Innovative methods in pediatric hematopoietic stem cell transplantation

Ph.D. Thesis

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1. INTRODUCTION

1.1. General introduction

Hematopoietic stem cell transplantation is the only curative treatment to several lethal hematologic, oncologic, immunologic and inborn disorders. A dramatic improvement in the outcome of these procedures has been noticed in the last years, changing even the indications. The current paper summarizes the result of the research activities of the author's team in the last two decades.

1.2. Acquired bone marrow failure syndromes

Acquired bone marrow failure syndromes are rare but fatal diseases in childhood. Since 2013 Hungary has been participating as a full member in the work of the European Working Group on uniform diagnostics and therapy in patients with acquired bone marrow failure syndromes. Hypocellular refractory cytopenia of childhood has been emphasized as a frequent entity, transplanted by reduced intensity conditioning with excellent outcomes.

1.3. Virus-specific T-cell therapy

Viral reactivation is a frequent complication of allogeneic hematopoietic stem cell transplantation especially in children. For refractory cases, rapid virus-specific T-cell therapy would be ideally implemented within a few days.

1.4. Antithymocyte globulin and bradycardia

In antithymocyte globulin (ATG) treated patients occasionally bradycardia has been noticed. Therefore, we retrospectively analyzed the occurrence of bradycardia in ATG-treated children.

1.5. Cord blood transplantation in inborn errors

1.5.1. Lesch-Nyhan syndrome

Lesch-Nyhan syndrome (LNS) is a chronic, progressive neurodevelopmental disorder causing motor and behavioral dysfunction due to decreased synthesis of the enzyme hypoxantin-guanine phosphoribosyl-transferase (HPRT). Affected boys have mental retardation, delayed development, extrapyramidal motor

disturbances and self-injuring behavior. No effective treatment strategy has been observed till now.

1.5.1. WHIM syndrome

WHIM syndrome is a rare primary immunodeficiency disorder with autosomal dominant inheritance caused by a severe chemokine signalization defect due to truncation mutations of the CXCR4 chemokine receptor gene. Clinically WHIM syndrome is characterized by *w*arts, *h*ypogammaglobulinemia, *i*nfections, and *m*yelokathexis.

2. AIMS

2.1. Acquired bone marrow failure syndromes

Our aim was to analyze and compare the results of treatment before and after our joining to the international network of EWOG. We analyze the engraftment, incidence of acute and chronic graft versus host disease and the overall survival stratified by disease type.

2.2. Virus-specific T-cell therapy

Our aim was to analyze the outcome of the treatment of the refractory viral reactivations with virus-specific T-cells. We would like to prove the efficacy of this approach.

2.3. Antithymocyte globulin and bradycardia

Our aim is to show, that heart rate is significantly lower after antithymocyte-globulin treatment. We will analyze other vital parameters and consequences as well.

2.4. Cord blood transplantation in inborn errors

2.4.1. Lesch-Nyhan syndrome

As hematopoietic stem cell transplantation (HSCT) has been shown to be effective in several neurodevelopmental inborn errors, we hypothesized that it could be favorable in LNS as well. Our aim was to show the efficacy of this treatment.

2.4.2. WHIM syndrome

WHIM syndrome is an often fatal disease without succesfull supportive treatment. Our aim was to show, that

it can be cured with allogeneic hematopoietic stem cell transplantation.

3. METHODS

3.1. Acquired bone marrow failure syndromes

A total of 55 patients have been treated in the 8 centers of the Hungarian Pediatric Oncology Network during 5 years between 2013 and 2017 (severe aplastic anemia: 9, myelodysplastic syndrome: 41, juvenile myelomonocyter leukemia: 5 pts). Allogeneic hematopoietic stem cell transplantation was performed in severe aplastic anemia in 7 cases, while antithymocyte globulin was administered in 1 case and one patient died before diagnosis. In patients with myelodysplastic syndromes watch and wait strategy was applied in 4, while transplantation in 37 cases. Reduced intensity conditioning was used in 54 percent of these cases. Transplantation was the treatment of choice in all 5 patients with juvenile myelomonocyter leukemia.

3.2. Virus-specific T-cell therapy

Over the course of a year in our pediatric cohort of 43 allogeneic transplantation, 9 patients fulfilled criteria for virus-specific T-cell therapy. Viral infections were due to CMV in 3, EBV in 2 and AdV in 1 case, while more than one virus was detected in 3 cases. Viral diseases necessitating a T-cell therapy were CMV pneumonitis and colitis, AdV enteritis and cystitis and EBV induced PTLD. Cells were produced by the CliniMACS Prodigy® CCS (IFN-gamma) System within 24 hours after mononuclear leukapheresis.

3.3. Antithymocyte globulin and bradycardia

Using medical records between 2007 and 2012 we identified children undergoing a combined therapy with ATG and glucocorticoids (ATG group, n=22). The incidence of bradycardia was compared to that registered in children treated with glucocorticoids alone (glucocorticoid alone group, n=21). Heart rates (HR) were registered before and on days 0-3, 4-7 and 8-14 after the ATG or steroid administration.

3.4. Cord blood transplantation in inborn errors

3.4.1. Lesch-Nyhan syndrome

Following a myeloablative conditioning regimen (busulfan 3.2mg/kg/day for 4 days, cyclophosphamide 60 mg/kg/day for 2 days with ATG Thymoglobin 2.5mg/kg/day for 4 days) an unrelated umbilical cord blood unit was transfused at the age of two years. The graft was a 6/6 HLA-matched at HLA-A, B loci by antigen level, and at DRB1 by allelic level typing. Infused total nucleated cell dose was 3.6 x 10e7 per kilogram body weight.

3.4.2. WHIM syndrome

Despite conservative treatment with G-CSF in combination with regular intravenous immunoglobulin (IVIG) substitution the patient still had invasive infections, worsening pulmonary functions and at the age of 9 she was transplanted after a myeloablative conditioning regimen with an umbilical cord blood stem cell graft from her HLA-identical newborn brother.

4. RESULTS

4.1. Acquired bone marrow failure syndromes

The time from diagnosis to treatment was median 92 (3-393) days in the whole patient cohort, while in severe aplastic anemia median 28 (3-327) days only. Grade II-IV acute graft versus host disease occurred in 22.6%, grade III-IV in 6.8% and chronic in 11.2%. All the patients treated with severe aplastic anemia are alive with complete remission (100%). The overall estimated survival rate is 85.1% in myelodysplastic syndrome, while 75% in juvenile myelomonocyter leukemia. The median follow-up was 30.4 (1.1-62.5) months. There was a remarkable increase in overall survival comparing the data before (1992-2012) and after (2013-) joining the international group, 70% vs 100% (P=0.133) in severe aplastic anemia and 31.3% vs 85,1% (P=0.000026) in myelodysplastic syndrome.

4.2. Virus-specific T-cell therapy

Eight patients became completely asymptomatic, while 7 also cleared the virus. Six patients are alive without viral illness or sequelae demonstrating viral DNA clearance in peripheral blood with a median follow up of

535 (350-786) days. One patient with CMV pneumonitis died of respiratory insufficiency. In 2 cases the viral illness improved or cleared, however, the patients died of invasive Aspergillosis. No cases of GvHD, rejection, organ toxicity or recurrent infection were noticed.

4.3. Antithymocyte globulin and bradycardia

The rate of bradycardic episodes was higher during ATG therapy than in the steroid alone group, while severe bradycardia occurred only in the ATG group (97 versus 32, p=0.0037, and 13 versus 0, p=0.0029, respectively). There was an interaction between the time and treatment group on HR (p=0.046). Heart rates in ATG and steroid alone groups differed significantly on day 0-3 and day 4-7 (p=0.046, p=0.006, respectively). Within the ATG group HR was lower on days 4-7 compared to the days before and the days 8-14 values (p<0.001, 95%CI: 0.020-0.074).

4.4. Cord blood transplantation in inborn errors

4.4.1. Lesch-Nyhan syndrome

Serum HPRT levels reached normal values by the end of the sixth month post-transplant. Slow

neurodevelopmental improvement has been seen during the three-year follow-up and the missing of the self-injuring behavior.

4.4.2. WHIM syndrome

After ten-year post-HSCT follow-up period our patient showed a good clinical recovery and immuno-reconstitution with almost full donor chimerism. She lived a normal and not disabled life during the last 10 years.

5. CONCLUSIONS

5.1. Acquired bone marrow failure syndromes

We showed that the fast reference diagnostics and unified treatment protocols can dramatically improve outcome. Due to a change in paradigm of the conditioning regimen in hypocellular refractory cytopenia of childhood, the appliance of the TCR alpha-beta negative selection method and busulfan therapeutic drug monitoring, the overall survival rate has significantly increased.

5.2. Virus-specific T-cell therapy

We showed the efficacy of this approach to virus-specific T-cell therapy amongst the first research groups. Virus-specific T-cell therapy implemented by the CliniMACS Prodigy® CCS (IFN-gamma) System is an automated, fast, safe, and effective way to control resistant viral diseases after pediatric hematopoietic stem cell transplantation. We observed the full cure of the viral disease in all but one case. We showed that HLA match is not mandatory in the process of donor selection.

5.3. Antithymocyte globulin and bradycardia

These findings indicate that transient asymptomatic bradycardia is probably more common with ATG therapy than previously reported. HR should be closely monitored during and after ATG therapy.

5.4. Cord blood transplantation in inborn errors

5.4.1. Lesch-Nyhan syndrome

The slow neurodevelopmental improvement seen during the three-year follow-up and the missing self-injuring behavior can be considered as a proof for the

presence of enzyme-competent cells behind the blood-brain barrier.

5.4.2. WHIM syndrome

We reported the first successful allogeneic hematopoietic stem cell transplantation (HSCT) in a 9-year-old girl diagnosed at 6.5 years of age as having WHIM syndrome. HSCT is an effective treatment of this disease.

6. LIST OF PUBLICATIONS

6.1. Publications related to this thesis:

Impact factors summarized: 17,639

1. **Kállay K**, Csomor J, Ádám E, Bödör C, Kassa C, Simon R, Kovács G, Péter G, Ottóffy G, Bartyik K, Kiss C, Masát P, Réti M, Tóth B, Kriván G. (2018) [Change in paradigm in the treatment of pediatric acquired bone marrow failure syndromes in Hungary]. Orv Hetil, 159: 1710-1719.
2. **Kállay K**, Kassa C, Réti M, Karászi É, Sinkó J, Goda V, Stréhn A, Csordás K, Horváth O, Szederjesi

A, Tasnády S, Hardi A, Kriván G. (2018) Early Experience With CliniMACS Prodigy CCS (IFN-gamma) System in Selection of Virus-specific T Cells From Third-party Donors for Pediatric Patients With Severe Viral Infections After Hematopoietic Stem Cell Transplantation. J Immunother, 41: 158-163.

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4. **Kállay K**, Zakariás D, Csordás K, Benyó G, Kassa C, Sinkó J, Stréhn A, Horváth O, Vásárhelyi B, Kriván G. (2019) Antithymocyte Globuline Therapy and Bradycardia in Children. Pathol Oncol Res, 25: 487-492.
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7. Kotmayer L, Kiss, R; Király, PA ; Csomor, J ; **Kállay, K**; Alpár, D; Bödör, Cs. (2018) Familiáris myelodysplasiás szindrómában szenvedő család genomikus kópiaszám-változásainak vizsgálata multiplex ligatiofüggő szondaamplifikációval. Hematológia-Transzfúziológia, 51: 214-220.
8. Tawana K, Wang J, Király PA, **Kállay K**, Benyó G, Zombori M, Csomor J, Al Seraihi A, Rio-Machin A, Matolcsy A, Chelala C, Cavenagh J, Fitzgibbon J, Bödör C. (2017) Recurrent somatic JAK-STAT pathway variants within a RUNX1-mutated pedigree. Eur J Hum Genet, 25: 1020-1024.
9. Király PA, **Kállay K**, Marosvári D, Benyó G, Szőke A, Csomor J, Bödör C. (2016) [Clinical and genetic background of familial myelodysplasia and acute myeloid leukemia]. Orv Hetil, 157: 283-289.

6.2. Other publications:

Impact factors summarized: 12,255

1. Bader P, Kuçi Z, Bakhtiar S, Basu O, Bug G, Dennis M, Greil J, Barta A, **Kállay K**, Lang P, Lucchini G, Pol R, Schulz A, Sykora KW, von Luettichau I, Herter-Sprie G, Uddin MA, Jenkin P, Alsultan A, Buechner J, Stein J, Kelemen A, Jarisch A, Soerensen J, Salzmann-Manrique E, Hutter M, Schäfer R, Seifried E, Klingebiel T, Bonig H, Kuçi S. (2018) Effective treatment of steroid and therapy-refractory acute graft-versus-host disease with a novel mesenchymal stromal cell product (MSC-FFM). Bone Marrow Transplant, 53: 852-862.
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5. Kriván G, Szabó D, **Kállay K**, Benyó G, Kassa C, Sinkó J, Goda V, Arató A, Veres G. (2014) [Successful autologous haematopoietic stem cell transplantation in severe, therapy-resistant child-hood Crohn's disease. Report on the first case in Hungary]. Orv Hetil, 155: 789-792.
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cell histiocytosis and Erdheim-Chester disease. Onco Targets Ther, 10: 521-526.

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9. Horváth O, **Kállay K**, Kriván G. (2015) A vashiányos anaemia korszerű kezelése gyermekkorban. Gyermekgyógyászati Tovább-képző Szemle, 20: 211-213.

10. Horváth O, **Kállay K**, Kriván G. (2016) A góckérdés napjainkban. Gyermekgyógyászati Továbbképző Szemle, 21: 103-104.
11. **Kállay K**, Fekete F, Szever Z, Majorosi G. (2001) Guillain-Barré-szindróma kezelése napjainkban : irodalmi áttekintés egy eset kapcsán. Gyermekgyógyászat, 52: 374-378.
12. **Kállay K**, Liptai Z, Benyó G, Kassa C, Stréhn A, Goda V, Sinkó J, Rásonyi R, Réti M, Kriván G. (2012) A boxbajnok - avagy kihívások egy X-hez kötött adrenoleukodystrophiában szenvedő gyermek transzplantációjakor. Hematológia-Transzfúziológia, 45: 23-26.
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