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Hypomorphic RAG deficiency: impact of disease burden on survival and thymic recovery argues for early diagnosis and HSCT

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Abstract:
Patients with hypomorphic mutations in *RAG1* or *RAG2* genes present as either Omenn syndrome or atypical combined immunodeficiency (CID) with a wide phenotypic range. Hematopoietic stem cell transplantation (HSCT) is potentially curative, but data are scarce. We report on a worldwide cohort of 60 patients with hypomorphic *RAG* variants who underwent HSCT, 78% of whom experienced infections (29% active at HSCT), 72% autoimmunity and 18% granulomas pre-transplant. These complications were frequently associated with organ damage. Eight individuals (13%) were diagnosed by newborn screening or family history. HSCT was performed at a median of 3.4 years (range 0.3 - 42.9 years) from matched unrelated donors (MUD), matched sibling or matched family donors (MSD/MFD) or mismatched donors in 48%, 22% and 30% of the patients, respectively. Grafts were T-cell depleted in 15 cases (25%). Overall survival at 1 and 4 years was 77.5 and 67.5% (median follow-up 39 months). Infection was the main cause of death. In univariable analysis, active infection, organ damage pre-HSCT, T-cell depletion of the graft and transplant from a MMFD were predictive of worse outcome, while organ damage and T-cell depletion remained significant in multivariable analysis (HR=6.01, HR=8.46, respectively). All patients diagnosed by newborn screening or family history survived. Cumulative incidences (CI) of acute and chronic GvHD were 35% and 22% respectively. CI of new-onset autoimmunity was 15%. Immune reconstitution, particularly recovery of naïve $CD4^+$ T-cells was faster and more robust in patients transplanted before 3.5 years and without organ damage. These findings support the indication for early transplantation. PIDTC clinical protocols NCT01186913 and NCT01346150

Conflict of interest: COI declared - see note

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Figure 1 (A-C) – Overall survival according to age, newborn screening/family history

Figure 2 (A-D) – Overall survival according to organ damage prior to HSCT, active infections at HSCT, T-cell depleted grafts and donor

Figure 3 – forest plot for univariable cox regression

Figure 4 – cumulative incidence of aGvHD, cGvHD, de novo/relapse autoimmunity

Figure 5A – $CD4^+$ T-cell reconstitution by age at HSCT

Figure 5B – $CD4^+CD45RA^+$ T-cell reconstitution by age at HSCT

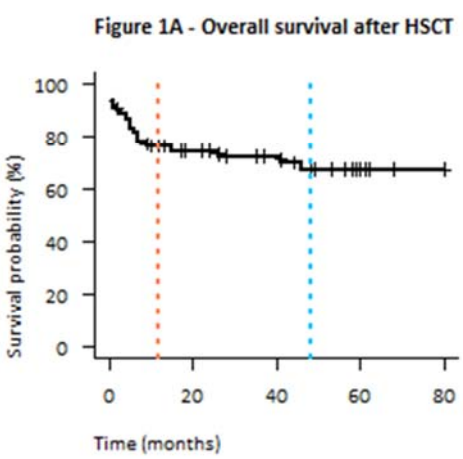
Figure 5C – $CD4^+$ T-cell reconstitution by presence of infections

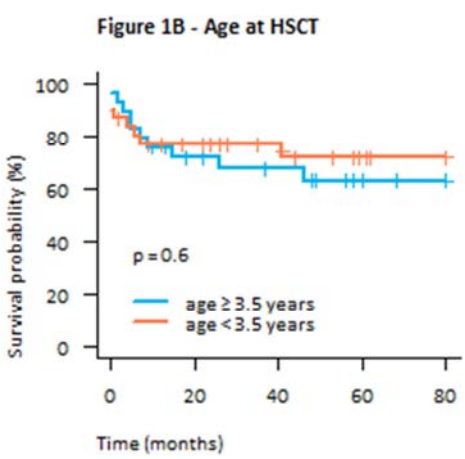
Figure 5D – $CD4^+$ T-cell reconstitution by presence of autoimmunity

Figure 5E – $CD4^+CD45RA^+$ T-cell reconstitution by presence of infections

Figure 5F – $CD4^+CD45RA^+$ T-cell reconstitution by presence of autoimmunity

Figure 5G – $CD4^+CD45RA^+$ T-cell reconstitution by presence of organ damage





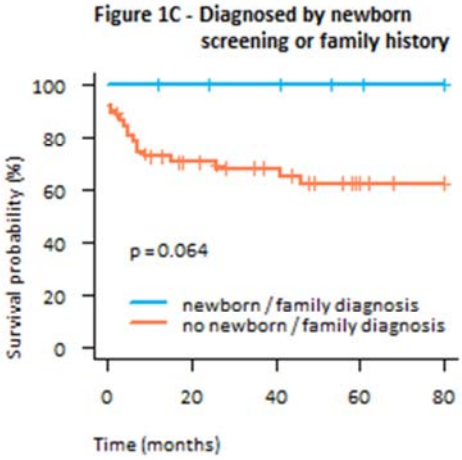


Figure 1A – Kaplan-Meier estimates of survival for the entire cohort after HSCT. *NB.* The curve starts at 93% as 4 events occurred at time point 0 (7%), **Age at HSCT (cut off defined by the median age at 3.5 years) had no influence on overall survival (OS, Fig. 1B). No events occurred in patients diagnosed by newborn screening or family history (Fig. 1C).**

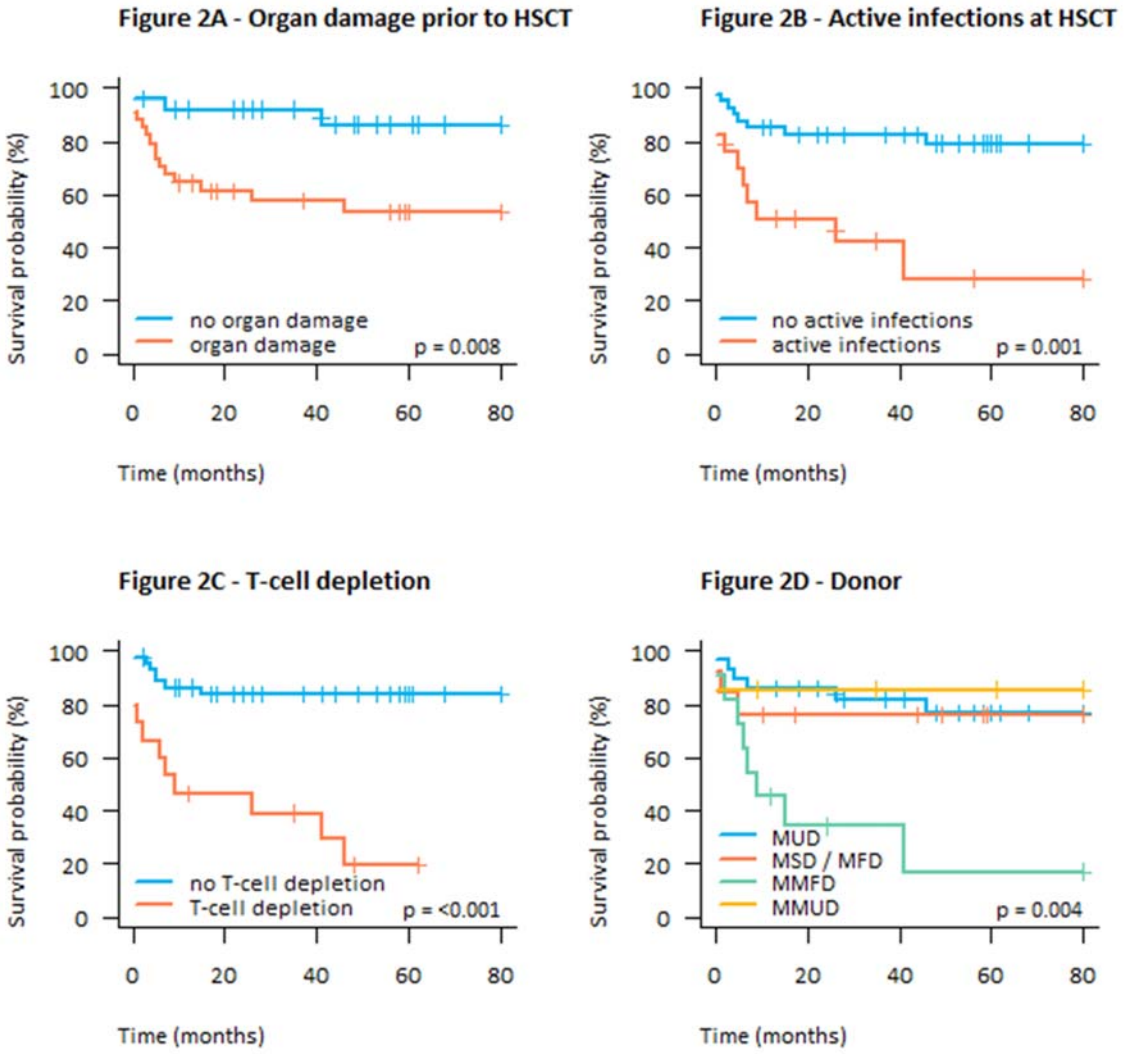


Figure 2 A - The survival probability with or without organ damage prior HSCT was 65% versus 92% after 12 months, and 55% versus 87% at 4 years post-HSCT (Logrank Test, p=0.008). Autoimmunity prior HSCT had no influence on survival (yet indirectly via organ damage – as shown by logistic regression to be a determinant for organ damage (p=0.003, Table 4). Figure 2B – Impact of active infection at HSCT on survival (p=0.001). Figure 2C – The survival probability of patients with or without T-cell depletion is 19% vs. >80% at 4 years post-HSCT (Logrank test, p <0.001). Figure 2D - The survival probability of patients transplanted with MMFD donor was inferior as compared to other donors (45% and 18% at 12 months and 4 years post-HSCT, p=0.004). *NB*: only 2 of 7 grafts from MMUD were T-cell depleted.

Variable	(n)	HR	[95% CI]	*p*
infection prior to HSCT	(47)	5.71	[0.76 - 43.01]	0.091
active infection at HSCT	(17)	4.57	[1.77 - 11.79]	0.002
autoimmunity prior to HSCT	(43)	1.45	[0.48 - 4.40]	0.515
autoimmunity and/or granuloma prior to HSCT	(47)	2.28	[0.52 - 9.92]	0.272
active autoimmunity and/or granuloma at HSCT	(32)	1.12	[0.43 - 2.96]	0.812
organ damage prior to HSCT	(34)	4.58	[1.32 - 15.83]	0.016
year of HSCT (mean 2014)	-	0.99	[0.88 - 1.11]	0.862
age at HSCT (mean 3.4)	-	1.01	[0.97 - 1.05]	0.682
bone marrow	(35)	0.44	[0.17 - 1.13]	0.089
cord blood	(7)	0.37	[0.05 - 2.76]	0.33
peripheral blood stem cells	(18)	3.42	[1.34 - 8.73]	0.01
mismatched family donor (MMFD)	(11)	4.97	[1.91 - 12.90]	0.001
in vitro T-cell depletion	(15)	6.79	[2.61 - 17.64]	<0.001
myeloablative conditioning (MAC)	(19)	0.76	[0.27 - 2.14]	0.605
reduced intensity conditioning (RIC)	(18)	0.92	[0.33 - 2.57]	0.867
reduced toxicity conditioning (RTC)	(22)	1.1	[0.43 - 2.84]	0.843
serotherapy	(49)	1.14	[0.33 - 3.96]	0.832

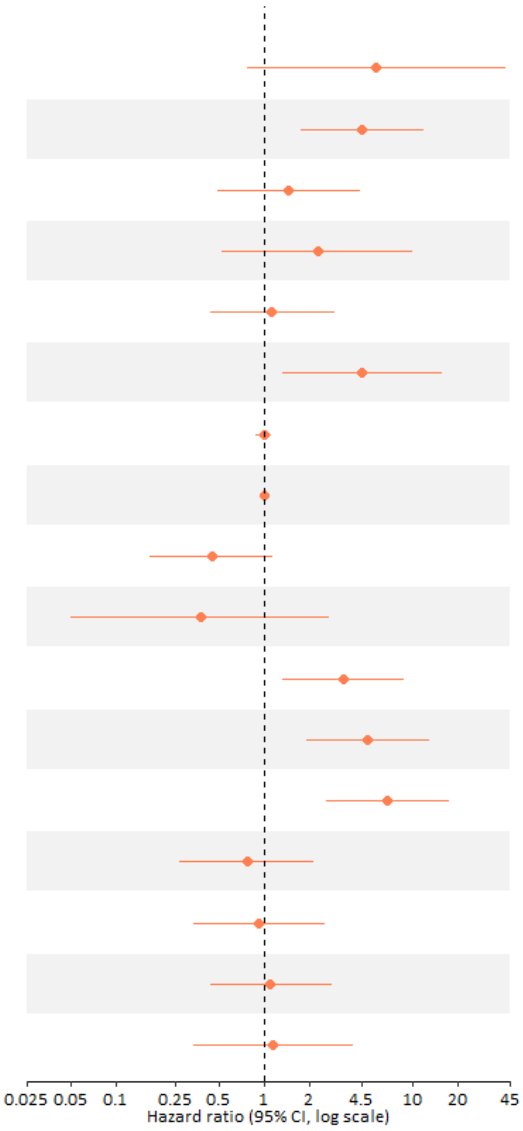


Figure 3 – Forest plot for univariable Cox-analysis of risk factors affecting OS. *NB.* Most PBSC grafts were T-depleted (12 of 18), thus not significant in multivariable analysis.

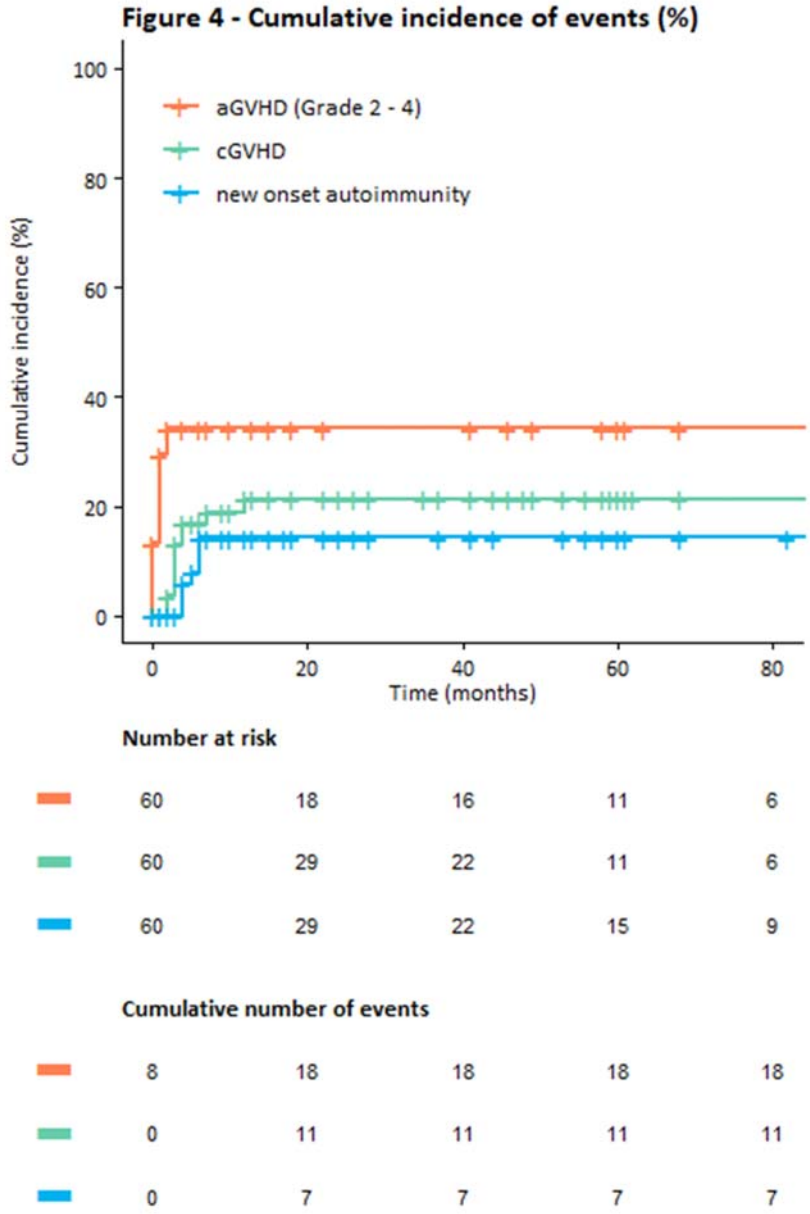
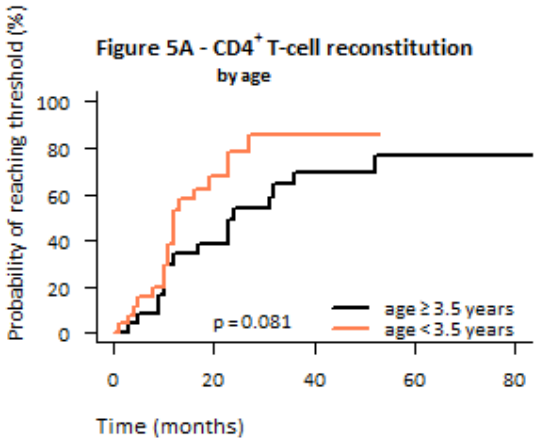
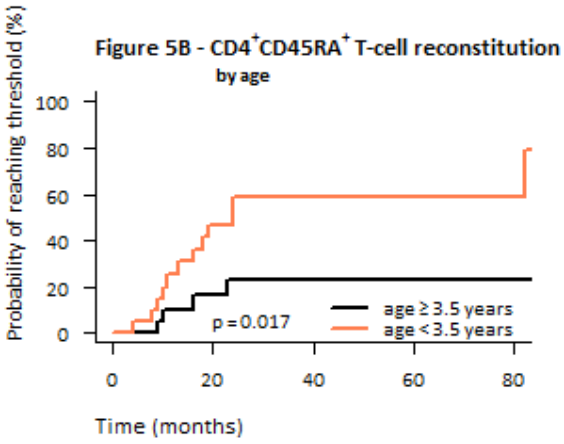
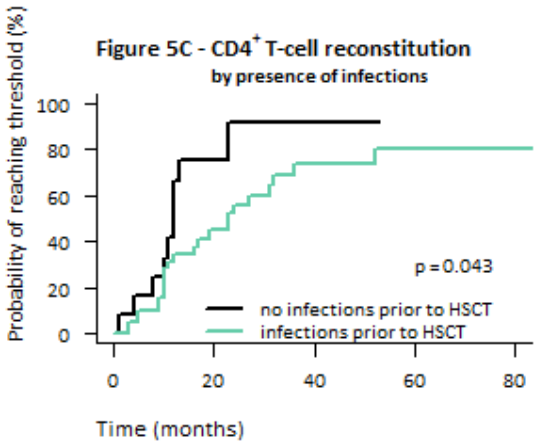
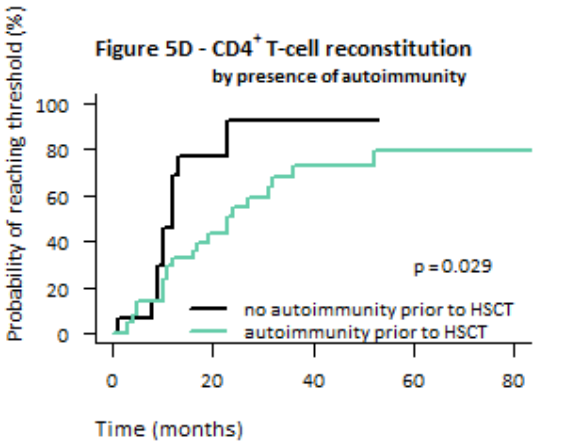


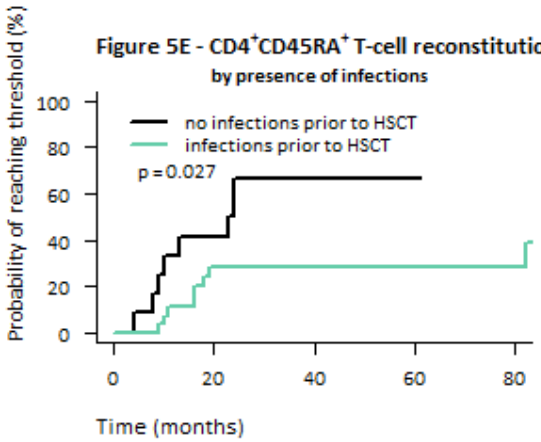
Figure 4 – Cumulative risk of aGvHD, cGVHD and new onset autoimmunity. The last follow-up time point before the end of the study was used for censoring.

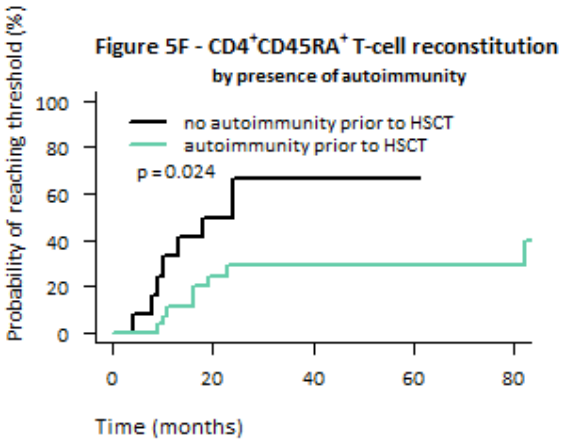












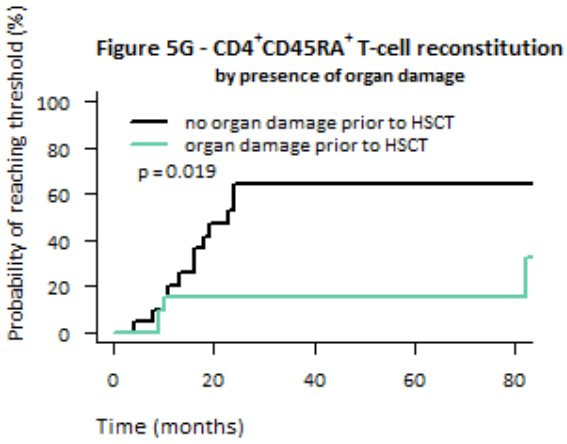
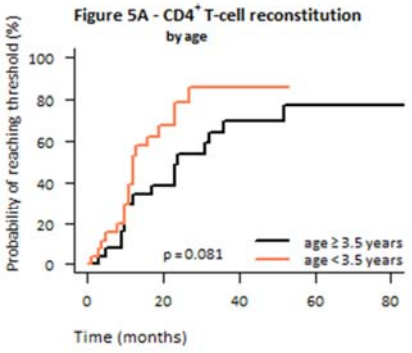
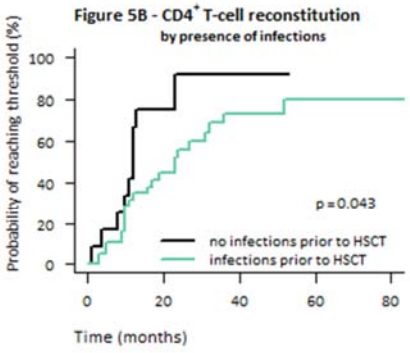
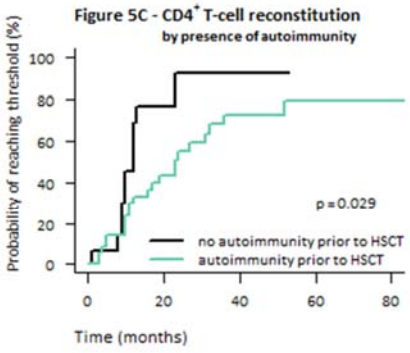
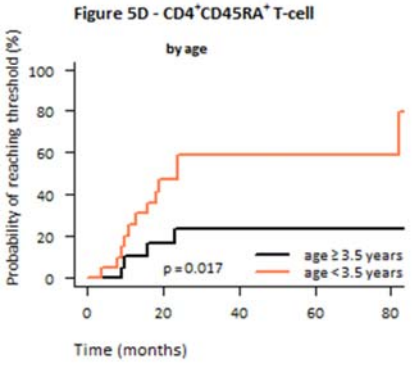


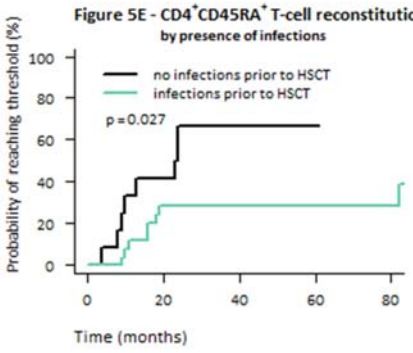
Figure 5A-B – Difference of Immune reconstitution by age at HSCT. Cumulative incidence function and Gray's test were used. An event was defined as having reached a cell count above a given threshold: Age-adjusted between 400 and 1200/µL for 1 to >10 years of age for $CD4^+$ T-cells. Naive CD4 T-cells ($CD4^+CD45RA^+$) were also normalized to age (between 43-72%). In case of censoring, the last follow-up time point of measurement of this parameter was used. $CD4^+CD45RA^+$ T-cell count was faster and more robust in patients undergoing HSCT prior the age 3.5 years (p=0.017). Figure 5C-D – Patients without infections or autoimmunity prior to HSCT show a faster and more robust immune reconstitution of $CD4^+$ T-cells. Cumulative incidence function and Gray's test were used. An event was defined as having reached a cell count above the threshold between 400 and 1200/µL for 1 to >10 years of age for $CD4^+$ T-cells. Figure 5 E-G – Patients without infections, autoimmunity or organ damage prior to HSCT show a faster and more robust immune reconstitution of $CD4^+CD45RA^+$ T-cells. Cumulative incidence function and Gray's test were used. An event was defined as having reached a cell count above the age-adjusted threshold.

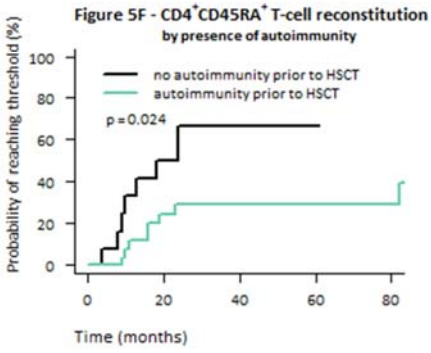


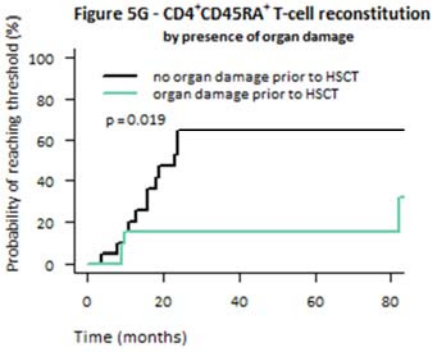












Hypomorphic RAG deficiency: impact of disease burden on survival and thymic recovery argues for early diagnosis and HSCT

short title for running head: HSCT for hypomorphic RAG deficiency

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on behalf of the Inborn Errors Working Party (IEWP) of the European Society for Immunodeficiencies (ESID) and European society for Blood and Marrow Transplantation (EBMT) and the Primary Immune Deficiency Treatment Consortium (PIDTC)

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Figures 1-5/Tables 1-4
Supplementary Tables 6-7
Supplementary Figures 1-3

Key points

- Infections, autoimmunity and granulomatous inflammation before HSCT predispose to organ damage thereby compromising survival and quality of immune reconstitution.
- In patients with hypomorphic RAG deficiency, HSCT with T-cell depleted grafts show poor outcome.

Explanation of novelty

At variance with severe forms of RAG deficiency, hypomorphic forms are often detected in late childhood or adulthood because of their atypical presentations. Allogeneic hematopoietic stem cell transplantation is potentially curative, but very limited data on outcome and potential risk factors are available in the literature. Here, we report on an international study allowing retrospective analysis of 60 patients worldwide who have received HSCT for hypomorphic RAG deficiencies. While pre-HSCT autoimmunity mostly resolved after HSCT, new onset autoimmunity early after HSCT was noticed with a cumulative incidence of 15% suggesting impaired and/or delayed restoration of tolerance.

Abstract

Patients with hypomorphic mutations in *RAG1* or *RAG2* genes present as either Omenn syndrome or atypical combined immunodeficiency (CID) with a wide phenotypic range. Hematopoietic stem cell transplantation (HSCT) is potentially curative, but data are scarce. We report on a worldwide cohort of 60 patients with hypomorphic *RAG* variants who underwent HSCT, 78% of whom experienced infections (29% active at HSCT), 72% autoimmunity and 18% granulomas pre-transplant. These complications were frequently associated with organ damage. Eight individuals (13%) were diagnosed by newborn screening or family history. HSCT was performed at a median of 3.4 years (range 0.3 – 42.9 years) from matched unrelated donors (MUD), matched sibling or matched family donors (MSD/MFD) or mismatched donors in 48%, 22% and 30% of the patients, respectively. Grafts were T-cell depleted in 15 cases (25%). Overall survival at 1 and 4 years was 77.5 and 67.5% (median follow-up 39 months). Infection was the main cause of death. In univariable analysis, active infection, organ damage pre-HSCT, T-cell depletion of the graft and transplant from a MMFD were predictive of worse outcome, while organ damage and T-cell depletion remained significant in multivariable analysis (HR=6.01, HR=8.46, respectively). All patients diagnosed by newborn screening or family history survived. Cumulative incidences (CI) of acute and chronic GvHD were 35% and 22% respectively. CI of new-onset autoimmunity was 15%. Immune reconstitution, particularly recovery of naïve $CD4^+$ T-cells was faster and more robust in patients transplanted before 3.5 years and without organ damage. These findings support the indication for early transplantation.

Introduction

Variants in genes essential for V(D)J recombination lead to a developmental arrest of T and B lymphocytes. These genes encode components of the recombination activation complex, including the recombination activating gene 1 (RAG1) and RAG2 proteins, and factors of the nonhomologous end joining pathway of DNA double-strand break repair (1).

Loss of function variants in *RAG1* and *RAG2* genes impede V(D)J recombination leading to severe combined immunodeficiency (SCID) characterized by absent or severely reduced T and B cells, but normal NK-cell numbers. In contrast, individuals harboring hypomorphic variants in the *RAG* genes, usually present as either Omenn syndrome, or atypical combined immunodeficiency (CID) with residual T and B cells but decreased naïve T cells and a spectrum of clinical and immunological phenotypes. Patients with atypical CID may experience frequent and opportunistic infections, including severe infections with *herpesviridae* and human papillomavirus (HPV), and a wide range of autoimmune manifestations, including cytopenias and granulomatous lesions of the skin (2). Autoimmune and autoinflammatory manifestations frequently predominate over infections. CID patients with hypomorphic *RAG* variants who undergo hematopoietic stem cell transplantation (HSCT) are often diagnosed in childhood or teenage years if not identified by low levels of T-cell receptor excision circles (TRECs) at newborn screening or by a positive family history. Although HSCT is potentially curative, data on clinical and immunological outcome including resolution of immune dysregulatory manifestations are scarce. Patients with hypomorphic *RAG* variants often manifest infections, autoimmunity and/or organ damage at the time of HSCT. Compared to patients with null *RAG* mutations presenting with SCID, they are at higher risk of graft rejection due to their residual T-cell function. In addition, thymic abnormalities affecting mechanisms of immune tolerance have been described in patients with hypomorphic *RAG* variants. How these abnormalities impact on the capacity of the thymus to sustain immune reconstitution and restore tolerance following HSCT is unknown (3-5). Here we report on a worldwide cohort of 60 patients with hypomorphic *RAG* variants focusing on the natural course prior to HSCT, HSCT characteristics and complications, as well as immunological outcomes following HSCT.

Methods

Inclusion criteria: Patients were eligible if they had received their first transplant between 2004 and 2019 for a confirmed RAG1/RAG2 deficiency, had >300 autologous T cells at diagnosis of immunodeficiency or <300 T cells but received no HSCT prior to the age of 18 months. RAG1/RAG2 deficiency presenting as typical SCID or Omenn phenotype were excluded.

Data source: The retrospective study was approved by the scientific review board of the Inborn Errors Working Party (IEWP) of the European Society for Immunodeficiencies (ESID), European Society for Blood and Marrow Transplantation (EBMT), the Primary Immune Deficiency Treatment Consortium (PIDTC) Steering Committee and the local IRB (TU Dresden BO-EK-372072021). A specific questionnaire for data collection and analysis was sent to the participating centers. Data of all patients registered at the EBMT office were in compliance with the General Data Protection Regulation (GDPR 2016/679). Data for patients previously enrolled in PIDTC studies in the US and Canada, obtained under approved Institutional Review Board protocols (NCT01346150) were retrieved and shared in irreversibly de-identified form. Data collected included clinical, genetic and immunological characteristics before transplant, characteristics of HSCT, and outcome regarding engraftment, immunological reconstitution, and clinical status.

Definitions: Intensity of the conditioning regimen (CR) was categorized into 3 groups; myeloablative (MAC), reduced toxicity (RTC), reduced intensity (RIC)/non-myeloablative regimen (NMA) as defined in the last IEWP guidelines (6) and shown in Suppl. Table 1.

Based on HLA compatibility, donors were grouped into 4 categories: Matched unrelated donor (MUD) defined as 10/10 identical unrelated donor or 6/6 unrelated cord blood, Matched sibling donor and matched family donor (MSD/MFD) as 10/10 or 6/6 HLA identical relatives, mismatched family donor (MMFD), and mismatched MUD (MMUD) as ≤9/10 HLA-matched. Engraftment definitions were in accordance with the EBMT handbook (7). Acute and chronic GvHD were graded according to modified Seattle and National Institute of Health criteria respectively (8). Organ damage was documented with respect to the affected organ: lung (e.g. chronic bronchitis, bronchiectasis, interstitial pneumonitis), liver (e.g. viral hepatitis, cholestasis, hepatic siderosis), kidney (e.g. glomerular or tubular damage), other (e.g. colitis).

Immune reconstitution was assessed at different timepoints after transplant: ≥6-≤12 months, >12-≤18 months, >18-≤24 months, >2-≤5 years, >5-≤10 years, and >10 years post HSCT. For immune reconstitution, cumulative incidence function was used. An event was defined as having reached a cell count above a given threshold at a given timepoint. In case of censoring, the last follow-up time point of measurement of this parameter was used. Gray's test was used to compare the cumulative incidences by age.

Immunophenotyping included absolute numbers of $CD3^+$, $CD4^+$, $CD8^+$, $CD19^+$ cells/µl. Percentages of naïve $CD4^+$ T cells defined as $CD3^+CD4^+CD45RA^+$/total $CD3^+CD4^+$ cells were also collected. Normal immune reconstitution was defined as $CD3^+$ T cells >1000/µl, $CD8^+$ T cells >300/µl and $CD4^+$ T cells age-adjusted between 400 and 1200/µl for 1 to >10 years of age. Normal percentages of naive $CD4^+$ T cells were also age adjusted (between 43-72%). Chimerism, performed as per centre protocols on

whole blood or lineage-specific where available, was retrieved at engraftment and later during follow-up. Donor engraftment was documented as full donor chimerism (>90%), mixed chimerism as any value between 10% and 90% donor), or graft failure (<10% donor). B-cell subset phenotyping, ongoing immunoglobulin substitution, and T-cell functions were incomplete or unavailable for the majority of individuals.

Measurement of V(D)J recombination activity

Measurement of V(D)J recombination activity of *RAG1* or *RAG2* variants was performed with an assay based on a v-Abl RAG1/RAG2$^{-/-}$ pro-B cell line containing a single pMX-INV integrated cassette, as described (9, 10). The list of *RAG* variants identified and their respective levels of recombination activity are shown in Suppl. Table 2.

Statistical analysis

Endpoints were overall survival (OS) and quality of immune reconstitution with a focus on naïve CD4+ T cells. Statistical analysis was performed using the program "RStudio". Overall survival (OS) was estimated via Kaplan-Meier (KM) analysis. Survival analyses were censored as the last follow-up time point before the end of the study on February 8th 2021. Logrank test was used to compare survival curves regarding certain variables. Risk factors for death were calculated via Cox regression with the time of death as end point. Hazard ratios were calculated by univariable Cox regression for pre-transplant, transplant variables and post-transplant variables. The maximum number of variables to insert in the Cox-regression model depended on the number of events (1 predictor per 10 events)(11). For multivariable Cox regression a stepwise selection was performed in a sense of forward selection due to the small number of events (18 events). Only variables significant on the p=0.05-significance level in the univariable model were added successively to the multivariable model and eliminated stepwise. The remaining variables with the highest impact were included in the final adjusted model. For Cox modelling the proportional hazards assumption was checked via Schoenfeld and scaled Schoenfeld residuals test. Logistic regression was performed to find determinants for significant variables resulting from the Cox model.

To define the variable importance a random forest algorithm was used. According to Breiman, random forests are a combination of tree predictors: each tree depends on the values of a random vector sampled independently and with the same distribution for all trees in the forest (12).

By permuting data only for the variable of interest during the calculation of decision trees the increase of prediction error can be used to estimate the importance of the variable. (13)

Missing data had been imputed by predictive mean matching.

Results

Population and HSCT characteristics

Sixty patients with homozygous or compound heterozygous variants in either *RAG1* (n=46) or *RAG2* (n=14) fulfilled the inclusion criteria and were included in the study. The median age at symptom onset was 1.4 years (range 0-15.4 years). Median age at genetic diagnosis was 3.3 years (range 0-40 years), with 8 patients (13% of the cohort) diagnosed neonatally via either newborn screening or positive family history, all free of symptoms at transplant. Median age at HSCT is shown in Suppl. Figure 1. Characteristics of the cohort including pre-HSCT complications are detailed in Table 1. Infections were documented in 78% of patients (29% with active infection at HSCT), granulomata in 18% (all active at HSCT) and autoimmunity in 72%. Forty-three patients experienced 91 autoimmune manifestations (median of 2 manifestations per patient, range 1 to 5 for those with autoimmunity). Cytopenias occurred in 33 patients who had 50 episodes of AIHA (n=21), ITP (n=15), neutropenia (n=13), and pure red cell aplasia (n=1). Twenty-one patients experienced 41 episodes of autoimmune organ involvement, the most frequent being skin disease (dermatitis, vitiligo, alopecia areata), followed by colitis, myositis and myasthenia gravis. Autoimmunity was active at the time of HSCT in 30 of 37 patients for whom information was available (Suppl. Table 3). Malignancies were a rare complication; two patients had developed lymphoma prior to HSCT: one EBV-associated lymphoma, and one diffuse large B-cell lymphoma. Pre-HSCT complications resulted in organ damage in 57% of patients, mostly affecting the lungs (50%) or liver (17%) (Suppl. Table 4).

A total of 62 HSCTs were performed in this cohort of 60 patients between 2004 and 2019 at 31 different centres (40 patients in Europe, 18 in North America and 2 in Australia). The characteristics of HSCT detailed in Table 2 are given for the first HSCTs. Median age was 3.4 (0-43) years. Donors were MUD, MSD/MFD, MMFD and MMUD in 48%, 22%, 18% and 12% of cases, respectively. Ex vivo T-cell depletion of the graft was performed in 15 cases (25%), with $CD34^+$ positive selection (n=5), TCR$\alpha\beta$/CD19 depletion (n=6), or $CD45RA^+$ depletion ($CD34^+$ selection followed by $CD45RA^+$ depletion of the negative fraction) (n=4). Donors were MMFD, n=7; MMUD, n=2; MUD, n=5 and MFD, n=1. There was an equal distribution between myeloablative (MAC) vs. reduced toxicity (RT) vs. reduced intensity conditioning (RIC) (Table 2). Nonmyeloablative conditioning with fludarabine only was applied in a single patient. The sources of stem cells for HSCT were bone marrow, PBSC and cord blood, in 58%, 30% and 12% of cases, respectively. All patients (98%) received GvHD prophylaxis with one agent (n=19) or a combination of immunosuppressive drugs (n= 41). Serotherapy was given to 49 patients (rabbit ATG n= 32, alemtuzumab n= 17).

Survival analyses

Median follow-up was 39 months (57 months for survivors). Forty-two patients were alive at last follow-up. Most patient deaths occurred within the first 12 months, while 4 patients died between 12 and 48 months post-HSCT. Estimated overall survival (OS) at 1 and 4 years were 77.5% and 67.5%, respectively (Fig. 1A). Eighteen patients (30%) died at a median interval of 5 months post HSCT (range 0-46 months). Main causes of death were infections (n=10), GvHD plus infections (n=2), graft rejection (n=2), and post-transplant autoimmunity (n=1). Late deaths (>12 months post-transplant) were related to infection (n=2), GvHD and new-onset autoimmunity, each in one case.

In univariable analysis, age at HSCT (with a cut off defined by the median age of 3.5 years) did not influence OS (Fig 1B). Survival of patients diagnosed by newborn screening or family history was 100% (Fig. 1C).

In particular, the survival probability with or without organ damage prior HSCT was 65% vs. 92% after 12 months and 55% vs. 87% at 4 years post-HSCT (p=0.008, Fig. 2A). Any type of active infection at HSCT had a negative impact (p=0.001, Fig. 2B) while autoimmunity before HSCT had no impact on survival (Fig. 3). In vitro T-depletion of the graft had a strong negative impact with an estimated OS at 12 months and 4 years respectively of 46% and 19% as compared to 87% and 85% for patients transplanted with an unmanipulated graft (p<0.001, Fig. 2C, Suppl. Table 5). Patients transplanted with MMFD donors had an inferior survival probability (45% at 12 months and 18% at 4 years post-HSCT, p<0.001, Fig 2D), as compared to other donor sources (e.g. MUD: 87% at 12 months and 76% at 4 years). Recipients of grafts from MMFD or T-cell depleted grafts did not differ for age at HSCT or morbidity pre-HSCT, and these variables did not confound each other. The choice of conditioning regimen had no impact on survival (Figure 3). All variables with significance or borderline significance had the maximum drop in survival probability within the first 12 months after HSCT (Fig. 3).

Multivariable analysis revealed that organ damage prior to HSCT and T-cell depletion of the graft were the major predictors for death (HR=6.01, HR=8.46, respectively), while age at HSCT, infections before HSCT and active infectious burden at HSCT were not significant predictors. Random forest analysis showed the highest variable importance for T-cell depletion (2.39), followed by mismatched family donor (MMFD) (1.7) and organ damage prior to HSCT (1.13)(Table 3 and Suppl. Table 6).

Due to the strong negative impact of pre-HSCT organ damage on OS, we examined parameters that may be associated with its occurrence. Logistic regression revealed that autoimmunity and or/granulomas prior to HSCT (p=0.003), age at HSCT ≥3.5 years (p=0.005), infection prior to HSCT

(p=0.01), and a delay of >12 months between birth and diagnosis were significant determinants for organ damage prior to HSCT (Table 4).

Engraftment, chimerism and post-transplant complications

Five patients died before engraftment. Graft failure after the first procedure occurred in 4 patients (7%), of whom 3 died. A second procedure was attempted in 2 individuals, but despite engraftment in both cases, one patient died of infection. Full chimerism (>90% donor) was documented in 75% of patients who engrafted. Sinusoidal obstructive syndrome, mostly mild, occurred in 6 patients (10%) (Seattle grade 3 in one individual). Acute and chronic GvHD were documented in 28 and 11 patients, respectively, a 12-month cumulative risk of 35% for Grade II-IV aGvHD and 22% cGVHD (Fig 4). Median onset of aGvHD was 1 month (range 0-2.4 months) post HSCT; it was mainly mild (grade ≤2) while grades 3 and 4 aGvHD were documented in 5 and 2 patients, respectively. Median onset of cGvHD was 4 months. Post-transplant infections, the main cause of death, occurred in 26 patients (43%), 20 of viral and 12 of non-viral origin, some in combination. Post-HSCT outcome of pre-existing autoimmunity, documented in 43 patients prior to HSCT, was available for 33 of them (Suppl Table 3). Autoimmune cytopenias including pure red cell aplasia resolved in all cases. Uveitis (n=1) recurred in the early post-HSCT period, but then resolved. Myositis, evaluable in 1 patient, improved. Outcome of myasthenia gravis could be assessed in one long-term survivor in whom anti-cholinesterase treatment was slowly tapered and stopped 4 years later without recurrence of symptoms, despite persistence of autoantibodies. Alopecia areata resolved in all evaluable patients, vitiligo had variable outcomes with resolution or improvement in 3 of 5 evaluable patients. Granuloma resolved in all survivors. In addition, 9 new-onset autoimmune manifestations occurred in 7 patients a median of 4 months after HSCT (range 1 to 6) (Fig. 4). These were AIHA (n=3), autoimmune hyperthyroidism (n=2), and myositis, myasthenia gravis, sclerosing cholangitis and coeliac disease (in one patient each). None of the patients with new-onset autoimmune manifestations had cGvHD, and only one of them had mixed donor chimerism.

Immune reconstitution

The probability of $CD3^+$ T-cell counts reaching >1000/µl within the first 12 months post HSCT was 55%, increasing to 79% at 4 years. $CD8^+$ T-cells >300/µl were reached in 60% of patients within 12 months post HSCT, in 90% at 4 years. The probability of $CD4^+$ T-cells reaching the age-adjusted reference ranges was 40% after 12 months, and 80% within 4 years. Immune reconstitution defined by naïve $CD4^+$ T cell counts above an age-adjusted threshold was seen in 18% at 12 months post HSCT, and rose to 42% of all patients at 4 years.

When analyzing immune reconstitution in this cohort, naïve $CD4^+$ T-cell recovery was significantly different in patients younger or older than the median age of 3.5 years at HSCT: $CD4^+CD45RA^+$ T-cell count rose faster in patients undergoing HSCT prior to age 3.5 years (p=0.017). The probability of naïve $CD4^+$ T cells reaching the age-adjusted reference range was 60% for the group transplanted <3.5 years, but only 20% in those ≥3.5 y/o at HSCT. Over the follow-up period $CD3^+$ and $CD8^+$ T-cell counts were lower in the older HSCT group (p=0.01 and p=0.03, respectively), while $CD4^+$ T-cells were not different (p=0.081, Fig. 5A-B).

The influence of the following variables on $CD4^+$ T cells and $CD4^+CD45RA^+$ naïve T cells reconstitution were analyzed: pretransplant infections, autoimmunity and organ damage; conditioning (MAC, RT, RIC, suppl. Fig. 2), donor type, T-cell depletion of grafts, GvHD, and new onset or relapsed autoimmunity post HSCT. For $CD4^+$ T cells, infections and autoimmunity prior to HSCT showed a significant adverse influence on immune reconstitution (Fig. 5C-D). In patients without infections, autoimmunity or organ damage prior to HSCT, immune reconstitution as measured by $CD4^+CD45RA^+$ T-cell numbers was faster and more robust (Fig. 5E-G).

Finally, more than 80% of the patients had achieved independence from immunoglobulin replacement therapy by 5 years after HSCT (Suppl. Figure 3). The type of CR and age at HSCT had no significant impact on reconstitution of humoral immunity (Suppl. Table 7).

Discussion

This is the largest retrospective study of outcomes of HSCT in patients with hypomorphic RAG1/RAG2 deficiencies. Only sporadic cases or small series were published previously (14, 15). Our study highlights the deleterious impact of pretransplant infections, autoimmunity and organ damage for both survival and immune reconstitution post-HSCT. The cumulative disease burden pre-HSCT in individuals with hypomorphic RAG deficiencies observed beyond early childhood supports early diagnosis and intervention by HSCT.

The pre-transplant characteristics of the cohort recapitulates the large spectrum of complications recognized in patients with hypomorphic *RAG* variants (16-18), including infection susceptibility, high rates of autoimmunity and granulomatous inflammation predisposing to organ damage. Symptoms often first manifested beyond infancy or early childhood up to 15 years of age, but molecular diagnosis was delayed with a surprisingly wide range up to mid-adulthood. This delay may both be related to the nonspecific nature of disease manifestations of hypomorphic RAG deficiency, and to the retrospective nature of the study (including patients born 1976- 2017). In fact, the age of diagnosis was significantly lower in patients born from 2010 onwards, perhaps due to introduction of NBS, but maybe also due to better recognition of the disease and improved access to next generation sequencing.

The overall survival at 1- and 4-years post-transplant was comparable to previously published data for typical SCID due to RAG1/2 defects (19-21), and for primary immune regulatory disorders (22). The presence of active infection at HSCT was predictive of unfavorable outcome in univariable analysis, as repeatedly shown for typical SCID in both the large North American and the European patient series (19, 23). Organ damage at HSCT was a strong predictor of dismal outcome both in uni- and multivariable analyses, similar to available large series of HSCT outcomes for other inborn errors of immunity (IEI) (6, 24). History of infection, autoimmunity and granuloma that typically occurred early in the course of hypomorphic RAG deficiency were all predictive of organ damage. Young age at transplant has been correlated with favorable outcome in various IEIs (6, 24, 25); however, median age at HSCT in this present cohort was not associated with overall survival. Interestingly, in the small group of 8 patients diagnosed very early by NBS or due to a positive family history, all were successfully transplanted prior any symptoms from either MUD, MMFD or MMUD. NBS by TREC measurement has been set up in several countries worldwide, and offers the potential to identify patients with typical SCID early in life, permitting prompt definitive diagnosis and treatment if possible before onset of symptoms (26). The ability of NBS to reliably identify patients suffering from hypomorphic RAG deficiency needs to be verified.

In this series, HSCT performed following ex vivo T-cell depletion of the graft including from a MMFD had a significantly poorer outcome. HSCT from MMFD in SCID have commonly been associated with inferior OS and event free survival (EFS) (16, 21, 27). Large series of patients with CIDs also highlighted the inferior outcome with MMFD mainly following $CD34^+$ selection of the graft, e.g. in CD40L or MHCII-deficiencies (24), (28), the reasons for dismal outcome being mostly infections. Recent studies with new strategies of *ex vivo* depletion of the graft such as $TCR\alpha\beta^+/CD19^+$ depletion showed improved outcome in IEIs (29) provided patients had not suffered from active infection at transplant (30, 31). $CD45RA^+$ depletion of the graft might be an alternative that could allow early anti-viral response with limited risk of GvHD (32). Strategies using post-transplant cyclophosphamide (PTCY) as GvHD prophylaxis have also shown promising results (33, 34). In our present series, various *ex vivo* manipulations of the graft were performed in 15 patients - not only in HLA- mismatched transplants, but also in 5 MUD and 1 MSD - most likely to avoid the risk of graft-versus-host disease in a nonmalignant setting. The outcome for T-cell depleted grafts in this cohort, however, was very poor especially after $CD34^+$ selection (0/5 survival) and $TCR\alpha\beta^+/CD19^+$ depletion (1/6 survival). Infections were the main cause of death documented in 9/11 patients who died, including 6 cases with active infections at the time of HSCT. These numbers are too small to draw firm conclusions, but they suggest that strategies allowing early immune reconstitution should be adopted when possible.

The burden of infection before and after HSCT results in significant morbidity and mortality, with infections being the leading cause of death after HSCT, frequently but not exclusively documented in patients who received T-cell depleted graft. The high frequency of neutralizing anti-type I interferon antibodies in pre-transplant hypomorphic RAG 1/2 deficient patients (35) could contribute to this peri-transplant risk factor, a hypothesis that could not be tested in this retrospective series.

Autoimmunity is one of the most frequent manifestations pre- HSCT in patients with hypomorphic RAG deficiencies. The pathophysiology is related to loss of central and peripheral tolerance at various stages of development (36-39). Previous studies have shown that expression of AIRE, a transcriptional activator that allows expression of tissue restricted antigens in the thymus enabling deletion of self-reactive T cells (40), is markedly reduced in thymi of RAG deficient patients with Omenn syndrome and late-onset combined immunodeficiency (3-5). Additionally, deficiency of $FOXP3^+$ regulatory T-cells was also documented in these conditions (5). Consistent with this, peripheral T-cells from patients with hypomorphic *RAG* mutations have been shown to display molecular signatures of self-reactive T-cell repertoire (41), indicating impaired purging of autoreactive T cells in the thymus. Furthermore, it has been demonstrated that patients with hypomorphic RAG mutations carry numerical and functional abnormalities of the Treg compartment (37), suggesting that defects of peripheral tolerance may also contribute to the immune

dysregulation of this condition. New-onset autoimmunity was also observed early after transplant in 7 patients of our cohort at a median of 4 months. These were autoimmune cytopenias and autoimmune thyroiditis - which are not rare events after HSCT (42, 43). However, unusual de novo immune dysregulation such as myasthenia gravis, myositis and sclerosing cholangitis also occurred post-HSCT. The critical role of thymic medulla regeneration early after HSCT to restore tolerance and prevent autoimmunity was demonstrated in experimental mouse models (44). One can speculate that pretransplant damage of the thymic medulla interferes with efficient reconstitution, and may predispose to autoimmunity. Alternatively, it is possible that delayed or incomplete reconstitution of the Treg compartment may also contribute to the increased rate of post-transplant autoimmunity observed in this cohort. These hypotheses will need to be evaluated in future studies.

The goal of HSCT in patients with IEI is to achieve a rapid and durable restoration of immune function while avoiding GVHD and limiting toxicities. In this cohort, our analysis of immune reconstitution focused on $CD4^+$ and naive $CD4^+$ T cell counts. In particular, the number of CD4+ CD45RA+ cells post-HSCT has been shown to represent a valid surrogate marker of thymic output (45) and a biomarker predictive of long-term immune reconstitution (21). Our data showed that T-cell reconstitution was slower and incomplete in patients with pre-transplant organ damage and in those ≥3.5 years of age. As the thymus is the central organ where T-cell reconstitution occurs it is not surprising that thymic damage through both pre-HSCT autoimmunity, inflammation and infections may hamper immunological reconstitution, especially when patients are diagnosed late and transplanted beyond infancy. In this series, with the exception of a single patient who received unconditioned HSCT, all other patients received a variety of conditioning regimens (RIC, RTC, and MAC), but no significant effects of these different regimens on either T- or B-cell reconstitution were observed (Suppl. Figure 2 and Suppl. Table 7).

Conclusion

Poor pre-HSCT clinical status predicted an unfavorable HSCT outcome and slower naïve T-cell recovery. These findings advocate for early detection and early definitive treatment of patients with hypomorphic RAG1/RAG2 deficiencies. The completeness of ascertainment via NBS to identify these patients is currently unknown, but NBS may facilitate earlier diagnosis and improve outcome. Overall survival of patients transplanted with *ex vivo* manipulation of the graft and from MMFD was poor, mainly due to infections. In addition, unusual *de novo* autoimmunity observed in this series might be related to thymic damage pre/during HSCT and persistent defects in central and/or peripheral tolerance early after HSCT.

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Authorship Contributions

C. Schuetz and B. Neven designed the study, interpreted compiled data, wrote the manuscript and managed patients. J. Gerke and M. Ege provided statistical analyses, interpreted data and revised the manuscript.

J. Puck and A. Lankester contributed to the study design, interpreted data and edited the manuscript.

L. D. Notarangelo contributed to the study design, supervised V(D)J recombination assays, and reviewed the manuscript. T. Kawai performed V(D)J recombination assays.

All other co-authors contributed their patients' data and reviewed the manuscript.

Conflict of Interest Disclosures

M.J. Cowan receives royalties from UpToDate and is on the Scientific Board with stock interest of Homology Medicines, Inc.

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Table 1: characteristics of cohort
Table 2: characteristics of HSCT
Table 3: overall survival - multivariable cox regression
Table 4: determinants for organ damage prior to HSCT

Table 1: characteristics of cohort

population characteristics	**total patient number n = 60**
sex, n (%)	
male	27 (45)
female	33 (55)
median year of birth	2008 (1976 - 2017)
mutation type, n (%)	
RAG 1	46 (77)
RAG 2	14 (23)
homozygous	19 (32)
compound heterozygous	41 (68)
median age at first symptoms, yrs (range)	1.4 (0 - 15.4)
median age at diagnosis, yrs (range)	3.3 (0 - 39.9)
diagnosed by newborn screening or family screening, n (%)	8 (13)
infection prior to HSCT, n (%)	47 (78)
active infection prior to HSCT, n (%)	17 (29) out of 58
presence of autoimmunity and / or granuloma prior to HSCT, n (%)	47 (78)
autoimmunity	43 (72)
autoimmune cytopenia	33 (55)
other autoimmune disease	24 (41)
granuloma	11 (18)
active autoimmunity and / or granuloma prior to HSCT, n (%)	32 (58) out of 55
malignancy/lymphoma , n (%)	2 (3)
organ damage, n (%)	34 (57)
lung	30 (50)
liver	10 (17)
kidney	6 (10)
other	9 (15)

„other" organ damage refers to gastrointestinal complications (n=7), steroid-induced diabetes (n=1) and vasculitis-associated epilepsy (n=1). For details of organ damage please refer to **Suppl. Table 6**.

Table 2: characteristics of HSCT

transplant characteristics	**total patient number n = 60**
median year of HSCT (range)	2014 (2004 - 2019)
median age at HSCT, yrs (range)	3.4 (0.3 - 42.9)
age < 3.5	31 (52)
age ≥ 3.5	29 (48)
donor, n (%)	
matched sibling donor (MSD) or matched family donor (MFD)	13 (22)
mismatched family donor (MMFD)	11 (18)
matched unrelated donor (MUD)	29 (48)
mismatched unrelated donor (MMUD)	7 (12)
graft, n (%)	
bone marrow	35 (58)
cord blood	7 (12)
PBSC	18 (30)
in vitro T-cell depletion, n (%)	15 (25)
conditioning regimen, n (%)	
myeloablative conditioning (MAC)	19 (32)
reduced intensity conditioning (RIC)	18 (30)
reduced toxicity conditioning (RTC)	22 (37)
serotherapy, n (%)	
ATG	32 (53)
Alemtuzumab	17 (28)

Table 3: overall survival - multivariable cox regression

Determinant		Univariable Model			Stepwise multivariable Model		
	n (%)	**HR**	**[95% CI]**	***p***	**HR**	**[95% CI]**	***p***
infection prior to HSCT	47 (78.3)	5.71	[0.76-43.01]	0.091			
active infection at HSCT	17 (29.3)	4.57	[1.77-11.79]	0.002			
organ damage	34 (56.7)	4.58	[1.32-15.83]	0.016	6.01	[1.72-21.00]	**0.005**
mismatched family donor (MMFD)	11 (18.3)	4.97	[1.91-12.90]	0.001			
T-cell depletion	15 (25.0)	6.79	[2.61-17.64]	<0.001	8.46	[3.22-22.24]	**<0.001**

HR= hazard ratio, CI= confidence interval

Table 4: determinants for organ damage prior to HSCT

		organ damage prior to HSCT, n (%)		
Variable		**no**	**yes**	**OR (univariable)***
autoimmunity and/or granuloma prior to HSCT	no	11 (42.3)	2 (5.9)	-
	yes	15 (57.7)	32 (94.1)	11.73 (2.73-82.27, **p=0.003**)
> 12 months between birth and diagnosis	no	7 (30.4)	2 (6.2)	-
	yes	16 (69.6)	30 (93.8)	6.56 (1.40-47.67, **p=0.029**)
infection prior to HSCT	no	10 (38.5)	3 (8.8)	-
	yes	16 (61.5)	31 (91.2)	6.46 (1.70-31.95, **p=0.010**)
age at HSCT ≥ 3.5 years	no	19 (73.1)	12 (35.3)	-
	yes	7 (26.9)	22 (64.7)	4.98 (1.69-16.04, **p=0.005**)

* OR = Odd's ratio (95% confidence interval)

Figures

Figure 1 (A-C) – Overall survival according to age, newborn screening/family history

Figure 2 (A-D) – Overall survival according to organ damage prior to HSCT, active infections at HSCT, T-cell depleted grafts and donor

Figure 3 – forest plot for univariable cox regression

Figure 4 – cumulative incidence of aGvHD, cGvHD, de novo/relapse autoimmunity

Figure 5A – $CD4^+$ T-cell reconstitution by age at HSCT

Figure 5B – $CD4^+CD45RA^+$ T-cell reconstitution by age at HSCT

Figure 5C – $CD4^+$ T-cell reconstitution by presence of infections

Figure 5D – $CD4^+$ T-cell reconstitution by presence of autoimmunity

Figure 5E – $CD4^+CD45RA^+$ T-cell reconstitution by presence of infections

Figure 5F – $CD4^+CD45RA^+$ T-cell reconstitution by presence of autoimmunity

Figure 5G – $CD4^+CD45RA^+$ T-cell reconstitution by presence of organ damage

References

1. Dvorak CC, Cowan MJ. Radiosensitive severe combined immunodeficiency disease. Immunol Allergy Clin North Am. 2010;30(1):125-42.
2. Delmonte OM, Schuetz C, Notarangelo LD. RAG Deficiency: Two Genes, Many Diseases. J Clin Immunol. 2018;38(6):646-55.
3. De Ravin SS, Cowen EW, Zarember KA, Whiting-Theobald NL, Kuhns DB, Sandler NG, et al. Hypomorphic Rag mutations can cause destructive midline granulomatous disease. Blood. 2010;116(8):1263-71.
4. Cavadini P, Vermi W, Facchetti F, Fontana S, Nagafuchi S, Mazzolari E, et al. AIRE deficiency in thymus of 2 patients with Omenn syndrome. J Clin Invest. 2005;115(3):728-32.
5. Poliani PL, Facchetti F, Ravanini M, Gennery AR, Villa A, Roifman CM, et al. Early defects in human T-cell development severely affect distribution and maturation of thymic stromal cells: possible implications for the pathophysiology of Omenn syndrome. Blood. 2009;114(1):105-8.
6. Albert MH, Sirait T, Eikema DJ, Bakunina K, Wehr C, Suarez F, et al. Hematopoietic stem cell transplantation for adolescents and adults with inborn errors of immunity, an EBMT IEWP study. Blood. 2022.
7. Carreras E DC, Mohty M, Kröger N, editors. The EBMT Handbook: Hematopoietic Stem Cell Transplantation and Cellular Therapies [Internet]. 7th ed. Cham (CH): Springer; 2019. Chapter 42. PMID: 32091796. 2019.
8. Jagasia MH, Greinix HT, Arora M, Williams KM, Wolff D, Cowen EW, et al. National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: I. The 2014 Diagnosis and Staging Working Group report. Biol Blood Marrow Transplant. 2015;21(3):389-401 e1.
9. Tirosh I, Yamazaki Y, Frugoni F, Ververs FA, Allenspach EJ, Zhang Y, et al. Recombination activity of human recombination-activating gene 2 (RAG2) mutations and correlation with clinical phenotype. J Allergy Clin Immun. 2019;143(2):726-35.
10. Lee YN. A systematic analysis of recombination activity and genotype-phenotype correlation in human recombination-activating gene 1 deficiency. . J Allergy Clin Immunol. 2014;133(4):1099-108.
11. Peduzzi P, Concato J, Feinstein AR, Holford TR. Importance of events per independent variable in proportional hazards regression analysis .2. Accuracy and precision of regression estimates. J Clin Epidemiol. 1995;48(12):1503-10.
12. Breiman L. Random forests. Mach Learn. 2001;45(1):5-32.
13. Liaw A. Classification and regression by randomForest. R news. 2002;2(3):18-22.
14. Westermann-Clark E, Grossi A, Fioredda F, Giardino S, Cappelli E, Terranova P, et al. RAG deficiency with ALPS features successfully treated with TCRalphabeta/CD19 cell depleted haploidentical stem cell transplant. Clin Immunol. 2018;187:102-3.
15. John T, Walter JE, Schuetz C, Chen K, Abraham RS, Bonfim C, et al. Unrelated Hematopoietic Cell Transplantation in a Patient with Combined Immunodeficiency with Granulomatous Disease and Autoimmunity Secondary to RAG Deficiency. J Clin Immunol. 2016;36(7):725-32.
16. Chen K, Wu W, Mathew D, Zhang Y, Browne SK, Rosen LB, et al. Autoimmunity due to RAG deficiency and estimated disease incidence in RAG1/2 mutations. J Allergy Clin Immunol. 2014;133(3):880-2 e10.
17. Farmer JR, Foldvari Z, Ujhazi B, De Ravin SS, Chen K, Bleesing JJH, et al. Outcomes and Treatment Strategies for Autoimmunity and Hyperinflammation in Patients with RAG Deficiency. J Allergy Clin Immunol Pract. 2019;7(6):1970-85 e4.
18. Schuetz C, Huck K, Gudowius S, Megahed M, Feyen O, Hubner B, et al. An immunodeficiency disease with RAG mutations and granulomas. N Engl J Med. 2008;358(19):2030-8.
19. Lankester AC, Neven B, Mahlaoui N, von Asmuth EGJ, Courteille V, Alligon M, et al. Hematopoietic cell transplantation in severe combined immunodeficiency: The SCETIDE 2006-2014 European cohort. J Allergy Clin Immunol. 2022;149(5):1744-54 e8.

20. Schuetz C, Neven B, Dvorak CC. SCID patients with ARTEMIS vs RAG deficiencies following HCT: increased risk of late toxicity in ARTEMIS-deficient SCID (vol 123, pg 281, 2014). Blood. 2018;132(23):2527-.
21. Haddad E, Logan BR, Griffith LM, Buckley RH, Parrott RE, Prockop SE, et al. SCID genotype and 6-month posttransplant CD4 count predict survival and immune recovery. Blood. 2018;132(17):1737-49.
22. Chan AY, Leiding JW, Liu XR, Logan BR, Burroughs LM, Allenspach EJ, et al. Hematopoietic Cell Transplantation in Patients With Primary Immune Regulatory Disorders (PIRD): A Primary Immune Deficiency Treatment Consortium (PIDTC) Survey. Front Immunol. 2020;11.
23. Pai SY, Logan BR, Griffith LM, Buckley RH, Parrott RE, Dvorak CC, et al. Transplantation Outcomes for Severe Combined Immunodeficiency, 2000-2009. New Engl J Med. 2014;371(5):434-46.
24. Ferrua F, Galimberti S, Courteille V, Slatter MA, Booth C, Moshous D, et al. Hematopoietic stem cell transplantation for CD40 ligand deficiency: Results from an EBMT/ESID-IEWP-SCETIDE-PIDTC study. J Allergy Clin Immunol. 2019;143(6):2238-53.
25. Burroughs LM, Petrovic A, Brazauskas R, Liu XR, Griffith LM, Ochs HD, et al. Excellent outcomes following hematopoietic cell transplantation for Wiskott-Aldrich syndrome: a PIDTC report. Blood. 2020;135(23):2094-105.
26. Currier R, Puck JM. SCID newborn screening: What we've learned. J Allergy Clin Immunol. 2021;147(2):417-26.
27. Ege MJ, Schuetz C, Jacobsen EM, Muller-Langer SM, Furlan I, Sirin M, et al. Late thymic deficiency after HLA-haploidentical hematopoietic stem cell transplantation for severe combined immunodeficiency. J Allergy Clin Immun. 2019;143(4):1623-+.
28. Lum SH, Neven B, Slatter MA, Gennery AR. Hematopoietic Cell Transplantation for MHC Class II Deficiency. Front Pediatr. 2019;7:516.
29. Lum SH, Greener S, Perez-Heras I, Drozdov D, Payne RP, Watson H, et al. T-replete HLA-matched grafts vs T-depleted HLA-mismatched grafts in inborn errors of immunity. Blood Adv. 2022;6(4):1319-28.
30. Elfeky R, Shah RM, Unni MNM, Ottaviano G, Rao K, Chiesa R, et al. New graft manipulation strategies improve the outcome of mismatched stem cell transplantation in children with primary immunodeficiencies. J Allergy Clin Immun. 2019;144(1):280-93.
31. Shah RM, Elfeky R, Nademi Z, Qasim W, Amrolia P, Chiesa R, et al. T-cell receptor alpha beta(+) and CD19(+) cell-depleted haploidentical and mismatched hematopoietic stem cell transplantation in primary immune deficiency. J Allergy Clin Immun. 2018;141(4):1417-+.
32. Touzot F, Neven B, Dal-Cortivo L, Gabrion A, Moshous D, Cros G, et al. CD45RA depletion in HLA-mismatched allogeneic hematopoietic stem cell transplantation for primary combined immunodeficiency: A preliminary study. J Allergy Clin Immun. 2015;135(5):1303-U728.
33. Fernandes JF, Nichele S, Arcuri LJ, Ribeiro L, Zamperlini-Netto G, Loth G, et al. Outcomes after Haploidentical Stem Cell Transplantation with Post-Transplantation Cyclophosphamide in Patients with Primary Immunodeficiency Diseases. Biol Blood Marrow Tr. 2020;26(10):1923-9.
34. Neven B, Diana JS, Castelle M, Magnani A, Rosain J, Touzot F, et al. Haploidentical HSCT with post-transplant cyclophosphamide for primary immunodeficiencies and inherited disorders in children. Bone Marrow Transpl. 2019;54:95-6.
35. Walter JE, Rosen LB, Csomos K, Rosenberg JM, Mathew D, Keszei M, et al. Broad-spectrum antibodies against self-antigens and cytokines in RAG deficiency (vol 125, pg 4135, 2015). J Clin Invest. 2016;126(11):4389-.
36. Delmonte OM, Villa A, Notarangelo LD. Immune dysregulation in patients with RAG deficiency and other forms of combined immune deficiency. Blood. 2020;135(9):610-9.
37. Rowe JH, Stadinski BD, Henderson LA, Ott de Bruin L, Delmonte O, Lee YN, et al. Abnormalities of T-cell receptor repertoire in CD4(+) regulatory and conventional T cells in patients with RAG mutations: Implications for autoimmunity. J Allergy Clin Immunol. 2017;140(6):1739-43 e7.

38. Lee YN, Frugoni F, Dobbs K, Tirosh I, Du L, Ververs FA, et al. Characterization of T and B cell repertoire diversity in patients with RAG deficiency. Sci Immunol. 2016;1(6).
39. Cassani B, Poliani PL, Moratto D, Sobacchi C, Marrella V, Imperatori L, et al. Defect of regulatory T cells in patients with Omenn syndrome. J Allergy Clin Immunol. 2010;125(1):209-16.
40. Perniola R. Twenty Years of AIRE. Front Immunol. 2018;9:98.
41. Daley SR, Koay HF, Dobbs K, Bosticardo M, Wirasinha RC, Pala F, et al. Cysteine and hydrophobic residues in CDR3 serve as distinct T-cell self-reactivity indices. J Allergy Clin Immun. 2019;144(1):333-6.
42. Lum SH, Elfeky R, Achini FR, Margarit-Soler A, Cinicola B, Perez-Heras I, et al. Outcome of Non-hematological Autoimmunity After Hematopoietic Cell Transplantation in Children with Primary Immunodeficiency. J Clin Immunol. 2021;41(1):171-84.
43. Lum SH, Selvarajah S, Deya-Martinez A, McNaughton P, Sobh A, Waugh S, et al. Outcome of autoimmune cytopenia after hematopoietic cell transplantation in primary immunodeficiency. J Allergy Clin Immunol. 2020;146(2):406-16.
44. Takahama Y. mTEC damage risks immune recovery. J Exp Med. 2022;219(2).
45. Cavazzana-Calvo M, Carlier F, Le Deist F, Morillon E, Taupin P, Gautier D, et al. Long-term T-cell reconstitution after hematopoietic stem-cell transplantation in primary T-cell-immunodeficient patients is associated with myeloid chimerism and possibly the primary disease phenotype. Blood. 2007;109(10):4575-81.