 and −5). The HLA haplotypes of the donor were *A*02:01-B*54:01-C*01:02-DRB1*15:02/A*26:01-B*40:02-C*03:04-DRB1*09:01* and the HLA haplotypes of the patient were A2/A24-B54/B61-Cw1/Cw9-DRB1*15:02/DRB1*09:01, which were estimated to be *A*02:01-B*54:01-C*01:02-DRB1*15:02/A*24:02-B*40:02-C*03:03-DRB1*09:01* based on the HLA frequency in the Japanese population. Neutrophil engraftment occurred on day 19, and transfusion independence was achieved on day 91 after bone marrow transplantation. She developed chronic graft-versus-host disease (cGVHD) of the skin, liver, and lungs on day 366 and required long-term treatment with CsA and prednisolone until August 2010. When CsA was discontinued at the age of 51, her white blood cell count was 9.4 × 10^9/L, hemoglobin level was 12.2 g/dL, and platelet count was 92 × 10^9/L. Six years later, pancytopenia developed without any signs of infection and recurrence of cGVHD. The complete blood counts were as follows: white blood cells 2.9 × 10^9/L with 78% neutrophils and 7.5% lymphocytes, hemoglobin level 9.6 g/dL, platelets 12 × 10^9/L, and reticulocytes 65 × 10^9/L. Bone marrow examination showed hypocellularity without abnormal cells and dysplastic signs. Sex chromosome analyses of her peripheral blood granulocytes and T cells showed complete donor chimerism. LGF with complete donor chimerism was diagnosed. A high-sensitivity flow cytometry assay revealed glycosylphosphatidyl-inositol-anchored protein (GPI-AP)-deficiency in 0.082% granulocytes and 0.006% erythrocytes (Fig. 1a), suggesting that the patient's LGF was immune-mediated [3].

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Escape hematopoiesis by HLA-B5401-lacking hematopoietic stem progenitor cells in men with acquired aplastic anemia

Leukocytes that lack HLA class I alleles derived from hematopoietic stem and progenitor cells (HSPC) that undergo copy number-neutral loss of heterozygosity of the short arm of chromosome 6 (6pLOH) or HLA allelic mutations are often detected in patients with aplastic anemia (AA). The presence of HLA class I allele-lacking leukocytes provides compelling evidence that cytotoxic T-lymphocytes (CTL) are involved in the development of AA,[1-5] but the precise mechanisms underlying HLA lack and clonal hematopoiesis by such HLA(–) HSPC is unknown.

We recently showed that *B*54:01* was one of three HLA alleles that were most likely to be possessed by 6pLOH$^+$ patients [29% (5/17)] when only patients not carrying *HLA-B*40:02* were analyzed.[5] To gain insight into the mechanism underlying clonal hematopoiesis by HLA-B5401-lacking HSPC, we studied the role of *HLA-B*54:01* in the pathogenesis of AA in a larger number of patients as well as HSPC derived from induced pluripotent stem cells (iPSC) that were generated from an AA patient whose monocytes lacked B5401.

A total of 733 AA patients were enrolled in an observational study to determine the prevalence of HLA class I allele-lacking leukocytes by GeneChip 500 K arrays (Affymetrix, Japan) and droplet digital polymerase chain reaction using a QX200 AutoDG Droplet Digital PCR System (Bio-Rad, Hercules, CA, USA) or a next-generation sequencer (MiSeq; Illumina, San Diego, CA, USA) as previously described.[1,5] Informed consent was obtained from the study participants for the genetic analyses and iPSC generation. The diagnosis and severity of AA were determined according to standard criteria.[6] The characteristics of the AA patient (KANA6) who was selected to generate iPSC from monocytes are described in the *Online Supplementary Material* and in *Online Supplementary Figures S1* and *S2* and *Online Supplementary Tables S1* and *S2*. This study was approved by the ethics committee of the Kanazawa University Institute of Medical, Pharmaceutical, and Health Sciences.

The generation of iPSC (*Online Supplementary Figure S3*), induction of HSPC from iPSC, and transplantation of the induced iPSC-HSPC human CD34$^+$ cells into sublethally (150 cGy) irradiated 57BL/6.Rag2^{null}Il2rg^{null} NOD-Sirpa (BRGS) young mice were carried out according to the methods we described previously. Details are provided in the *Online Supplementary Methods* and the monoclonal antibodies and primer sets used for this study are listed in *Online Supplementary Tables S3-S6*. Statistical analyses of the patients' clinical parameters, 6pLOH determination and calculation were performed as described previously.[1,5] For all iPSC experiments, statistical analyses were performed using the GraphPad Prism software package, version 5.02 (San Diego, CA, USA). The results were analyzed using a Student *t*-test.

The presence of 6pLOH was evaluable in 618 (84.3%) of 733 patients with AA, and 6pLOH was detected in 107 (17.3%) of the patients. The 6pLOH$^+$ patients were assessed for the allelic frequency in their lost haplotypes. Consistent with our previous report,[5] *HLA-B*40:02* was most strongly involved in 6pLOH (46.7%), and *HLA-B*54:01* (10.3%) was the second most frequent HLA-B allele in the lost haplotype (*Online Supplementary Table S7*). When only 6pLOH$^+$ patients not carrying *HLA-B*40:02* were analyzed, the frequency of *HLA-B*54:01* (19.3%) was the highest among all HLA-B alleles included in the lost haplotype (*Online Supplementary Table S8*).

Of the 733 patients with AA in this study cohort, 115 (15.7%) had *HLA-B*54:01*, which is a significantly higher frequency than that previously reported in a general

Table 1. Clinical characteristics of aplastic anemia patients with loss of heterozygosity of 6p carrying *HLA-B*54:01*.

Case	Age (years)	Sex	Severity	Treatments	Response to IST	Missing haplotype				Retained haplotype				PNH cells
						A	B	C	DRB1	A	B	C	DRB1	
1	65	M	VSAA	ATG/CsA	NR	24:02	54:01	01:02	04:05	31:01	15:01	03:03	08:02	(+)
2	71	M	NSAA	AS	NA	11:01	54:01	01:02	04:05	02:06	35:01	03:03	15:01	(+)
3	69	M	NSAA	ATG/CsA/AS	CR	11:01	54:01	01:02	04:05	02:01	40:01	04:01	15:01	(+)
4	77	M	SAA	ATG/CsA	PR	24:02	54:01	01:02	04:05	24:02	15:01	03:03	15:01	(+)
5	71	M	SAA	NA	NA	24:02	54:01	08:01	09:01	02:01	40:06	01:02	15:01	(-)
6 (KANA6)	53	M	NSAA	CsA/AS	CR	24:02	54:01	01:02	04:01	01:01	37:01	06:02	01:01	(+)
7	81	M	SAA	NA	NR	24:02	54:01	01:02	01:01	24:02	52:01	12:02	15:02	NT
8	80	M	NSAA	CsA/ELT	PR	24:02	54:01	03:03	15:02	02:06	35:01	01:02	04:05	(+)
9	18	M	SAA	ATG/CsA	NA	11:01	54:01	03:04	04:05	26:05	40:06	15:02	09:01	(+)
10	55	M	SAA	ATG/CsA/ELT	PR	02:07	54:01	01:02	04:05	24:02	15:18	07:04	12:01	(+)
11	77	F	NSAA	CsA	NA	24:02	54:01	01:02	15:02	24:02	15:07	03:03	04:03	(+)
12	32	M	VSAA	ATG/CsA	PR	11:01	40:02	01:02	08:03	24:02	54:01	03:04	12:01	(-)
13	11	M	SAA	BMT	NR	02:01	40:02	03:04	09:01	24:02	54:01	01:02	04:05	NT
14	30	M	SAA	BMT	NR	31:01	40:02	03:04	15:01	24:02	54:01	01:02	04:05	NT
15	20	F	SAA	ATG/CsA	PR	02:06	39:01	08:03	15:01	24:02	54:01	07:02	04:05	(+)
16	51	F	NSAA	Romiplostim	PR	02:06	46:01	01:02	09:01	24:02	54:01	01:03	15:01	(-)

PNH cells: paroxysmal nocturnal hemoglobinuria cells (glycosylphosphatidylinositol-anchored protein deficient, GPI-AP$^-$ cells); IST: immunosuppressive therapy; M: male; F: female; VSAA: very severe aplastic anemia; NSAA: non-severe aplastic anemia; SAA: severe aplastic anemia; ATG: antithymocyte globulin; CsA: cyclosporine A; AS: anabolic steroids; ELT: eltrombopag; BMT: bone marrow transplantation; NR: no response; NA: not available; CR: complete response; PR: partial response; NT: not tested; B5401(-) leukocytes, leukocytes that lost B5402 expression as a result of 6pLOH or *B*54:01* mutations..

ARTICLE

HLA discrepancy between graft and host rather than that graft and first donor impact the second transplant outcome

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Ferrata Storti Foundation

Haematologica 2019
Volume 104(5):1055-1061

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Received: August 20, 2018.

Accepted: November 23, 2018.

Pre-published: December 6, 2018.

doi:10.3324/haematol.2018.204438

Check the online version for the most updated information on this article, online supplements, and information on authorship & disclosures: www.haematologica.org/content/104/5/1055

ABSTRACT

Second allogeneic hematopoietic stem cell transplantation is a curative treatment option for patients with hematologic malignancies. However, it is unclear whether HLA discrepancy between graft and first donor has an impact on the outcome of second transplantation. We retrospectively analyzed 646 patients receiving second transplantation after an initial HLA mismatched transplantation. With regard to graft-*versus*-host, the one-allele mismatch (1 mismatch) group (SHR, 1.88; 95%CI: 0.79-4.45; *P*=0.163) and more than one-allele mismatch group (≥ 2 mismatch) (SHR, 1.84; 95%CI, 0.75–4.51; *P*=0.182) had higher risks of grade III–IV acute graft-*versus*-host disease (GvHD) compared to the HLA-matched (0 mismatch) group. In contrast, no difference in risk of acute GvHD was found among the 0, 1, and ≥ 2 mismatch group with respect to graft-*versus*-first donor. With regard to graft-*versus*-host, the ≥ 2 mismatch group showed a significantly higher risk of treatment-related mortality (SHR, 1.90; 95%CI, 1.04–3.50; *P*=0.038) compared to the 0 mismatch group, while the risk of relapse was slightly lower in the ≥ 2 mismatch group (SHR, 068; 95%CI, 0.44–1.06; *P*=0.086). In contrast, with regard to graft-*versus*-first donor, there were no significant differences in treatment-related mortality or relapse among the three groups. These findings suggested that HLA discrepancy between graft and host induces transplant-related immunological responses in second transplantation leading to an increase in treatment-related mortality, in contrast, the biological effects of HLA discrepancy between graft and first donor on outcome may be negligible.

SCIENTIFIC REPORTS

OPEN

High-throughput sequencing of IgG B-cell receptors reveals frequent usage of the rearranged IGHV4–28/IGHJ4 gene in primary immune thrombocytopenia

Received: 3 January 2019
Accepted: 4 June 2019
Published online: 14 June 2019

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Primary immune thrombocytopenia (ITP) is an acquired form of thrombocytopenia caused by IgG anti-platelet autoantibodies and represents an organ-specific autoimmune disorder. Although the glycoprotein (GP)IIb/IIIa and GPIb/IX have been shown to be targets for autoantibodies, the antigen specificity of autoantibodies is not fully elucidated. To identify the characteristics of IgG B-cell receptor (BCR) repertoires in ITP, we took advantage of adaptor-ligation PCR and high-throughput DNA sequencing methods for analyzing the clone-based repertoires of IgG-expressing peripheral blood B cells. A total of 2,009,943 in-frame and 315,469 unique reads for IGH (immunoglobulin heavy) were obtained from twenty blood samples. Comparison of the IGHV repertoires between patients and controls revealed an increased usage of IGHV4–28 in ITP patients. One hundred eighty-six distinct IGHV4–28-carrying sequences were identified in ITP patients and the majority of these clones used an IGHJ4 segment. The IGHV4–28/IGHJ4-carrying B-cell clones were found in all ITP patients. Oligoclonal expansions of IGHV4–28/IGHJ4-carrying B cells were accompanied by multiple related clones with single amino substitution in the CDR3 region suggesting somatic hypermutation. Taken together, the expansion of IGHV4–28/IGHJ4-carrying IgG-expressing B cells in ITP may be the result of certain antigenic pressure and may provide a clue for the immune pathophysiology of ITP.

Primary immune thrombocytopenia (ITP) is an acquired form of thrombocytopenia caused by anti-platelet autoantibodies. The underlying mechanism is thought to involve the production of IgG autoantibodies specific for platelet membrane antigens, such as glycoprotein (GP)IIb/IIIa and GPIb/IX, although anti-platelet autoantibody testing is less sensitive for the diagnosis[1,2]. The ASH and IWG guidelines for the management of ITP do not recommend routine testing of anti-platelet autoantibodies for the diagnosis of ITP, and thus diagnostic biomarkers for ITP need to be developed[3–5].

Although the principal pathophysiology of ITP is an IgG-mediated autoimmune disease, the B-cell receptor (BCR) repertoires associated with this disorder are largely unknown. The spleen is generally believed to be the primary site for the activation of T and B cells responsible for autoantibody production in primary ITP[6,7]. Interestingly, however, Kuwana *et al.* found that B cells secreting anti-GPIIb/IIIa or anti GPIb antibodies can be detected in the peripheral blood as well as spleen from primary ITP patients using an enzyme-linked immunospot (ELISPOT) assay[7–9]. In addition, others have reported that antigen-specific IgG-bearing memory B cells can be detected in circulating blood in humans[10].

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Biol Blood Marrow Transplant 25 (2019) 1536 1543

Biology of Blood and Marrow Transplantation

journal homepage: www.bbmt.org

Comparison of Outcomes of Allogeneic Transplantation for Primary Myelofibrosis among Hematopoietic Stem Cell Source Groups

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Article history:
Received 29 November 2018
Accepted 19 February 2019

Keywords:
Primary myelofibrosis
Allogeneic transplantation
Unrelated donor

A B S T R A C T

The choice of alternative donor is a major issue in allogeneic hematopoietic stem cell transplantation (HSCT) for patients with primary myelofibrosis (PMF) without an HLA-matched related donor. We conducted this retrospective study using the Japanese national registry data for 224 PMF patients to compare the outcomes of first allogeneic HSCT from HLA-matched related donor bone marrow (Rtd-BM), HLA-matched related donor peripheral blood stem cells (Rtd-PB), HLA-matched unrelated donor bone marrow (UR-BM), unrelated umbilical cord blood (UR-UCB), and other hematopoietic stem cell grafts. Nonrelapse mortality (NRM) rates at 1 year after Rtd-BM, Rtd-PB, UR-BM, UR-UCB, and other transplantations were 16%, 36%, 30%, 41%, and 48%, respectively. Multivariate analysis identified UR-UCB transplantation, other transplantation, frequent RBC transfusion before transplantation, and frequent platelet (PLT) transfusion before transplantation as predictive of higher NRM. Relapse rates at 1 year after Rtd-BM, Rtd-PB, UR-BM, UR-UCB, and other transplantation were 14%, 17%, 11%, 14%, and 15%, respectively. No specific factor was associated with the incidence of relapse. Overall survival (OS) at 1 and 4 years after Rtd-BM, Rtd-PB, UR-BM, UR-UCB, and other transplantation were 81% and 71%, 58% and 52%, 61% and 46%, 48% and 27%, and 48% and 41%, respectively. Multivariate analysis identified older patient age, frequent RBC transfusion before transplantation, and frequent PLT transfusion before transplantation as predictive of lower OS. In conclusion, UR-UCB transplantation, as well as UR-BM transplantation, can be selected for PMF patients without an HLA-identical related donor. However, careful management is required for patients after UR-UCB transplantation because of the high NRM. Further studies including more patients after HLA-haploidentical related donor and HLA-mismatched unrelated donor transplantation would provide more valuable information for patients with PMF when making decisions regarding the choice of alternative donor.

INTRODUCTION

It was initially anticipated that the use of hematopoietic stem cell transplantation (HSCT) to treat primary myelofibrosis (PMF) might decrease due to the widespread availability of Janus kinase (JAK) inhibitors [1]. However, so far JAK inhibitor therapy has not resulted in the induction of complete or partial

Financial disclosure: See Acknowledgments on page 1542.

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https://doi.org/10.1016/j.bbmt.2019.02.019
1083-8791/

Complement and inflammasome overactivation mediates paroxysmal nocturnal hemoglobinuria with autoinflammation

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Patients with paroxysmal nocturnal hemoglobinuria (PNH) have a clonal population of blood cells deficient in glycosylphosphatidylinositol-anchored (GPI-anchored) proteins, resulting from a mutation in the X-linked gene *PIGA*. Here we report on a set of patients in whom PNH results instead from biallelic mutation of *PIGT* on chromosome 20. These PIGT-PNH patients have clinically typical PNH, but they have in addition prominent autoinflammatory features, including recurrent attacks of aseptic meningitis. In all these patients we find a germ-line point mutation in one *PIGT* allele, whereas the other *PIGT* allele is removed by somatic deletion of a 20q region comprising maternally imprinted genes implicated in myeloproliferative syndromes. Unlike in PIGA-PNH cells, GPI is synthesized in PIGT-PNH cells and, since its attachment to proteins is blocked, free GPI is expressed on the cell surface. From studies of patients' leukocytes and of *PIGT*-KO THP-1 cells we show that, through increased IL-1β secretion, activation of the lectin pathway of complement and generation of C5b-9 complexes, free GPI is the agent of autoinflammation. Eculizumab treatment abrogates not only intravascular hemolysis, but also autoinflammation. Thus, PIGT-PNH differs from PIGA-PNH both in the mechanism of clonal expansion and in clinical manifestations.

Introduction

Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired hematopoietic stem cell (HSC) disorder characterized by complement-mediated hemolysis, thrombosis, and bone marrow failure (1, 2). Affected cells harbor a somatic mutation in the *PIGA* gene, essential for the initial step in glycosylphosphatidylinositol (GPI) biosynthesis that occurs in the endoplasmic reticulum (ER) (Figure 1A, top and ref. 3). Loss of GPI biosynthesis results in the defective expression of GPI-anchored proteins (GPI-APs), including complement inhibitors CD59 and DAF/CD55 (Figure 1A, middle). The affected stem cells generate large numbers of abnormal blood cells after clonal expansion that occurs under bone marrow failure. The affected erythrocytes are defective in complement regulation and destroyed by the membrane attack complex (MAC or C5b-9) upon complement activation (1). Eculizumab, an anti-complement component 5 (C5) monoclonal antibody (mAb), has been used to prevent intravascular hemolysis and thrombosis (4, 5). Eculizumab binds to C5 and inhibits its activation and subsequent generation of C5b-9 complexes.

Among more than 20 genes involved in GPI biosynthesis and transfer to proteins, *PIGA* is X-linked whereas all others are autosomal (6). Because of X-linkage, one somatic mutation in *PIGA* causes GPI deficiency in both males and females (3). In contrast, 2 mutations are required for an autosomal gene, but the probability of somatic mutations in both alleles at the same locus is extremely low, which explains why GPI deficiency in most patients with PNH is caused by *PIGA* somatic mutations. Recently, we reported 2 patients with PNH whose GPI-AP deficiency was caused by germline and somatic mutations in the *PIGT* gene localized on chromosome 20q (7, 8). Both patients had a heterozygous germline loss-of-function mutation in *PIGT*, along with loss of the normal allele of *PIGT* by a deletion of 8 Mb or 18 Mb occurring in HSCs (7, 8). PIGT, forming a GPI transamidase complex with PIGK, PIGS, PIGU, and GPAA1, acts in the transfer of preassembled GPI to

▶ Related Commentary: p. 5074

Authorship note: BH, YM, and MO contributed equally to this paper. HS, PMK, and TK contributed equally to this paper.
Conflict of interest: HS receives research support through the University of Ulm from Novartis and Alexion Pharmaceuticals.

Submitted: July 19, 2018; **Accepted:** August 16, 2019; **Published:** October 22, 2019.
Reference information: *J Clin Invest*. 2019;129(12):5123–5136.
https://doi.org/10.1172/JCI123501.

International Journal of Hematology (2019) 110:411–418
https://doi.org/10.1007/s12185-019-02699-7

ORIGINAL ARTICLE

Comparative study on baseline clinical characteristics of Asian versus non-Asian patients with paroxysmal nocturnal hemoglobinuria

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Received: 5 February 2019 / Revised: 26 June 2019 / Accepted: 26 June 2019 / Published online: 5 July 2019

Abstract

A difference in clinical manifestations of paroxysmal nocturnal hemoglobinuria (PNH) among different races has been suggested. The aim of this study was to clarify whether the clinical characteristics of patients with PNH in the International PNH Registry differ by ethnic background. Patients, who were eculizumab naïve at baseline and had ≥ 1% PNH clone size, were eligible for this analysis. Totally, 1793 patients were enrolled and divided into two cohorts, Asian ($N=246$) and non-Asian ($N=1547$). The Asian cohort was further divided into Asians in Asia cohort ($N=202$) and Asians in non-Asia cohort ($N=44$), based on geographical region. The Asian cohort had significantly higher PNH clone size in granulocytes, higher lactate dehydrogenase levels, and lower hemoglobin levels. However, the frequencies of symptoms including abdominal pain, backache, easy bleeding, fatigue and headache at baseline were significantly lower in the Asian cohort. The proportion of patients with a history of thromboembolism (TE) was significantly lower in the Asian than in the non-Asian cohort (3.6% vs. 8.9%, $P<0.01$); however, there was no difference between Asians in Asia and Asians in non-Asia (3.3% vs. 4.9%, $P=0.61$). These findings suggested that genetic factors may play a stronger role in developing TE than lifestyle factors in PNH patients.

Keywords Paroxysmal nocturnal hemoglobinuria · Asian · Thromboembolism · International PNH Registry

Introduction

Paroxysmal nocturnal hemoglobinuria (PNH) is a rare, acquired hematopoietic stem cell disease characterized by intravascular hemolysis, bone marrow failure and thrombosis [1]. PNH arises from a somatic mutation of

Statement of Prior Presentation: This work was presented at the 59th Annual Meeting of the American Society of Hematology, December 9–12, 2017, Atlanta, GA.

Masatoshi Sakurai and Jun Ho Jang contributed equally to this study, and both should be regarded as the first authors.

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Successful treatment of a PNH patient non-responsive to eculizumab with the novel complement C5 inhibitor coversin (nomacopan)

Paroxysmal Nocturnal Haemoglobinuria (PNH) is a rare acquired life-threatening disease characterised by complement-induced haemolysis and a high incidence of thrombosis. The monoclonal antibody eculizumab binds to complement C5 and prevents its activation and cleavage into C5a and C5b. Treatment of PNH patients with eculizumab decreases haemolysis, transfusion requirements and the risk of thrombosis (Hillmen *et al.*, 2004, 2006, 2007; Brodsky *et al.*, 2008; Kelly *et al.*, 2011). Failure to respond to eculizumab has been reported in a subgroup of Asian PNH patients (Nishimura *et al.*, 2014). These patients had a genetic variant of C5, a missense mutation leading to c.2654G>A (p.Arg885His), which occurs in approximately 3·5% of Japanese and 1% of Chinese Han populations. This genetic variant interferes with the binding of eculizumab to C5 (Nishimura *et al.*, 2014). For PNH patients with this genetic variant there is no effective treatment.

A new small (17 kDa) protein complement inhibitor named coversin is now in phase 2 clinical development. Like eculizumab, coversin prevents cleavage and activation of C5, but it binds C5 at a different site (Jore *et al.*, 2016). Administration to healthy volunteers proved safe and demonstrated inhibition of terminal complement activation. We report safety, efficacy, pharmacokinetic and pharmacodynamic data from the first ever PNH patient treated with coversin. The patient, a 30-year-old Caucasian man (BMI 21 kg/m^2) with a stable PNH granulocyte clone of 90% (monocyte and erythrocyte clone sizes shown in Table SI), suffered from severe haemolysis. LDH (lactate dehydrogenase) and haemoglobin levels before start of treatment are shown in Fig 1A. There were several episodes of very severe haemolysis with LDH levels >2500 U/l (ULN 250 U/l). Three episodes of increased haemolysis were accompanied by a transient deterioration in kidney function. During one episode (LDH 2813 U/l), eGFR decreased to 24 ml/min. Main symptoms were extreme fatigue and muscle dystonia. He had no history of thrombosis, no prior transfusions and no underlying aplastic anaemia or myelodysplastic syndrome. He started treatment with eculizumab four years after diagnosis. Unexpectedly, he remained severely haemolytic despite adequate trough drug levels and no antidrug antibodies (ADA). Other causes of haemolysis were excluded. The PNH clone size had not changed (Table SI). Treatment was discontinued when, several months into treatment, the patient experienced increased haemolysis (LDH 9 × ULN) and macroscopic haemoglobinuria one day after receiving 900 mg eculizumab. DNA analysis of the coding region of C5 showed a heterozygous genetic variant in C5, c.2653C>A, which predicts p.Arg885Ser (Fig 2A). The amino acid substitution is different from that described previously (Nishimura *et al.*, 2014), but occurs with the same amino acid of the C5 protein. The same genetic variant was demonstrated in the DNA of the patient's healthy father and not in his healthy mother. *In vitro* studies confirmed a diminished response to eculizumab (measured by inhibition of CH50) compared to a normal control (NC), whereas coversin was equally effective in the patient and in the normal control, reducing CH50 to less than 8 U Eq/ml (Fig 2B)(the lower limit of quantification).

The AK578 study (ClinicalTrials.gov, NCT02591862) was an open label phase 2 study designed for this and potentially up to five other patients with genetic variants of C5, which rendered them unresponsive to treatment with Eculizumab (Data S1). The study was approved by the independent ethics committee of the Radboudumc and was conducted in agreement with Good Clinical Practice guidelines and according to the Declaration of Helsinki. When the inclusion period closed, the patient described here was the only participant in this trial.

Coversin was administered by subcutaneous injection at an ablating dose of 0·57 mg/kg/day on day 1, followed by a maintenance dose of 0·14 mg/kg/day thereafter. Protocol defined that blood samples were drawn for CH50 activity, free coversin levels and anti-drug antibodies (ADA). The primary efficacy goal was a reduction in serum LDH from day 0 (pre-dose) to day 28. Secondary goals were haemoglobin at days 28, 90 and 180 compared to baseline, LDH at days 90 and 180, haptoglobin level at days 28, 90 and 180 compared to baseline, transfusion independency and quality of life, assessed by the FACIT and EORTC QL-C30 instruments on days 0, 28, 90 and 180.

The patient started with the ablating dose of 0·57 mg/kg/day. There was a good initial response with CH50 decreasing from 100 U Eq/ml to <8 U Eq/ml (Fig 1B). He continued with the maintenance dose of 0·14 mg/kg every 24 h. Clinical symptoms and laboratory markers of haemolysis improved during the first five days of treatment. However, on day 6, the patient experienced haemolysis-associated symptoms with

doi: 10.1111/bjh.16305

www.oncotarget.com Oncotarget, 2019, Vol. 10, (No. 23), pp: 2270-2281

Research Paper

The augmented expression of the cytidine deaminase gene by 5-azacytidine predicts therapeutic efficacy in myelodysplastic syndromes

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Keywords: *myelodysplastic syndromes; cytidine deaminase; 5-azacytidine; DNA methylation; hypomethylating agent*

Received: *December 13, 2018* ***Accepted:*** *March 04, 2019* ***Published:*** *March 19, 2019*

ABSTRACT

5-Azacytidine (5AC), a hypomethylating agent, is clinically used for the treatment of patients with myelodysplastic syndromes (MDS). Cytidine deaminase (CDA) is a key enzyme in the detoxification of 5AC. We investigated whether the CDA expression could predict response to 5AC in MDS. Among leukemia-derived cell lines, MDS-L, an MDS-derived cell line with a relatively low CDA expression level, was found to be the most sensitive to 5AC. Combination with tetrahydrouridine, an inhibitor of CDA, synergistically potentiated the cytotoxic effect of 5AC. Treatment with 5AC markedly enhanced the expression level of CDA mRNA and showed demethylation at CpG sites in the 5′-flanking region of the CDA gene. We further compared the protein expression levels of CDA in matched clinical samples before and after treatment with 5AC in bone marrow cells from 8 MDS patients by an immunohistochemical analysis. The CDA expression level showed an approximately 2- to 3-fold increase after 5AC treatment in 3 of these cases, and these three patients with relatively higher CDA expression levels after 5AC treatment all showed better clinical responses to 5AC. In contrast, the 5 remaining patients, whose CDA expression showed no augmentation, observed no clinical benefit. Taken together, the optimized determination of the CDA expression levels before and after 5AC treatment, and the methylation status at CpG sites of 5′-flanking region of the CDA gene, may contribute to the development of precise 5AC therapy for MDS.

International Journal of Hematology
https://doi.org/10.1007/s12185-019-02683-1

ORIGINAL ARTICLE

Hematologic recovery induced by eltrombopag in Japanese patients with aplastic anemia refractory or intolerant to immunosuppressive therapy

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Received: 2 December 2018 / Revised: 3 June 2019 / Accepted: 4 June 2019

Abstract
Eltrombopag, an oral thrombopoietin-receptor agonist, stimulates hematopoiesis in patients with acquired aplastic anemia (AA) and has higher exposure in patients of East Asian origin. We evaluated the pharmacokinetics, efficacy, and safety of eltrombopag in Japanese patients with AA refractory or intolerant to immunosuppressive therapy (IST). Twenty-one patients (15 with non-severe AA, six with severe AA) with platelet counts < 30,000/μL received eltrombopag in a dose-escalation fashion (25, 50, 75, or 100 mg once daily) depending on individual platelet responses; the responders continued eltrombopag treatment beyond 6 months. The primary endpoint was hematologic response at 6 months, defined as improvements in blood counts or transfusion requirements. Ten (48%) patients achieved hematologic responses in at least one lineage at 6 months. Six patients achieved tri- and/or bi-lineage responses with continuation of eltrombopag treatment, with two patients no longer requiring eltrombopag treatment. The most common adverse events were nasopharyngitis and abnormal hepatic function, with the majority being grade 1 or 2. Cytogenetic abnormalities were observed in three patients; however, no progression to myelodysplastic syndrome/other malignancy was observed. Eltrombopag can safely restore multi-lineage hematopoiesis in Japanese patients with AA refractory or intolerant to IST.
Clinical Trial registration NCT02148133.

Keywords Eltrombopag · Japanese patients · Aplastic anemia · Inter-ethnic difference

Introduction

Acquired aplastic anemia (AA) is a rare bone-marrow failure disease mainly caused by immune-mediated suppression of hematopoiesis, and is characterized by peripheral pancytopenia and marrow hypoplasia [1–3]. In Japan, AA is designated as a disease for which care and research are subsidized through the Specified Disease Treatment Research Program supported by the Ministry of Health, Labour, and Welfare. The number of patients with AA was estimated to be

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Published online: 10 June 2019

日本臨牀　77巻 月刊号9（2019年9月1日発行）別刷

特集：骨髄異形成症候群（MDS）

—基礎・臨床の最新動向—

III. MDSの診断

細胞形態学的所見

松田　晃

International Journal of Hematology (2019) 110:533–542
https://doi.org/10.1007/s12185-019-02710-1

ORIGINAL ARTICLE

Serum ferritin levels at diagnosis predict prognosis in patients with low blast count myelodysplastic syndromes

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Received: 9 April 2019 / Revised: 12 July 2019 / Accepted: 17 July 2019 / Published online: 29 July 2019

Abstract
Serum ferritin, a marker of systemic iron status, is considered a prognostic factor for patients with myelodysplastic syndromes (MDS), despite the lack of supporting evidence. We investigated the association between serum ferritin levels at diagnosis and the prognoses of Japanese MDS patients with bone marrow blasts < 5% and peripheral blood blasts < 2%. Three hundred and ninety patients with cytopenia were registered prospectively in the multicenter database, among whom 107 patients with MDS (72 males and 35 females, with a median age of 70 years) met the eligibility criteria. The median serum ferritin level at diagnosis was 204 ng/mL; we divided the cohort into low ($n = 56$) and high ($n = 51$) ferritin groups using a cutoff of 210 ng/mL. Kaplan–Meier analyses revealed that the 3-year overall survival (OS) of the high ferritin group was significantly shorter than that of the low ferritin group (66% and 79%, respectively). The cumulative incidences of leukemic progression were similar between the groups. On multivariate analysis, age, blast percentage, cytogenetic abnormalities, and serum ferritin levels at diagnosis were independently associated with OS in our patients. Thus, modest elevations of ferritin levels at diagnosis may influence the prognoses of patients with MDS who have low blast counts.

Keywords Myelodysplastic syndromes · Ferritin · Iron overload · Prognosis

Introduction

Myelodysplastic syndromes (MDS) are a group of disorders characterized by ineffective hematopoiesis that leads to cytopenia as well as a high susceptibility to leukemic transformation [1]. The clinical course of MDS is highly variable; some patients rapidly progress to leukemia while others remain stable for years. As such, disease risk stratification at presentation is important for selecting optimal treatments for individual patients. A number of prognostic scoring systems including the International Prognostic Scoring System (IPSS) [2, 3], World Health Organization prognostic scoring system [4], MD Anderson risk model score for MDS [5], and revised IPSS (IPSS-R) [6] have been developed; these systems incorporate chromosomal abnormalities, degrees of cytopenia, and bone marrow (BM) blast percentages into their criteria. Notably, the serum level of ferritin, an iron storage protein in the cytoplasm that is actively secreted by macrophages [7], is routinely used for evaluating systemic iron status in clinical practice and is regarded as a negative prognostic factor when elevated in patients with hematopoietic malignancies including MDS [8]. Indeed, a number of studies demonstrated that elevated pre-transplant serum ferritin levels predict inferior overall survival (OS) in patients with hematologic malignancies undergoing allogeneic hematopoietic stem cell transplantation [9–11]. Furthermore, some retrospective studies showed that elevated serum ferritin levels at diagnosis or presentation are associated with shorter OS in patients with MDS [6, 12–14]. However, most such studies investigated patients both with and without excess blasts (EB), and serum ferritin

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s12185-019-02710-1) contains supplementary material, which is available to authorized users.

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Internal Medicine
The Japanese Society of Internal Medicine

doi: 10.2169/internalmedicine.3770-19
Intern Med Advance Publication
http://internmed.jp

[CASE REPORT]

Secondary Pulmonary Alveolar Proteinosis Following Treatment With Azacitidine for Myelodysplastic Syndrome

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Abstract:

Secondary pulmonary alveolar proteinosis (sPAP) is a complication of myelodysplastic syndrome (MDS). A 60-year-old woman was diagnosed with MDS with excess blasts-1. Fifty-four months after the initial diagnosis, treatment with azacitidine was initiated. Seventy-three months after the diagnosis, a bone marrow examination revealed increased myeloblasts, at which time computed tomography showed diffuse ground-glass opacities and interlobular septal thickening in the bilateral lower lung fields. A lung biopsy revealed the presence of PAP; therefore, the clinical diagnosis of MDS/sPAP was confirmed. Careful attention should be paid to the development of sPAP in MDS patients with pulmonary lesions during azacitidine treatment.

Key words: myelodysplastic syndromes, secondary pulmonary alveolar proteinosis, azacitidine, umbilical cord blood transplantation

(Intern Med Advance Publication)
(DOI: 10.2169/internalmedicine.3770-19)

Introduction

Pulmonary alveolar proteinosis (PAP) is a rare lung disease characterized by the abnormal accumulation of surfactant proteins and lipids within alveolar spaces, resulting in respiratory failure (1). PAP is classified into three subtypes: primary, congenital, and secondary. Primary PAP is an autoimmune disorder mediated by an antibody against granulocyte macrophage colony-stimulating factor (GM-CSF) (2). Germline mutations in the *GATA2* gene have been reported to include a predisposition to myelodysplastic syndromes (MDS)/acute myeloid leukemia and PAP (3, 4). Secondary PAP (sPAP) is associated with underlying diseases, such as hematological neoplasms, cancers, infections, and the inhalation of silica or titanium, and the anti-GM-CSF antibody is not detected. MDS is the most frequent primary disease; 26 out of 40 sPAP cases (65.0%) were MDS patients (5). Although the cause of sPAP in MDS (MDS/sPAP) remains unclear, the abnormal numbers and functions of alveolar macrophages that originate from MDS cells are considered to result in impaired surfactant clearance (6, 7).

The emergence of sPAP is observed not only at the diagnosis of MDS but also under various clinical conditions (8-12). A previous study showed the development of sPAP at the time of disease progression after treatment with cytotoxic agents (10); however, limited information is currently available on the emergence of sPAP in MDS patients treated

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Received: August 7, 2019; Accepted: October 23, 2019; Advance Publication by J-STAGE: December 26, 2019
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LEUKEMIA & LYMPHOMA
https://doi.org/10.1080/10428194.2020.1734595

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

Nationwide epidemiological survey of familial myelodysplastic syndromes/acute myeloid leukemia in Japan: a multicenter retrospective study

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ABSTRACT

Although several pedigrees of familial myelodysplastic syndromes/acute myeloid leukemia (fMDS/AML) have been reported, the epidemiology and clinical features has been poorly understood. To explore the epidemiology of this entity, we performed a retrospective nationwide epidemiological survey in Japan using questionnaire sheets. The questionnaire was sent to 561 institutions or hospitals certified by Japanese Society of Hematology, unearthing the existence of 41 pedigrees of fMDS/AML. Among them, we obtained the clinical information of 31 patients in 20 pedigrees. The median age of the initial diagnosis was 51 years (range 9–88 years) and the WHO classification 2008 ranged from refractory anemia (RA) to AML. Focusing on the familial MDS patients, refractory anemia with excess blasts (RAEB)-2 was the largest group (27.3%). The median overall survival (OS) of fMDS and fAML in this study were 71.6 and 12.4 months, and the five-year OS were 61.3 and 50%, respectively.

ARTICLE HISTORY
Received 7 November 2019
Accepted 16 February 2020

KEYWORDS
Myelodysplastic syndromes; familial MDS; familial AML; nationwide survey; retrospective study

Introduction

The myelodysplastic syndromes (MDS) are a group of clonal disorders of hematopoietic stem cells and are defined by ineffective hematopoiesis and/or bone marrow dysplasia [1–3]. More than 30,000 new cases of MDS occur in the US every year, and approximately 30% of MDS patients eventually progress to acute myeloid leukemia (AML) [4,5]. The median overall survival (OS) of MDS patients with International Prognostic Scoring System (IPSS) 'high' is around 0.4 years [6], thus, it is still challenging for physicians to treat severe MDS cases. Although allogeneic hematopoietic stem cell transplantation (HSCT) is still the only treatment to cure MDS, very limited MDS patients are considered an indication for HSCT due to the elderly [7]. Moreover, it is reported that five-year OS after allogeneic HSCT was 28% in the patients with refractory anemia with excess blasts (RAEB)-2 [8].

MDS or AML is mostly a sporadic disease, however, familial cases have been also recognized recently. Holme et al. [1] reported the large cohort of 27 families with familial MDS/AML (fMDS/AML) from the UK. Since many of the fMDS/AML cases have been recently defined, the guidelines for genetic analysis and clinical treatment are currently based on expert opinions [9]. In clinical settings, clinicians are required to take a detailed family history and consult with a certified genetic counselor when necessary [10]. The curative treatment for fMDS/AML is allogeneic HSCT only, same as the sporadic MDS [11]. Although epidemiology and disease characteristics of sporadic MDS/AML have been gradually uncovered, little is

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Supplemental data for this article can be accessed here.

International Journal of Hematology (2019) 110:237–243
https://doi.org/10.1007/s12185-019-02673-3

ORIGINAL ARTICLE

Characteristics of palliative home care for patients with hematological tumors compared to those of patients with solid tumors

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Received: 8 January 2019 / Revised: 16 May 2019 / Accepted: 21 May 2019 / Published online: 31 May 2019

Abstract

Home care medicine is a platform for providing supportive care for end-stage cancers. However, for undefined reasons, patients with hematological tumors (HTs) often fail to receive opportunities for home care. We, therefore, sought to delineate the clinical differences between solid tumors (STs) and HTs and to determine whether home care is effective for patients with HTs, as well as those with STs. We retrospectively analyzed the treatments, prognosis, and places of death of patients with STs ($n=99$) and HTs ($n=20$) who received palliative home care in our clinic and subsequently died between May 2016 and May 2018. Patients with HTs commonly required intravenous antibiotics, platelet transfusion, and red blood cell transfusion, while patients with STs tended to more frequently require the use of opioids. Importantly, there were no significant differences between the cohorts with respect to survival time and frequency of emergent visits to patients after their referral to us. Furthermore, most patients in both groups died at home. More than 50% of patients were not admitted to hospitals during our follow-up. Collectively, while therapeutic approaches sometimes differ, this study provides clinical evidence that palliative home care can be feasible even for patients with HTs.

Keywords Home care · Home visiting · Palliative care · Hematological tumors · Transfusions at home · Frail patients

Abbreviations

HTs	Hematological tumors
STs	Solid tumors
MDS	Myelodysplastic syndromes
QoL	Quality of life

Introduction

With recent advancements in communication technologies, medical devices and various drugs, home care medicine is becoming more familiar as a platform of best supportive care to patients with intractable tumors. Patients have more treatment options at home than before, including intravenous antibiotics, transfusions, opioids and oxygen therapies. The development of electronic devices such as mobile phones, tablet computers and electronic clinical charts has also facilitated smooth communications between patients and medical staffs. Moreover, because of the increasing medical care expenditures especially for the elderly, the social health-care system is facilitating a transition of medical care from hospitals to patients' homes.

While home care can sometimes impose physical or mental burdens on caregivers, there are many advantages to home care such as reduced frequencies of hospital visits as well as facilitation of patient relaxation at home and increased time spent with family members, pets, and favorite surroundings. Furthermore, even for patients eligible for chemotherapy or radiation therapy, the incorporation of home care into outpatient clinics also helps continue ongoing treatments by reducing the frequencies

Takashi Ishida and Kota Ohashi contributed equally to this work.

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Biol Blood Marrow Transplant 25 (2019) 1576–1585

Biology of Blood and Marrow Transplantation

journal homepage: www.bbmt.org

Safety and Seropositivity after Live Attenuated Vaccine in Adult Patients Receiving Hematopoietic Stem Cell Transplantation

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Article history:
Received 20 January 2019
Accepted 1 April 2019

Key Words:
Vaccination
HSCT
Adult
Measles
Rubella
Mumps
VZV

A B S T R A C T

Vaccination against vaccine-preventable diseases (VPDs) is highly recommended for hematopoietic stem cell transplantation (HSCT) recipients by several guidelines; however, the safety and seropositivity after live attenuated vaccines remain unclear in adult HSCT recipients. We analyzed titers of antibodies against measles, rubella, mumps, and varicella zoster virus (VZV) from Japanese adult patients who underwent allogeneic HSCT (allo-HSCT) (n = 74), autologous HSCT (auto-HSCT) (n = 39), or chemotherapy (n = 93). The seropositive rates for measles, rubella, mumps, and VZV in allo-HSCT recipients were 20.2%, 36.4%, 5.4%, and 55.4%, respectively. These rates were equivalent to those in auto-HSCT recipients but were significantly lower than those in patients receiving chemotherapy. Antibody titers tended to gradually decrease with time. Twenty-nine allo-HSCT recipients and 8 auto-HSCT recipients received live attenuated vaccines against VPDs for which they tested seronegative. The titers of antibodies against measles, rubella, and mumps significantly increased after 2 shots of vaccine, and the seropositive rate increased up to 19%, 30%, and 27%, respectively. Three patients (8.1%) experienced mild adverse events, which resolved promptly, indicating safe administration of the live attenuated vaccines. In multivariate analysis, history of chronic graft-versus-host disease was significantly associated with high seropositivity for measles as well as high seroconversion rate for measles after vaccination. Live attenuated vaccines against VPDs were safely administered in seronegative adult HSCT recipients. A further observational study is crucial to evaluate the efficacy of vaccination in seronegative HSCT patients.

INTRODUCTION

Several guidelines for vaccination of the immunocompromised host recommend that recipients of autologous and allogeneic hematopoietic stem cell transplantation (HSCT) receive vaccination against vaccine-preventable diseases (VPDs) [1–4]. Long-term survivors after HSCT can eventually develop life-threatening infectious complications as a result of the loss of their immunity, although the hematologic diseases remain cured. Therefore, it becomes more important to manage infections adequately, especially if they can be prevented in advance. In addition, several outbreaks of measles have been documented around the world [5,6], and severe or fatal measles infections have been reported in HSCT recipients [7,8]. Previous reports have demonstrated the safety and efficacy of immunization against VPDs such as measles, rubella, mumps, and varicella zoster virus (VZV) in pediatric HSCT recipients [9–11]; however, few studies have evaluated the seropositivity for VPDs and safety of live attenuated vaccines in adult HSCT recipients. In addition to HSCT, the emergence of new, more effective therapies, such as molecular-targeted therapy and cancer immunotherapy, has increasingly benefited patients with hematologic malignancies. However, it is still unknown whether immunization against VPDs is needed for patients who receive the newly developed innovative treatments. In

Financial disclosure: See Acknowledgments on page 1585.

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https://doi.org/10.1016/j.bbmt.2019.04.006
1083-8791/

Annals of Hematology (2019) 98:2579–2591
https://doi.org/10.1007/s00277-019-03801-z

ORIGINAL ARTICLE

Comparison of calcineurin inhibitors in combination with conventional methotrexate, reduced methotrexate, or mycophenolate mofetil for prophylaxis of graft-versus-host disease after umbilical cord blood transplantation

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Received: 11 May 2019 / Accepted: 11 September 2019 / Published online: 18 October 2019

Abstract
Umbilical cord blood transplantation (UCBT) is a curative treatment for hematological malignancies. However, appropriate prophylaxis against graft-versus-host disease (GVHD), aimed at obtaining rapid and stable engraftment and avoiding toxicity, remains controversial in UCBT. We retrospectively compared outcomes in 409 patients who received calcineurin inhibitors (CIs) plus conventional-dose methotrexate (conv-MTX/CIs, $n = 77$; methotrexate, 10 mg/m^2 on day 1, 7 mg/m^2 on days 3 and 6) with those who received CIs plus reduced-dose methotrexate (reduced-MTX/CIs, $n = 209$; methotrexate, 5 mg/m^2 or 5 mg/body on days 1, 3, and 6) or CIs with mycophenolate mofetil (MMF/CIs, $n = 123$) for GVHD prophylaxis after UCBT. The cumulative incidence of neutrophil engraftment was significantly higher in the reduced-MTX/CI (82.3%) and MMF/CI (86.6%) groups than the conv-MTX/CI (71.4%) group ($p = 0.014$), although there were no differences in platelet recovery or infectious complications among the three groups. The incidence and severity of GVHD were comparable among the three groups, and there were no significant differences in transplantation-related mortality among the three groups. In conclusion, GVHD prophylaxis with reduced-dose methotrexate and MMF was closely associated with high incidence of neutrophil engraftment without an effect on the incidence and severity of GVHD, which was compared to GVHD prophylaxis with conventional-dose methotrexate.

Keywords GVHD · Prophylaxis · Reduced-dose · Methotrexate · MMF · UCBT

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Introduction

During the past decade, umbilical cord blood (UCB) has been increasingly used as an alternative hematopoietic stem cell source for allogeneic bone marrow (BM) or peripheral blood stem cells (PBSC) because of its potential advantages of rapid availability and lower risk of graft-versus-host disease (GVHD), which has permitted less stringent human leukocyte antigen (HLA) matching [1]. However, UCB transplantation (UCBT) is associated with delayed neutrophil and platelet recovery and a higher incidence of engraftment failure compared with BM or PBSC, which is a major issue that remains to be solved. In addition, GVHD is a major complication after UCBT as well. Thus, appropriate prophylaxis with immunosuppressants is important to obtain rapid engraftment to further improve outcomes in patients undergoing UCBT [2–4].

Received: 20 August 2019 | Revised: 29 November 2019 | Accepted: 2 December 2019

DOI: 10.1002/ajh.25693

RESEARCH ARTICLE

Allogeneic hematopoietic stem cell transplantation for aplastic anemia with pre-transplant conditioning using fludarabine, reduced-dose cyclophosphamide, and low-dose thymoglobulin: A KSGCT prospective study

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Abstract

The optimal pre-transplant conditioning for aplastic anemia (AA) remains unclear. We performed a prospective study on allogeneic transplantation from a related or unrelated donor for adult patients with AA. We assessed whether reduced-dose cyclophosphamide (CY) could decrease toxicity while maintaining engraftment, and low-dose thymoglobulin could safely prevent graft-vs-host disease (GVHD). The pre-transplant conditioning regimen consisted of fludarabine 120 mg/m^2, CY 100 mg/kg, and thymoglobulin 2.5 mg/kg with or without 2 Gy of total body irradiation. Twenty-seven patients with a median age of 36 years were analyzed. Sixteen patients received graft from related donors. The stem cell source was bone marrow in

Biol Blood Marrow Transplant 26 (2020) 445–450

Biology of Blood and Marrow Transplantation

journal homepage: www.bbmt.org

Allogeneic – Adult

Mixed Chimerism and Secondary Graft Failure in Allogeneic Hematopoietic Stem Cell Transplantation for Aplastic Anemia

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Article history:
Received 6 August 2019
Accepted 1 October 2019

Keywords:
Aplastic anemia
Allogeneic hematopoietic stem cell transplantation
Mixed chimerism
Secondary graft failure

A B S T R A C T

Mixed chimerism (MC) and/or secondary graft failure (SGF) with recipient- or donor-type chimerism is a major obstacle in allogeneic transplantation for aplastic anemia (AA). From a registry database in Japan, patients with AA age >15 years who underwent a first allogeneic bone marrow or peripheral blood stem cell transplantation between 2000 and 2014 and achieved engraftment were included in this study. MC that did not require either granulocyte-colony stimulating factor (G-CSF) or transfusion support (group 1), MC (not SGF) that required G-CSF and/or transfusion support (group 2), SGF with MC or complete recipient-type chimerism (group 3), and SGF with complete donor-type chimerism (group 4) developed in 26, 16, 19, and 17 patients, respectively. The overall median duration of follow-up for survivors was 1727 days. The overall survival (OS) was 90.4% at 1 year and 83.5% at 5 years in patients without MC or SGF (n = 340), which was not different from the OS in groups 1 and 2. However, inferior OS was observed in group 3 (1 year, 52.1%; 5 years, 52.1%) and group 4 (1 year, 82.4%; 5 years, 56.3%). In multivariate analyses, the use of fludarabine (Flu) and the absence of irradiation in conditioning were associated with the development of SGF with MC or complete recipient-type chimerism, and the use of Flu in conditioning was associated with SGF with complete donor-type chimerism. In conclusion, the use of Flu may affect the occurrence of SGF with both recipient-type and donor-type chimerism.

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) is an effective and curative treatment option for patients with aplastic anemia (AA). High-dose cyclophosphamide (Cy) (200 mg/kg) combined with antithymocyte globulin (ATG) is frequently used as a conditioning regimen and has shown excellent outcomes, especially in HSCT from an HLA-matched sibling [1]. However, the cardiotoxicity of high-dose Cy is a major problem [2], because patients with AA often have

Financial disclosure: See Acknowledgments on page 450.

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https://doi.org/10.1016/j.bbmt.2019.10.004
1083-8791/

International Journal of Hematology (2019) 109:711–717
https://doi.org/10.1007/s12185-019-02644-8

ORIGINAL ARTICLE

Outcome of allogeneic hematopoietic stem cell transplantation in adult patients with hepatitis-associated aplastic anemia

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Received: 21 December 2018 / Revised: 1 April 2019 / Accepted: 2 April 2019 / Published online: 8 April 2019

Abstract

Outcomes of allogeneic hematopoietic stem cell transplantation (HSCT) for hepatitis-associated aplastic anemia have not been fully evaluated. In the present study, the outcomes of 37 adult patients with hepatitis-associated aplastic anemia who underwent allogeneic HSCT were retrospectively analyzed using the registry database of Japan Society for Hematopoietic Cell Transplantation. The median age of the patients was 24 years (range, 16–61). The median period between diagnosis of hepatitis-associated aplastic anemia and HSCT was 6.0 months (range, 0.5–430.8). Stem cell sources were bone marrow ($N=19$) or peripheral blood stem cells ($N=5$) from an HLA-identical sibling or bone marrow ($N=11$) and cord blood ($N=2$) from an unrelated donor. The majority of conditioning regimens were fludarabine-based or high-dose cyclophosphamide-based. In all but 2 cases of early death, neutrophil engraftment was achieved. At the time of analysis, 32 patients were alive, with a median follow-up of 54.1 months. Five-year overall and failure-free survival rates were 86.0% (95% CI, 69.4–93.9%) and 75.0% (95% CI, 57.4–86.2%), respectively. Despite the heterogeneity in transplant procedures in a small number of patients, these results suggest that allogeneic HSCT is safe for use in hepatitis-associated aplastic anemia with a low rate of transplant-related mortality.

Keywords Hepatitis-associated aplastic anemia · Allogeneic hematopoietic stem cell transplantation · Conditioning · Engraftment · Transplant-related mortality

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Received: 17 October 2018 | Accepted: 23 October 2018
DOI: 10.1002/hon.2566

ORIGINAL RESEARCH ARTICLE

WILEY

Induction chemotherapy followed by allogeneic HCT versus upfront allogeneic HCT for advanced myelodysplastic syndrome: A propensity score matched analysis

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Abstract

To reduce post-transplant relapse, acute myeloid leukemia (AML) type remission induction chemotherapy has been attempted to reduce disease burden before allogeneic hematopoietic cell transplantation (HCT) in patients with advanced myelodysplastic syndrome (MDS). However, the efficacy of induction chemotherapy before HCT is unclear. We retrospectively analyzed the Japanese registration data of 605 adult patients, who had received allogeneic HCT for advanced MDS between 2001 and 2016, to compare the post-transplant relapse between patients who received induction chemotherapy followed by allogeneic HCT and those who received upfront HCT. Propensity score matching identified 230 patients from each cohort. There were no significant differences in overall survival and non-relapse mortality between the two groups. The cumulative incidence of relapse was significantly higher in patients who received induction chemotherapy than those who received upfront HCT. In the subgroup analyses, upfront HCT had a significantly reduced relapse incidence among patients with poor cytogenetics, those with higher international prognostic scoring system at diagnosis, and those who received reduced-intensity conditioning. Our results suggested that AML type remission induction chemotherapy before HCT did not improve post-transplant relapse and survival for adult patients with advanced MDS. Upfront HCT is preferable for patients with a poor karyotype.

KEYWORDS

myelodysplastic syndrome, allogeneic hematopoietic cell transplantation, induction chemotherapy, cytoreductive treatment, relapse, propensity score matched analysis

Leukemia (2019) 33:1773–1782
https://doi.org/10.1038/s41375-019-0385-0

ARTICLE

Myelodysplastic syndrome

A germline *HLTF* mutation in familial MDS induces DNA damage accumulation through impaired PCNA polyubiquitination

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Received: 9 July 2018 / Revised: 27 November 2018 / Accepted: 27 December 2018 / Published online: 29 January 2019

Abstract

Although several causal genes of familial myelodysplastic syndromes (MDS) have been identified, the genetic landscape and the molecular pathogenesis are not totally understood. To explore novel driver genes and their pathogenetic significance, we performed whole-exome sequence analysis of four individuals from a familial MDS pedigree and 10 candidate single-nucleotide variants (*C9orf43, CYP7B1, EFHB, ENTPD7, FAM160B2, HELZ2, HLTF, INPP5J, ITPKB,* and *RYK*) were identified. Knockdown screening revealed that *Hltf* downregulation enhanced colony-forming capacity of primary murine bone marrow (BM) stem/progenitor cells. γH2AX immunofluorescent staining assay revealed increased DNA damage in a human acute myeloid leukemia (AML) cell line ectopically expressing HLTF E259K, which was not observed in cells expressing wild-type HLTF. Silencing of *HLTF* in human AML cells also led to DNA damage, indicating that *HLTF* E259K is a loss-of-function mutation. Molecularly, we found that an E259K mutation reduced the binding capacity of HLTF with ubiquitin-conjugating enzymes, methanesulfonate sensitive 2 and ubiquitin-conjugating enzyme E2N, resulting in impaired polyubiquitination of proliferating cell nuclear antigen (PCNA) in *HLTF* E259K-transduced cells. In summary, our results indicate that a familial MDS-associated *HLTF* E259K germline mutation induces accumulation of DNA double-strand breaks, possibly through impaired PCNA polyubiquitination.

Introduction

The myelodysplastic syndromes (MDS) are a group of clonal disorders of hematopoietic stem cells, characterized by bone marrow (BM) dysplasia and ineffective hematopoiesis [1, 2]. Previous comprehensive mutational analyses uncovered the genetic landscape of sporadic MDS, including frequent alterations in *Tet methylcytosine dioxygenase 2* (*TET2*), *splicing factor 3b subunit 1* (*SF3B1*), *additional sex combs like 1, transcriptional*

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Supplementary information The online version of this article (https://doi.org/10.1038/s41375-019-0385-0) contains supplementary material, which is available to authorized users.

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Received: 29 April 2019 | Revised: 3 July 2019 | Accepted: 4 July 2019

DOI: 10.1002/pbc.27937

Pediatric Blood & Cancer

SIOP INTERNATIONAL SOCIETY OF PAEDIATRIC ONCOLOGY

aspho The American Society of Pediatric Hematology/Oncology

WILEY

LETTER TO THE EDITOR

Pediatric patients with cancer predisposition in Japan: Results of a questionnaire survey

To the Editor:

Approximately 10% of children with cancer have germline pathogenic variants in cancer predisposition genes.[1–3] Recent reports have demonstrated that patients with cancer predisposition benefit from a particular treatment and that surveillance according to the risk of developing certain cancers contributes to better survival.[4–6] Accordingly, there is an urgent requirement for establishing clinical infrastructure in Japan. To address these issues, the Japanese Pediatric Hereditary Tumor Study Group was formed in 2017. The group investigated the current medical condition of pediatric patients with cancer predisposition because presently no data are available on the number of pediatric patients with cancer predisposition and on the associated diagnosis and management by pediatric oncologists in Japan. A questionnaire was mailed to 107 hospitals accredited by the Japanese Society of Pediatric Hematology/Oncology. The questionnaire comprised close-ended questions regarding (1) the number and age of the patients they followed up in a 1-year period, (2) the diagnosis of the patients, (3) the use of genetic testing, and (4) whether they provide any particular care in addition to routine care for the survivors. Responses were received from 82 (76.6%) hospitals. In total, 937 patients with cancer predisposition underwent follow-up within a 1-year period in 2017. Of them, 451 were being treated for or had a history of malignancy and 486 did not; diagnosis was confirmed through genetic testing in 153 (33.9%) of the former and 193 (39.7%) of the latter patients. The remaining patients were diagnosed clinically without genetic testing (Figures 1A and B). Twenty-eight percent of all patients were aged ≥15 years (Figure 1C); of all hospitals, 64% experienced <10 cases per year (Figure 1D). For performing genetic analysis, 63 hospitals used institutional laboratories and 10 outsourced the testing to commercial laboratories. The cost of testing was covered by research funding in 59 hospitals; however, in nine hospitals, the patients paid themselves. During follow-up of cancer survivors, 42 (51%) hospitals offered specific/additional care according to cancer risk. Open-ended questions related to the problems and needs revealed several issues, including "limited availability of genetic testing," "lack of insurance coverage for surveillance and genetic testing," "difficulty in transition to adult care," and "lack of clinicians and genetic counselors with knowledge of this field."

This survey has several limitations. First, diagnostic criteria are not standardized across physicians and the availability of genetic testing is limited. Therefore, it is possible that we underestimated the number of patients. Second, respondents were pediatric oncologists, and patients followed up by other specialists may not have been included. Importantly, however, our survey showed that in Japan, nearly 1000 children, adolescents, and young adults with cancer predisposition undergo follow-up. Most genetic testing was conducted in research settings with support from research funds. Surveillance strategies vary across institutions maybe because of the lack of insurance coverage for testing and the lack of a standard protocol of cancer surveillance in Japan. Furthermore, our results suggest that most patients and families are followed up by physicians with limited experience. Therefore, in addition to appropriate clinical care, promotion and standardization of genetic testing are needed to provide patients and practitioners with adequate information that comprises the pros and cons of this testing.

ACKNOWLEDGMENTS

We are grateful to physicians who responded to this survey, and we thank the members of our study group. The authors would like to thank Enago for the English language review. This work was supported by MHLW KAKENHI Grant Number 201708007A (T.K).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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ORIGINAL ARTICLE

Inhaled GM-CSF for Pulmonary Alveolar Proteinosis

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ABSTRACT

BACKGROUND

Pulmonary alveolar proteinosis is a disease characterized by abnormal accumulation of surfactant in the alveoli. Most cases are autoimmune and are associated with an autoantibody against granulocyte–macrophage colony-stimulating factor (GM-CSF) that prevents clearing of pulmonary surfactant by alveolar macrophages. An open-label, phase 2 study showed some therapeutic efficacy of inhaled recombinant human GM-CSF in patients with severe pulmonary alveolar proteinosis; however, the efficacy in patients with mild-to-moderate disease remains unclear.

METHODS

We conducted a double-blind, placebo-controlled trial of daily inhaled recombinant human GM-CSF (sargramostim), at a dose of 125 μg twice daily for 7 days, every other week for 24 weeks, or placebo in 64 patients with autoimmune pulmonary alveolar proteinosis who had a partial pressure of arterial oxygen (Pao_2) while breathing ambient air of less than 70 mm Hg (or <75 mm Hg in symptomatic patients). Patients with severe pulmonary alveolar proteinosis (Pao_2 <50 mm Hg) were excluded to avoid possible exacerbation of the disease in patients who were assigned to receive placebo. The primary end point was the change in the alveolar–arterial oxygen gradient between baseline and week 25.

RESULTS

The change in the mean (±SD) alveolar–arterial oxygen gradient was significantly better in the GM-CSF group (33 patients) than in the placebo group (30 patients) (mean change from baseline, −4.50±9.03 mm Hg vs. 0.17±10.50 mm Hg; P=0.02). The change between baseline and week 25 in the density of the lung field on computed tomography was also better in the GM-CSF group (between-group difference, −36.08 Hounsfield units; 95% confidence interval, −61.58 to −6.99, calculated with the use of the Mann–Whitney U test and the Hodges–Lehmann estimate of confidence intervals for pseudo-medians). Serious adverse events developed in 6 patients in the GM-CSF group and in 3 patients in the placebo group.

CONCLUSIONS

In this randomized, controlled trial, inhaled recombinant human GM-CSF was associated with a modest salutary effect on the laboratory outcome of arterial oxygen tension, and no clinical benefits were noted. (Funded by the Japan Agency for Medical Research and Development and the Ministry of Health, Labor, and Welfare of Japan; PAGE ClinicalTrials.gov number, NCT02835742; Japan Medical Association Center for Clinical Trials number, JMA-IIA00205.)

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N Engl J Med 2019;381:923-32.
DOI: 10.1056/NEJMoa1816216

JCI insight

MAPK mutations and cigarette smoke promote the pathogenesis of pulmonary Langerhans cell histiocytosis

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JCI Insight. 2020. https://doi.org/10.1172/jci.insight.132048.

Research In-Press Preview Immunology Pulmonology

Pulmonary Langerhans cell histiocytosis (PLCH) is a rare, smoking-related, lung disease characterized by dendritic cell (DC) accumulation, bronchiolocentric nodule formation, and cystic lung remodeling. Approximately 50% of PLCH patients harbor somatic BRAF-V600E mutations in cells of the myeloid/monocyte lineage. However, the rarity of the disease and lack of animal models has impeded the study of PLCH pathogenesis. Here, we established a cigarette smoke (CS)-exposed, BRAF-V600E mutant mouse model that recapitulates many hallmark characteristics of PLCH. We show that CD11c-targeted expression of BRAF-V600E increases DC responsiveness to stimuli, including the chemokine CCL20, and that mutant DC accumulation in the lungs of CS-exposed mice is due to both increased cellular viability and enhanced recruitment. Moreover, we report that the chemokine CCL7 is secreted from DCs and human peripheral blood monocytes in a BRAF-V600E-dependent manner, suggesting a possible mechanism for recruitment of cells known to dominate PLCH lesions. Inflammatory lesions and airspace dilation in BRAF-V600E mice in response to CS are attenuated by transitioning animals to filtered air and treatment with a BRAF-V600E inhibitor, PLX4720. Collectively, this model provides mechanistic insights into the role of DCs, the BRAF-V600E mutation and CS exposure in PLCH pathogenesis, and provides a platform to develop therapeutic targets.