HEMATOPOIETIC STEM CELL TRANSPLANTATION IN ARPC1B DEFICIENCY

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Abstract

Mutations in the component isoform ARPC1B of Human actin-related protein 2/3 complex have been recently associated with combined immunodeficiency related to impaired T-cell function, allergies, autoinflammation, and platelets abnormalities. Currently, indications on the management of this novel disease and information on its outcome are lacking.

We report the first case series of 7 children who underwent allogeneic-HSCT (allo-HSCT) with homozygous mutation in ARPC1B gene.

All patients presented an early clinical onset, complicated with neonatal hemorrhagic enteritis in 3 and macrophage activating syndrome in 2, characterized by recurrent infections, failure to thrive and gastrointestinal bleeding episodes. Allo-HSCT was performed at the median age of 3.54 years after a myeloablative conditioning regimen in all cases. Engraftment occurred in all patients with a full donor chimerism in 6 out of 7. The clinical course after engraftment was uneventful in 3 out of 7 children; two developed a grade 1-2 acute Graft-versus-Host Disease (GvHD), 1 of them a grade 1 chronic-GvHD. Progressive multifocal leukoencephalopathy JC virus-related was diagnosed in one patient 13 months after haploidentical-HSCT, successfully managed with donor-derived viral-specific T-cell infusion. Only one patient had a severe outcome with veno-occlusive disease and transplant-associated microangiopathy and died 3 months after HSCT because of a sepsis. At a median follow-up of 19 months (range 3 – 110), 6 out of 7 patients are alive and disease free.

The severe clinical phenotype at diagnosis and the high survival rate with limited transplant-related morbidity reported strongly support the indication to allo-HSCT for patients with this diagnosis.

Introduction

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) represents an established curative treatment for several primary immunodeficiencies (PIDs) and currently the survival rates reported are higher than 90% depending on diagnosis, age and overall patient status at the time of transplantation [1, 2, 3, 4]. These results, reached over the years due to the advances in donor selection, graft manipulation, conditioning regimens (CR) and treatment of complications, have led to HSCT indication in newly diagnosed patients with several forms of PIDs. Many novel genetic mutations associated with combined immunodeficiency have been described [5, 6] and some of these present a variable phenotype in which autoimmunity, auto-inflammatory pattern, and infection susceptibility are associated, in which immunosuppressive and anti-inflammatory therapies are administered [7]. Some of these inflammatory and immunological disorders are associated with defects of actin-binding molecules [8, 9, 10, 11], confirming actin cytoskeleton central role in almost all stages of immune system function [12, 13, 14]. Human actin-related protein 2/3 complex (Arp 2/3), required for actin filament branching, has two ARPC1 component isoforms, with ARPC1B prominently expressed in blood cells. ARPC1B mutation results in a combined immunodeficiency, allergy, and “auto-inflammation” that has been recently described, resembling Wiskott-Aldrich syndrome (WAS) [15], characterized by early clinical onset, recurrent infections related to impaired T-cell function and migration, allergic manifestations, bleeding tendency and platelet abnormalities [16, 17, 18, 19, 20, 21, 22]. In this emerging disease, although the indication for allogeneic-HSCT (allo-HSCT) should be considered as for other PIDs [23, 24], there is still no evidence regarding the transplant outcomes in terms of survival and quality of life.

We report the outcome of the first series of 7 children who underwent allo-HSCT because of homozygous mutation in ARPC1B gene.

Matherials And Methods

Seven patients with diagnosis of primary immune regulatory disorders related to *ARPC1B* mutation underwent allo-HSCT in 6 different Centres (Italy, Germany, Spain, USA, Israel, Greece), six of them have been reported in previous studies [16, 19, 21] in which this novel syndrome has been described.

Data have been retrospectively collected for each patient by a specific questionnaire distributed to participating centers about patients’ clinical features, genetic, pre-transplant treatments, conditioning regimen (CR), donor type, stem cell (SC) source, engraftment defined as neutrophils count > $0.5x10^9$/L for at least 3 consecutive days and platelet count > 50000/L without platelet transfusion in the previous 5-7 days, early and late toxicities, acute and chronic graft versus host disease (a- and c-GvHD), post-HSCT infections, donor chimerism and survival.

Results

Patients' demographic, clinical features and treatments performed before HSCT are reported in ***Table 1***, while ***Table 2*** summarizes HSCT features and outcomes.

All patients presented with very early clinical onset during the first months of life (range 9 days – 6 months) with life-threatening events occurring in 5, including neonatal hemorrhagic enteritis in 3 (*P2, P3, P6*) and macrophage activating syndrome (MAS) requiring Pediatric Intensive Care Unit admission in 2 patients. The first (P1) child, in which MAS occurred at the age of one month was triggered by CMV infection, received steroids and antiviral treatment (ganciclovir), while in the second patient (P4), MAS developed at the 9^{th} day of life and required multiple lines of therapies including methylprednisolone and anakinra followed by dexamethasone, tocilizumab, and etoposide.

Failure to thrive was a common feature in almost all patients, as well as the gastrointestinal bleeding episodes, with thrombocytopenia reported in 3 patients (*P2, P3, and P6*). Cutaneous leukocytoclastic vasculitis have been observed in P1 and P2, with histologic confirmation.

The clinical course before allo-HSCT was characterized by recurrent bacterial and viral infections in all patients, as showed in ***Table 1***, while no fungal infection has been reported. The patient who underwent allo-HSCT at the older age (*P2, 15 years*) developed a severe chronic lung disease secondary to

recurrent staphylococcus pulmonary infections with multiple bronchiectasis and large pneumatocele, that required surgical lobectomy, performed at the age of seven. This patient required immunosuppressive treatment with sirolimus and mycophenolate because of cutaneous leukocytoclastic vasculitis, ultimately achieving clinical control but likely contributed to an increase of infection incidence.

The median age at transplant was 1.83 years (range, 0.15 – 15.16). For all patients, the indication to allo-HSCT was the poor control of clinical autoimmune and autoinflammatory symptoms and the recurrence of infections events. P3, P6 and P7 underwent transplant without prior trial of alternative treatments, since they had an older brother with a similar phenotype who died due to severe complications represented by sepsis at 2 months of life during severe gastrointestinal bleeding (P3), adenoviral infection with a multi-organ failure while awaiting HSCT (P4), and an unidentified infection of the central nervous system at 3 years of age (P7), respectively.

The SC donor was a matched-related (MRD) in 3 transplants (P5, P6, and P7), a matched unrelated (MUD) in 2 (P3 and P4), while P1 and P2 received a TCR-ab^{+}CD19^{+}-depleted HSCT from an haploidentical parent. The CR was myeloablative in all transplants, busulfan-based in 4 and treosulfan-based in the remaining three.

Engraftment occurred in all patients after a median of 18 days neutrophils (range, 13-23 days) and 17 for platelets (range, 13-35 days) after allo-HSCT. No episodes of graft rejection have been reported and 5 out of 7 patients showed a stable full donor chimerism, a transient mixed chimerism has been reported in P7, while only P5 showed stable mixed chimerism, that was almost complete in T cells (CD3^{+} 93% donor-derived), but with only 5% granulocytes of donor origin at last follow-up ***(Table 2)***.

The clinical course after engraftment was uneventful in 3 out of 7 patients (P1, P3, and P5), with neither significant post-transplant infections nor GvHD. GvHD was reported in 2 children (P2 and P4) after engraftment, P2 developed grade 1 cutaneous acute-GvHD that did not require systemic treatments and, 6 months later, a grade 2 cutaneous c-GvHD successfully treated with systemic steroid. P4 developed a grade 2 cutaneous and gastrointestinal acute-GvHD, with a complete response after steroid therapy.

One patient (P6) had a severe outcome after HSCT, represented by the development of veno-occlusive disease (VOD) and transplant-associated thrombotic microangiopathy (TA-TAM), treated with defibrotide and eculizumab, respectively, obtaining only their partial control, this patient died 3 months after HSCT because of a Carbapenem-resistant Enterobacteriaceae sepsis.

Of note, P2 developed 13 months after HSCT, a severe JC virus-related encephalitis, with severe neurological impairment due to *progressive multifocal leukoencephalopathy*, that was successfully managed with donor-derived viral-specific T-cell infusions (CTL-infusion). This patient suffered neurological sequelae and at last follow up he showed ataxia and tremors [25].

At a median follow-up of 19 months (range 3 – 110), 6 to 7 patients are alive and disease free.

Discussion

In this report, we describe the clinical features, transplant details and outcomes of 7 patients who have undergone allo-HSCT for a PID associated with *ARPC1B* germline mutations. To the best of our knowledge, this is the first case series reported on this topic, which enables the community to derive useful information for the clinical management of this emerging and challenging diagnosis. The severe clinical phenotype at diagnosis and the high survival rate with limited transplant-related morbidity reported support the indication to allo-HSCT for patients with ARPC1B deficiency. The main limitations of our report include the small number of patients and the retrospective nature of the study, that excludes patients with ARPC1B deficiency that didn't reach diagnosis or transplant. However, the present data support the fundamental message of the feasibility and efficacy of allo-HSCT in ARPC1B deficiency, while the previous studies are limited to describing disease's features [16-22, 26].

We found that most patients underwent transplantation because of the severe phenotype characterized by life-threatening infective events or inflammatory and/or autoimmune presentations, in particular, in three of them, the indication was further strengthened by the presence of family history of death of a sibling due to complications of the same condition.

Allogeneic HSCT led to successful resolution of immunodeficit with sustained donor chimerism and excellent survival in 6 patients. Interestingly, the patient with mixed chimerism (P5) is also alive and free of symptoms. Only one patient died (P6) after HSCT because of sepsis from Gram-negative bacteria, this patient received allo-HSCT from MRD at 2 months of age after severe infections performed following a CR including busulphan administered at weight-adapted dose but without AUC-based dose adjustment, the latter [27], together with the age < 1 year [28], represent recognized risk factors for VOD, occurred in this patient. Differently, all the other patients received myeloablative CR at reduced toxicity profile Treosulfan-based or Busulphan-based with dose-adjusted on AUC.

The other major complication observed in these patients after HSCT has been JC encephalitis in P2 occurred during steroid treatment for c-GvHD, of note, this patient underwent T-depleted haploidentical transplant at 15 years old, after a long history of infections and in presence of chronic lung disease, these conditions increase the risk of GvHD [29] , also in T-depleted haploidentical HSCT, and likely contributed to delayed immune reconstitution and, therefore, to viral infection susceptibility.

Similarly to other PIDs patients, also for patients with ARPC1B deficiency the goal of allo-HSCT is to correct the dysregulation of immune system with the disappearance of autoinflammatory and autoimmune manifestations and with the control of infective events. The performance of allo-HSCT with the use of myeloablative CR with low early and late toxicity, as treosulfan, could allow reaching these results. In case of the absence of MRD and considering the relevance of an early allo-HSCT in improving the outcome, the choice of a haploidentical familiar donor, promptly available, should be considered in ARPC1B

as in other PIDs, in which different platforms of haplo-HSCT with graft manipulation for T-depletion [30,31,32] or without but using post-transplant Cyclophosphamide as GvHD prophylaxis [33] allow to achieve excellent results.

Conclusions

In conclusion, in this series of patients, we found that most patients with ARPC1B mutations tolerated transplant conditioning, with a high rate of engraftment, resolution of immunodeficiency, of autoinflammation and autoimmunity, and related manifestations. Active infections and clinically significant comorbidities at the time of transplant are the main potential risk factor contributing to adverse events in the acute post-transplant phase. More data are needed to confirm the indication and the timing for transplant and further to refine conditioning regimens as well as management of patients with significant inflammatory and autoimmune manifestations before HSCT. National and international immunodeficiency and transplant registries should be queried to examine reported outcomes in larger patient cohorts, comparing those of transplanted and not transplanted patients.

Declarations

Authors contributions: SG, SV, MF and MG contributed to the study conception and design. All the others authors contributed to the clinical management and data collection, each for patients belonging to their own center. Material preparation, data collection and analysis were performed by Stefano Giardino and Federica Lucioni. The first draft of the manuscript was written by Stefano Giardino and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability statemen: The datasets generated during the current study are available from the corresponding author on reasonable request.

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Ethics approval: This study, performed in line with the principles of the Declaration of Helsinki, is an observational retrospective study that collects pseudo-anonymized data. The Research Ethics Committee of each Centre involved has confirmed that no ethical approval is required.

Consent to Participate: Informed consent to participate in retrospective study was obtained from the parents of all individual participants included in the study.

Consent to Publish: not applicable

References

1. Shamriz O, Chandrakasan S. Update on Advances in Hematopoietic Cell Transplantation for Primary Immunodeficiency Disorders. Immunol Allergy Clin North Am. 2019 Feb;39(1):113-128. doi: 10.1016/j.iac.2018.08.003. Epub 2018 Nov 1. PMID: 30466768.
2. J. Heimall, B.R. Logan, M.J. Cowan, et al.Immune reconstitution and survival of 100 SCID patients post-hematopoietic cell transplant: a PIDTC natural history study. Blood, 130 (25) (2017), pp. 2718-2727
3. M. Ballow Historical perspectives in the diagnosis and treatment of primary immune deficiencies. Clin Rev Allergy Immunol, 46 (2) (2014), pp. 101-103
4. Morris EC. Allogeneic hematopoietic stem cell transplantation in adults with primary immunodeficiency. Hematology Am Soc Hematol Educ Program. 2020 Dec 4;2020(1):649-660. doi: 10.1182/hematology.2020000152. PMID: 33275750; PMCID: PMC7727582.
5. Tangye SG, Al-Herz W, Bousfiha A, Chatila T, Cunningham-Rundles C, Etzioni A, Franco JL, Holland SM, Klein C, Morio T, Ochs HD, Oksenhendler E, Picard C, Puck J, Torgerson TR, Casanova JL, Sullivan KE. Human Inborn Errors of Immunity: 2019 Update on the Classification from the International Union of Immunological Societies Expert Committee. J Clin Immunol. 2020 Jan;40(1):24-64. doi: 10.1007/s10875-019-00737-x. Epub 2020 Jan 17. Erratum in: J Clin Immunol. 2020 Feb 22;: PMID: 31953710; PMCID: PMC7082301.
6. Bousfiha A, Jeddane L, Picard C, Al-Herz W, Ailal F, Chatila T, Cunningham-Rundles C, Etzioni A, Franco JL, Holland SM, Klein C, Morio T, Ochs HD, Oksenhendler E, Puck J, Torgerson TR, Casanova JL, Sullivan KE, Tangye SG. Human Inborn Errors of Immunity: 2019 Update of the IUIS Phenotypical Classification. J Clin Immunol. 2020 Jan;40(1):66-81. doi: 10.1007/s10875-020-00758-x. Epub 2020 Feb 11. PMID: 32048120; PMCID: PMC7082388.
7. Arnold DE, Chellapandian D, Leiding JW. The Use of Biologic Modifiers as a Bridge to Hematopoietic Cell Transplantation in Primary Immune Regulatory Disorders. Front Immunol. 2021 Jun 24;12:692219. doi: 10.3389/fimmu.2021.692219. PMID: 34248986; PMCID: PMC8264452.
8. Papa R, Penco F, Volpi S, Gattorno M. Actin Remodeling Defects Leading to Autoinflammation and Immune Dysregulation. Front Immunol. 7 gennaio 2021;11:604206.
9. Firat-Karalar EN, Welch MD. New mechanisms and functions of actin nucleation. Curr Opin Cell Biol. febbraio 2011;23(1):4–13.
10. Pizarro-Cerdá J, Chorev DS, Geiger B, Cossart P. The Diverse Family of Arp2/3 Complexes. Trends Cell Biol. febbraio 2017;27(2):93–100.
11. Welch MD, DePace AH, Verma S, Iwamatsu A, Mitchison TJ. The Human Arp2/3 Complex Is Composed of Evolutionarily Conserved Subunits and Is Localized to Cellular Regions of Dynamic Actin Filament Assembly. J Cell Biol. 28 luglio 1997;138(2):375–84.
12. Moulding DA, Record J, Malinova D, Thrasher AJ. Actin cytoskeletal defects in immunodeficiency. Immunol Rev. novembre 2013;256(1):282–99.
13. Svitkina T. The Actin Cytoskeleton and Actin-Based Motility. Cold Spring Harb Perspect Biol. gennaio 2018;10(1):a018267.
14. Campellone KG, Welch MD. A Nucleator Arms Race: Cellular Control of Actin Assembly. 2010;31.

15. Tyler JJ, Allwood EG, Ayscough KR. WASP family proteins, more than Arp2/3 activators. Biochem Soc Trans. 15 ottobre 2016;44(5):1339–45.
16. Volpi S, Cicalese MP, Tuijnenburg P, Tool ATJ, Cuadrado E, Abu-Halaweh M, et al. A combined immunodeficiency with severe infections, inflammation, and allergy caused by ARPC1B deficiency. J Allergy Clin Immunol. giugno 2019;143(6):2296–9.
17. Kuijpers TW, Tool ATJ, van der Bijl I, de Boer M, van Houdt M, de Cuyper IM, et al. Combined immunodeficiency with severe inflammation and allergy caused by ARPC1B deficiency. J Allergy Clin Immunol. luglio 2017;140(1):273-277.e10.
18. Brigida I, Zoccolillo M, Cicalese MP, Pfajfer L, Barzaghi F, Scala S, et al. T-cell defects in patients with ARPC1B germline mutations account for combined immunodeficiency. Blood. 29 novembre 2018;132(22):2362–74.
19. Somech R, Lev A, Lee YN, Simon AJ, Barel O, Schiby G, et al. Disruption of Thrombocyte and T Lymphocyte Development by a Mutation in ARPC1B. J Immunol. 15 dicembre 2017;199(12):4036–45.
20. Kahr WHA. Loss of the Arp2/3 complex component ARPC1B causes platelet abnormalities and predisposes to inflammatory disease.
21. Randzavola LO, Strege K, Juzans M, Asano Y, Stinchcombe JC, Gawden-Bone CM, et al. Loss of ARPC1B impairs cytotoxic T lymphocyte maintenance and cytolytic activity. J Clin Invest. 11 novembre 2019;129(12):5600–14
22. Papadatou I, Marinakis N, Botsa E, et al. Case Report: A Novel Synonymous ARPC1B Gene Mutation Causes a Syndrome of Combined Immunodeficiency, Asthma, and Allergy With Significant Intrafamilial Clinical Heterogeneity. Front Immunol. 2021;12:634313. Published 2021 Feb 19. doi:10.3389/fimmu.2021.634313
23. Rivers E, Worth A, Thrasher AJ, Burns SO. How I manage patients with Wiskott Aldrich syndrome. Br J Haematol. maggio 2019;185(4):647–55.
24. Slatter MA, Gennery AR. Hematopoietic cell transplantation in primary immunodeficiency - conventional and emerging indications. Expert Rev Clin Immunol. 2018 Feb;14(2):103-114. doi: 10.1080/1744666X.2018.1424627. Epub 2018 Jan 16. PMID: 29300535.
25. Berzero G, Basso S, Stoppini L, Palermo A, Pichiecchio A, Paoletti M, Lucev F, Gerevini S, Rossi A, Vegezzi E, Diamanti L, Bini P, Gastaldi M, Delbue S, Perotti C, Seminari E, Faraci M, Luppi M, Baldanti F, Zecca M, Marchioni E, Comoli P. Adoptive Transfer of JC Virus-Specific T Lymphocytes for the Treatment of Progressive Multifocal Leukoencephalopathy. Ann Neurol. 2021 Apr;89(4):769-779. doi: 10.1002/ana.26020. Epub 2021 Feb 10. PMID: 33459417; PMCID: PMC8248385.
26. Castano-Jaramillo LM, Yamazaki-Nakashimada MA, Scheffler Mendoza SC, Bustamante-Ogando JC, Espinosa-Padilla SE, Lugo Reyes SO. A male infant with COVID-19 in the context of ARPC1B deficiency. Pediatr Allergy Immunol. 2021 Jan;32(1):199-201. doi: 10.1111/pai.13322. Epub 2020 Sep 2. PMID: 32683750; PMCID: PMC7405203.
27. Kloehn J, Brodt G, Ernst J, Gruhn B. Analysis of risk factors for hepatic sinusoidal obstruction syndrome following allogeneic hematopoietic stem cell transplantation in pediatric patients. J Cancer Res Clin Oncol. 2021 Jul 13. doi: 10.1007/s00432-021-03732-1. Epub ahead of print. PMID: 34255148.
28. Faraci M, Bertaina A, Luksch R, Calore E, Lanino E, Saglio F, Prete A, Menconi M, De Simone G, Tintori V, Cesaro S, Santarone S, Orofino MG, Locatelli F, Zecca M. Sinusoidal Obstruction Syndrome/Veno-Occlusive Disease after Autologous or Allogeneic Hematopoietic Stem Cell Transplantation in Children: a retrospective study of the Italian Hematology-Oncology Association-Hematopoietic Stem Cell Transplantation Group. Biol Blood Marrow Transplant. 2019 Feb;25(2):313-320. doi: 10.1016/j.bbmt.2018.09.027. Epub 2018 Sep 26. PMID: 30266674.
29. Ghimire S, Weber D, Mavin E, Wang XN, Dickinson AM, Holler E. Pathophysiology of GvHD and Other HSCT-Related Major Complications. Front Immunol. 2017 Mar 20;8:79. doi: 10.3389/fimmu.2017.00079. PMID: 28373870; PMCID: PMC5357769.
30. Balashov D, Shcherbina A, Maschan M, Trakhtman P, Skvortsova Y, Shelikhova L, et al. Single-center experience of unrelated and haploidentical stem celltransplantation with TCRalphabeta and CD19 depletion in children with primary immunodeficiency syndromes. Biol Blood Marrow Transplant 2015;21:1955-62.
31. Kharya G, Nademi Z, Leahy TR, Dunn J, Barge D, Schulz A, et al. Haploidentical T-cell alpha beta receptor and CD19-depleted stem cell transplant for Wiskott-Aldrich syndrome. J Allergy Clin Immunol 2014;134:1199-201.
32. Shah RM, Elfeky R, Nademi Z, Qasim W, Amrolia P, Chiesa R, Rao K, Lucchini G, Silva JMF, Worth A, Barge D, Ryan D, Conn J, Cant AJ, Skinner R, Abd Hamid IJ, Flood T, Abinun M, Hambleton S, Gennery AR, Veys P, Slatter M. T-cell receptor αβ+ and CD19+ cell-depleted haploidentical and mismatched hematopoietic stem cell transplantation in primary immune deficiency. J Allergy Clin Immunol. 2018 Apr;141(4):1417-1426.e1. doi: 10.1016/j.jaci.2017.07.008. Epub 2017 Aug 3. Erratum in: J Allergy Clin Immunol. 2019 May;143(5):1977. PMID: 28780238
33. Neven B, Diana JS, Castelle M, Magnani A, Rosain J, Touzot F, Moreira B, Fremond ML, Briand C, Bendavid M, Levy R, Morelle G, Vincent M, Magrin E, Bourget P, Chatenoud L, Picard C, Fischer A, Moshous D, Blanche S. Haploidentical Hematopoietic Stem Cell Transplantation with Post-Transplant Cyclophosphamide for Primary Immunodeficiencies and Inherited Disorders in Children. Biol Blood Marrow Transplant. 2019 Jul;25(7):1363-1373. doi: 10.1016/j.bbmt.2019.03.009. Epub 2019 Mar 12. PMID: 30876929

Tables

Table 1. Patients' features

N	Institution	Gender / Origin	Mutation	Age at symptoms onset	Age at genetic diagnosis	CLINICAL FEATURES		
						AT ONSET	OTHER	Infectious diseases episodes
P1	IRCSS G. Gaslini (ITALY)	Male / Italian	c.622G>T p.Val208Phe (missense)	2 months	5 years	• MAS (triggered by CMV): cytopenia, splenomegaly, maculopapular rash	• enterorrhagia, • growth failure, • enlarged lymphnodes and spleen • cutaneous rash, vasculitis skin	• Recurrent otitis (MDR Pseudomonas), • chronic CMV viremia
P2	IRCSS G. Gaslini (ITALY)	Male / Italian	c.64+1G>C (splice region)	1 month	15 years	• Neonatal hemorrhagic enteritis, • poor growth	• enterorrhagia/immune enteritis • thrombocytopenia • lung disease (multiple bronchiectasis, pneumatocele, lobectomy) • severe eczema • food allergy (cow milk protein intolerance), • inhalant allergy (asthma attacks) • vasculitis skin,	• recurrent pulmonary infections (staphylococcus spp.), • Salmonella typhi • Extensive warts
P3	Ann & Robert H. Lurie Children's Hospital of Chicago (USA)	Male / Somalian	c.392+2T>C IVS4+2T>C	2 weeks	14 months	• Neonatal hemorrhagic enteritis, • diffuse skin rash/eczema	• Multiple episodes of hematemesis • hemorrhagic gastritis • hematochezia requiring hospitalization and NJ feeds, • hypothyroidism, • atopic dermatitis, • failure to thrive, • autoimmune thrombocytopenia, • food allergy (milk/dairy with milk) • Brother died at 3 months of age with similar presenting symptoms ~5 years prior to his presentation	• Adenovirus enteritis, • Campylobacter enteritis, • central line infection, pneumonia and oral mucositis, • recurrent MSSA pustulosis/folliculitis, • pancreatitis
P4	Hospital Vall d'Hebron (SPAIN)	Female / Maroccan	c.491_495 del insC CTGCCC / p.Phe164Serfs*31	9 days	NA	• MAS requiring PICU admission for respiratory distress and anasarca	• Episode of DVT related to central line, • arterial hypertension, • axial hypotonia	• multiple septic events (candida spp., Enterobacter cloacae), • CMV infection
P5	University Medical Center Ulm (GERMANY)	Male / Maroccan	c.311G>C	1 month	NA	• Eczema, • RSV bronchopneumonia	• Growth failure	• Enteritis (Campylobacter), • Skin abscesses (P. aeruginosa + K. Pneumoniae),

								• otitis media (P.aeruginosa), • lymphadenitis with abscess, • erysipelas, • gross generalized molluscum contagiosum, • EBV infection
P6	Schniedr Children's medical center of Israel	Male / Persian Jew	c.G623 DEL-TC (p.V208fs)	1 month	2month	• Neonatal hemorrhagic enteritis • Severe eczema • failure to thrive	• GI bleeding colitis, • thrombocytopenia • Bleeding tendency	• multiple infections since 1 month of age: pneumonia, otitis, skin infections
P7	Aghia Sophia Children's Hospital, Athens (GREECE)	Male / Afghan	NM_005720.4: c.783G>A	6 months		• severe lower respiratory tract infection	• Eczema, food allergy, and allergic asthma (anaphylactic shock with formula milk), • Autoimmune hypothyroidism • failure to thrive	• recurrent bronchiolitis • pneumonia • skin abscess (perianal abscess due to Pseudomonas aeruginosa)

Legenda: *CMV – cytomegalovirus, DVT - deep vein thrombosis, MAS – macrophage activating syndrome, MDR – multi-drug resistant, MMF – mycophenola pediatric intensive care unit, RSV - respiratory syncytial virus, TMP-SMX - Trimethoprim/sulfamethoxazole*

Table 2. Transplant's features and outcome

N	HSCT features					Outcome					
	Age at HSCT (years)	Donor type	SC source *(BM,PBSC)*	Conditiong Regimen *(cumulative dose)*	Post-HSCT in vivo GvHD prophylaxis	Engratment *(day after HSCT)*		Chimerism *(% of donor-derived cells)*	Acute GvHD *(grade -organs involved)*	Chronic GvHD *(grade - organs involved)*	Infections Type of germ/infec
						Neutrophils	Platelets				
P1	5,25	HAPLO Father	PB	• TT 8 mg/m² • Treo 42 mg/m² • Fluda 160 mg/m² • Rituximab 200 mg/m² • ATG 12 mg/kg	none	13	13	DC *100%*	NO	NO	• Staphyloc epidermidis R *(blood cu* • CMV *viren* • Metapneu *(pharyngea*
P2	15,16	HAPLO mother	PB	• TT 8 mg/m² • Treo 42 mg/m² • Fluda 160 mg/m² • Rituximab 200 mg/m² • ATG 12 mg/kg	none	13	14	DC *100%*	1 Skin	2 Skin	• Staphyloc aureus *(blo cultures)* • CMV *viren* • EBV *virem* • BK virus *v* • JC virus *in* • HHV6 *vire*
P3	1,83	MUD (10/10)	PB	• Fluda 150 mg/m², • Bus dose adapted on AUC (target AUC: 5000ng·h/mL)	• PT-Cy • FK506 • MMF	16	17	DC *99%*	NO	NO	NO
P4	0,75	MUD (9/10)	BM	• Fluda 140 mg/m² • Bus dose adapted on AUC (target AUC: 7500 ng·h/mL) • ATG 10 mg/Kg	• CyA • MTX	18	35	DC *100%*	2 Skin	NO	• Klebsiella pneumonia *cultures)* • CMV *viren* • Metapneu *(pharyngea*
P5	5,75	MRD	BM	• TT 5 mg/m² • Treo 42 mg/m² • Fluda 160 mg/m² • Alemtuzumab 0,5 mg/kg	• CyA • MMF	22	20	SMC*	NO	NO	NO
P6	0,25	MRD	BM	• Bus (according to weight of patient) for 16 dose	• CyA • MMF	18	NA	DC *99%*	NO	NO	• Escheriac • Staphyloc epidermidis • Enterocco

				• Fluda 160mg/m² • ATG 10 mg/Kg							• Klebsiella Pneumonia • CMV *viren*
P7	1	MRD	BM	• Bus 16 mg/kg • Fluda 150 mg/m² • ATG (dose n.a.)	• CyA • MTX	23	18	TMC**	NO	NO	• Leuconos pseudomes • CMV *viren*

***** *% of donor-derived cells at the last follow up: CD3+ 93,6%, CD15+ 5,1%, CD3- 28%*

****** *% of donor-derived cells: 78% at 1 months, 98% from the second moat until the last follow up*

Legenda*: Bus - Busulfan, CMV – cytomegalovirus, CRE - Carbapenem-resistant Enterobacteriaceae, CSF - cerebrospinal fluid, DC – donor chimerism, Fluc cystitis, MAS – macrophage activating syndrome, MRD – matched related donor, MUD – matched unrelated donor, PICU – pediatric intensive care unit, SMC Thrombotic Microangiopathy, TMC – transient mixed chimerism, Treo – treosulfan, TT – thiotepa, VOD – veno-occlusive disease*