

## **Hematopoietic cell transplantation for treatment of primary immune deficiencies**

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### **Abstract**

Hematopoietic cell transplantation (HCT) has the potential to cure primary immune deficiency syndromes (PIDS) that are a group of disorders primarily affecting a single lineage, e.g., lymphoid or myeloid lineage. Generally, implementation of various conditioning regimens depends the type of IDS. Some syndromes that cause profound immune deficiency may not require a conditioning regimen. There appears to be a barrier even in cases of severe combined immune deficiency (SCID), particularly in the situation of HLA mismatched or haploidentical grafts. For example, donor B cell chimerism is less likely in  $\gamma$ -chain deficiency (X-SCID), as host cells persistently occupy the B lymphocyte niche, than in syndromes without B cells such as adenosine deaminase (ADA) deficiency. The immune defect may be corrected by partial reconstitution of normal immune cells, in other words full donor chimerism of the affected cell subset may not be required. This concept may add further rationale to limiting the intensity of the conditioning regimen.

SCID encompasses a broad range of inherited defects that individually cause a profound immune deficiency of both T and B cell function. The individual genetic defects give rise to various phenotypes, and, since the goal of HCT is to restore both T and B cell function, the SCID phenotype must be taken into consideration in addition to the degree of recipient-donor mismatch. Other biologic factors associated with the SCID phenotype may influence the barrier to engraftment, such as host NK cells, which may survive intensive conditioning regimens. One of the difficulties in analyzing outcome of HCT in SCID patients is the relative rarity of the condition, thus needing large multicentric studies. Recent studies show that the most important factor for improved survival after an HLA-identical sibling graft was younger age at time of HCT. Factors significantly associated with improved survival after haploidentical transplants were B+ SCID phenotype, protected environment, and lack of pulmonary infections before HCT. The advent of neonatal screening and in utero diagnosis has allowed early detection of SCID and therefore prompt intervention at an early age.

Primary T cell immunodeficiency (PTCD) syndromes may be differentiated from SCID by virtue of reduced but not completely absent T cell function, or absent T cell function with the presence of B lymphocyte or NK cell function. Allogeneic marrow transplantation remains the only curative therapy available for these disorders. Worse outcomes were seen in patients with PTCD compared to other types of immune deficiencies, regardless of donor. Although life-threatening infections may be less common early in life, children with PTCD often develop organ damage from chronic infections, particularly lung disease, prior to HCT.

In Wiskott-Aldrich syndrome, HCT offers significantly improved survival chances for patients. Achieving full donor chimerism was shown to be a favorable factor. In general, however, the studies suggest that low intensity regimens offer the potential for achieving donor cell engraftment with less morbidity than standard regimens, an important consideration for patients who currently may consider the risks of conventional transplants unacceptably high.

**Keywords:** Primary immune deficiencies, SCID, primary T cell deficiencies, hematopoietic stem cell transplantation, conditioning regimens, outcomes

## General principles of hematopoietic cell transplantation for primary immune deficiency diseases

The primitive hematopoietic stem cell (HSC) has the capability for self-renewal and differentiation, characteristics that allow transplantation of small numbers of HSC sufficient for complete restoration of the hematopoietic system of another individual. Transplanted HSC ultimately will differentiate into multiple lineages, including erythrocyte, monocyte/macrophage, granulocyte, megakaryocyte, and lymphoid cells. Thus hematopoietic cell transplantation (HCT) has the potential to cure disorders resulting from defects in the pluripotent progenitor cells as well as defects in single hematopoietic lineages. Primary immune deficiency syndromes are a group of disorders that primarily affect a single lineage, e.g., lymphoid or myeloid lineage, and can be cured with HCT. The goal of HCT for treatment of most primary immune deficiency disorders is to restore sufficient numbers of normal donor cells in the affected lineage(s); donor reconstitution of an unaffected lineage is not required for cure of the disease.

The barrier to successful allogeneic HCT is determined by differences in major or minor histocompatibility antigens between donor and recipient, resulting in bi-directional immunologically mediated graft-vs.-host (GVH) and host-vs.-graft (HVG) reactions. The barrier to engraftment is further determined by the capacity of host immune cells to generate a response to alloantigens. In addition, it has been postulated that host cell occupancy of a specific hematopoietic cell niche functions as a “space-occupying” barrier to engraftment. The strength of the opposing GVH reaction is determined by the number of alloreactive T cells in the graft. The conventional strategy to overcome this bi-directional barrier has relied upon three elements: first, elimination of host alloreactivity with agents capable of immunoablation and myeloablation; second, infusion of donor HSCs to rescue the patient from lethal myeloablation; and third, control of donor alloreactivity with post-transplant immune suppression or by elimination of T cells from the donor graft.

When these general principles are applied to the treatment of specific primary immune deficiency disorders, several distinct concepts emerge. First, syndromes that cause profound immune deficiency may not require a conditioning regimen, as there is no immunological resistance to engraftment. That said, there appears to be a barrier even in cases of severe combined immune deficiency (SCID), particularly when the MHC barrier is increased, i.e. in the situation of HLA-mismatched or haploidentical grafts. For example, donor B cell chimerism is less likely in  $\gamma$ -chain deficiency (X-SCID), as host cells persistently occupy the B lymphocyte niche, than in syndromes without B cells such as adenosine deaminase (ADA) deficiency. Accordingly, the intensity of the conditioning regimen generally is determined not only by the degree of human leukocyte antigen (HLA) disparity, but also by the magnitude of T cell deficiency and the occupancy of niches thought to be required for engraftment by host cells. Wiskott-Aldrich syndrome (WAS) exemplifies this concept in

a different way: a successful allograft must generate normal platelets as well as functional T cells; hence the conditioning regimen must provide some degree of myeloablation. Second, the immune defect may be corrected by partial reconstitution of normal immune cells — in other words full donor chimerism of the affected cell subset may not be required. A natural model is the absence of immune deficiency in most female carriers of X-linked immune deficiencies. This concept may add further justification for limiting the intensity of the conditioning regimen. Third, there is no potential benefit for GVH alloreactivity among patients with an immune deficiency, in contrast to patients with hematological malignancy in whom a graft-vs.-leukemia effect might be advantageous. Thus HCT protocols generally employ intensive post-grafting immune suppression or deplete alloreactive T cells from the graft, so as to minimize the risk of GVH disease (GVHD). The strong preference to avoid GVHD also forms the basis for the general disinclination to transplant peripheral blood stem cells, which contain approximately 10 times the number of T cells in the graft compared to bone marrow. The potential risk for life-threatening GVHD often enters into the determination of whether HCT should be undertaken in a specific patient, and the degree of disparate HLA or minor histocompatibility antigens among available donors must be balanced against the effectiveness of alternative therapies, such as enzyme replacement, intravenous immune globulin, prophylactic antibiotics, and other medical treatments.

### Severe combined immune deficiency (SCID)

SCID encompasses a broad range of inherited defects that individually cause a profound immune deficiency of both T and B cell function. The individual genetic defects give rise to various phenotypes in which the lymphocyte subsets may or may not be present, and if present, may or may not be functional, and which may pose a barrier to engraftment (Table 1) [1].

Lymphocyte phenotype			Mutation or deficiency
T	B	NK	
-	-	-	Adenosine deaminase
-	-	+	<i>RAG1</i> or <i>RAG2</i>
-	-	+	Artemis
-	+	-	Common $\gamma$ chain
-	+	-	<i>Jak3</i>
-	+	+/-	CD45 deficiency
-	+	+	IL-7R $\alpha$ -chain
-	+	+	CD3 $\gamma$ /CD3 $\epsilon$ /CD3 $\zeta$ deficiency

**Table 1.** Lymphocyte phenotype in the SCID syndromes

Since the goal of HCT is to restore both T and B cell function, the SCID phenotype must be taken into consideration in addition to the degree of recipient-donor mismatch. As mentioned above, elimination of occupancy of a B cell niche may require conditioning, particularly with haploidentical grafts; alternatively, establishment of “split” mixed chimerism (donor T / host B cells) may be sufficient to ameliorate the T cell dysfunction and allow the X-SCID patient to be supported with IVIG, similar to a patient with agammaglobulinemia. Lui et

al recently showed that resistance to B cell engraftment in B+ SCID appears to be at the level of the pro-B cell, thus suggesting a potential role for targeting early B cell progenitors in lieu of high intensity conditioning [2].

Other biological factors associated with the SCID phenotype may influence the barrier to engraftment. NK cells, which are relatively radio resistant and may survive intensive conditioning regimens, have been shown to mediate graft rejection [3,4]. The Perugia team has shown that donor NK alloreactivity is enhanced in the absence of T cells in the graft. Although this concept has been exploited to engineer donor grafts with enhanced anti-host NK activity, it may have implications for the reciprocal situation where T cells are lacking in the host. Particularly in the situation where the donor lacks inhibitory ligands, NK cells theoretically could pose an additional immune barrier to engraftment into a patient with SCID. This concept is supported by a murine model of Artemis deficiency, wherein NK-mediated graft rejection has been observed [5]. Another potential barrier is the presence of maternal T cells that have “engrafted” in the fetus in utero, often seen in patients with X-SCID. Maternal T cells can be detected using the standard methods for assessing chimerism, such as sex-chromosome specific fluorescent in situ hybridization (FISH) probes or polymerase chain reaction (PCR) detection of maternal-specific genetic polymorphisms, termed variable number tandem repeat (VNTR) sequences [6,7]. High-levels of maternal T cells have been associated with resistance to engraftment of haploidentical cells when no conditioning is given [8,9]. Thus, it is reasonable to consider use of a conditioning regimen in SCID patients with NK cells or maternal T cells. In the absence of a conditioning regimen, some reports have suggested that very large dose CD34+-selected peripheral blood stem cell (PBSC) grafts may help overcome graft rejection, although it may not facilitate B cell reconstitution [10].

One of the difficulties in analyzing the outcome of HCT in SCID patients is the relative rarity of the condition, which prohibits conducting large single center studies of a specific modality over a short period of time. Most reports with large numbers of patients represent either retrospective analyses of registry data, or single center studies conducted over a long period of time. Studies confined to a single phenotype or pilot studies of novel approaches generally have too few patients for meaningful assessment. The largest series of analyses has been reported by the European cooperative groups. In 1990 a retrospective study included 183 SCID patients given marrow transplants [11]. Among the 70 patients given grafts from HLA-identical siblings, 65 of whom were not given a preparative regimen, long term survival was 70%, and the majority of patients achieved stable engraftment of T and B lymphocytes. Factors that correlated with improved chances of survival included lack of infection prior to HCT and isolation of the patient in a protective environment. Clinical improvement was observed in all patients, including those with partial donor cell engraftment. In contrast to these results, less than 60% of patients given haploidentical grafts survived. The analysis definitively showed the important role of conditioning in establishing donor engraftment of T-depleted HLA-mismatched haploidentical marrow. Graft failure oc-

curred in 50% of unconditioned patients compared to 14% of conditioned patients, and the rate of engraftment improved proportionately with increased intensity of the regimen. Immunological recovery, including B cell recovery, was also facilitated by the preparative regimen, and the frequency of late deaths related to poor immunological reconstitution was reduced. However, the benefit of a preparative regimen for establishing donor cell engraftment was negated by an increase in regimen-related complications and did not improve overall survival.

Over time, the outcome of HCT for SCID has improved, as novel supportive care and alternative donor sources have been used. Survival for recipients of HLA-identical sibling grafts now approaches 90%, and in the 2003 retrospective study by the European Group for Blood and Marrow Transplantation (EBMT), outcome for recipients of HLA-phenotypically identical grafts was not statistically significantly different from that for genotypically HLA-identical donors [12]. This analysis also showed a significant improvement over time for recipients of T-depleted haploidentical grafts, from approximately 60% to almost 80% survival ( $p=.0007$ ) by the late 1990s. The most important factor for improved survival after an HLA-identical sibling graft was younger age at time of HCT, with a 2-fold increase in risk for patients aged 6–12 years, and an 8-fold increase in risk for patients >12 years when compared with patients given HCT before 6 years of age. Factors significantly associated with improved survival after haploidentical transplants were B+ SCID phenotype (64% vs. 36%,  $p=.0007$ ), protected environment (57% vs. 15%,  $p=.0001$ ), and lack of pulmonary infections before HCT (59% vs. 38%,  $p=.0001$ ). Use of a conditioning regimen was associated with improved survival for B- SCID patients, although the difference was not statistically significant. More recently, Fischer and colleagues analyzed a large cohort of SCID patients to determine factors that correlated with good clinical outcomes (survival and amelioration of clinical immune deficiency) [13]. Graft source was significant, and risk for poor outcome was 3.7-fold higher for recipients of haploidentical and 4.8-fold higher for phenotypically HLA-identical (related or unrelated) compared to HLA-identical sibling grafts. Establishment of donor myeloid cells correlated with better survival, presumably because donor chimerism of the myeloid lineage correlated with donor origin of lymphocytes. Lack of CD4+ cell reconstitution and persistent need for IVIG were both significantly associated with poor outcome. Finally, the type of SCID appears to affect outcome, as specifically those with Artemis mutations have a 6-fold higher risk for poor outcome. These patients had a higher incidence of chronic papilloma virus infections, malnutrition, and chronic GVHD. Other studies indicate that survival generally is higher among B+ compared to B- SCID patients, particularly after alternative donor HCT [14].

Most other centers report comparable results, although in smaller series. The general experience is that genotypically HLA-identical marrow transplantation restores T cell immunity in >90% of unconditioned SCID patients, although B cell reconstitution occurs in only half of these patients [8,15]. Despite the use of preparative regimens, recipients of T-depleted haploidentical marrow have delayed immune reconstitution,

with 3–6 months required for development of antigen responsive T cells, and commonly multiple marrow infusions are needed [16]. B lymphocyte reconstitution is generally sub-optimal and most patients require continued immunoglobulin support. The EBMT analysis found long-term functional T cells in about 80% of recipients of HLA-sibling grafts and 90% of B+ SCID recipients of haploidentical grafts; in contrast, functional T cells were observed in only 66% of B-SCID patients ( $p=.002$ ). Long-term B cell function was less robust in all cohorts: 88% of B+ and 63% of B- SCID recipients of HLA-identical sibling grafts, and 66% of B+ and 44% of B- SCID recipients of haploidentical grafts, respectively. Certain SCID phenotypes may require a preparative regimen, for example patients with reticular dysgenesis given T-depleted grafts appear to have improved outcome if given high intensity conditioning with subsequent reconstitution of normal myeloid cells [17]. B- SCID patients also appear to benefit from conditioning [14].

The role of conditioning for unrelated grafts is the same as for T-depleted haploidentical grafts, in that it may facilitate establishment of donor B lymphocyte chimerism, however, that benefit may be offset by regimen-related toxicities. In 1992 Filipovich and colleagues reported their initial experience with unrelated marrow grafts for 8 SCID patients, of whom 5 were given high intensity conditioning [18]. Graft rejection occurred in 2 of the 3 unconditioned patients, although both survived long term after a second transplant with high intensity conditioning. Among the conditioned patients, 2 died within the first 100 days after HCT, while the others survived long term with improved immune function. Subsequently larger series have shown excellent outcome after high intensity conditioning followed by HLA-matched unrelated grafts, with about 80% survival [19,20]. Compared to haploidentical recipients, unrelated marrow recipients had significantly lower risk for interstitial pneumonitis, graft failure, acute and chronic GVHD, and abnormal T cell receptor diversity [19]. Thus, immune reconstitution after T-replete unrelated donor transplantation appears to be faster compared to T-depleted haploidentical HCT, and often normal B cell function is achieved.

Outcomes for unrelated umbilical cord blood have been reported in small series and appears equivalent to that of matched unrelated marrow. Bhattacharya et al reported success in establishing well-matched (6/6 or 10/10 HLA match) umbilical cord blood grafts without conditioning [21]. The median time to achieving an absolute T cell count of more than 200 cells/ml among 5 surviving SCID patients was about 60 days, and mixed or full donor B cell engraftment was observed in 4 of these. Diaz reported 11 SCID patients, 9 given high intensity and 2 given reduced intensity conditioning, 7 and 1 of these, respectively, survive long term [22]. High intensity conditioning generally has been employed in recipients of HLA-mismatched cord blood units, with comparable results [20].

The condition of the patient before HCT correlates strongly with the risk of death after HCT irrespective of the graft source. In particular, infections and pulmonary disease are associated with significantly worse outcomes [12,14,23]. The most common cause of death in the first 6 months after HCT

is infection, thus it is not surprising that pre-existing infection is detrimental to success. Indeed, a pre-existing pulmonary infection confers a 2-to 3-fold increased risk of death following HCT [14]. Therefore, every patient with SCID should have a thorough evaluation for infection and occult pulmonary disease. Liver enzyme elevations may indicate maternal T cell-mediated GVHD or underlying liver dysfunction, the latter is commonly associated with untreated ADA deficiency. Enzyme replacement therapy with polyethylene glycol (PEG) conjugated adenosine deaminase improves T cell function [24]. Since functional T cells pose an immunological barrier to engraftment, the general practice has been to stop PEG-ADA several weeks before HCT in order to “T-deplete” the recipient. However PEG-ADA also prevents or improves the liver injury found in ADA deficiency, thus, early stopping may result in hepatitis and increase the risk for post-transplant liver toxicity during the time required to establish endogenous enzyme production. A reasonable approach is to continue PEG-ADA unless no conditioning is contemplated, in which case pre-transplant discontinuation is warranted. Age of the patient is another important factor associated with survival. The best outcomes are reported for SCID patients given allogeneic transplant within the first year of life [12]. Older patients are more likely to have infections and organ dysfunction that contribute to higher mortality.

The advent of neonatal screening and in utero diagnosis has allowed early detection of SCID and therefore prompt intervention at an early age. Buckley and colleagues reported better survival and the establishment of long-term functional T cells in patients with SCID who received HCT before 28 days of life (overall survival ((OS) 95%) compared to patients who were older (OS 74%) [25]. Survival of 21 SCID infants, given no conditioning and transplanted with haploidentical T cell depleted marrow grafts before 28 days of age (neonatal cohort), was 95% compared to 74% of 96 older infants. Thymopoiesis, as measured by T cell receptor excision circles (TREC), was improved in the neonatal transplant cohort, however, no comparative improvement in B cell function was observed. Acute GVHD grades III–IV developed in only 16% of patients who received grafts mismatched for 2 or 3 HLA antigens. Several factors probably contributed to the improved survival observed in patients less than 28 days old. In particular, younger patients are less likely to have opportunistic infections and subsequent co-morbidities, malnutrition, and failure to thrive, all of which have been associated with increased mortality following HCT, but may also contribute to decreased thymic function. These results show that T cell depleted haploidentical marrow grafts are feasible at a very early age and that there is little benefit in delaying HCT in order to identify a better matched donor.

Once established, donor progenitor cells develop and mature in the vestigial thymus of the SCID patient. Buckley and colleagues demonstrated that T cell receptor (TCR) gene rearrangement occurs in donor T cell precursors resulting in the generation of naïve T cells [25,26,27]. Measurements of TCR repertoire show development of a broad diversity within the donor T cell pool, which persists over time. Patel and colleagues reported that thymopoiesis, as measured by TRECs and naïve CD4 cells, declined exponentially over time after

HCT. Several studies have suggested that late graft failure or deterioration of thymopoiesis may jeopardize long-term T cell function [28]. In contrast, recent studies have shown that thymic output of T cells and T cell diversity remain normal for decades after HCT in approximately 80% of patients [29,30]. The degree of early T cell reconstitution appears to be strongly associated with the long-term stability of T cell function, thus, patients at most risk for poor T cell function or loss of T cell grafts are those with low numbers of TRECs and naïve-CD4 cells within the first few years after HCT [13,31]. Alain Fischer's team in Paris found that donor chimerism of the myeloid lineage correlated with higher CD4+ T cell counts long term, and that donor myeloid chimerism was limited to patients who received high intensity conditioning. Other factors that may be associated with higher risk for poor long-term T cell reconstitution are SCID phenotypes that lack B cells, presence of NK cells, and possibly young age at time of HCT. As noted above, long term B cell reconstitution is common only among patients given high intensity conditioning regimens [13,15].

### Primary T cell immunodeficiencies other than SCID

Primary T cell immunodeficiency (PTCD) syndromes may be differentiated from SCID by virtue of reduced but not completely absent T cell function, or absent T cell function with the presence of B lymphocyte or NK cell function. Nonetheless, the immune dysfunction leads to progressive decline from opportunistic infections, autoimmune phenomena, and a propensity to develop malignancies, particularly lymphoma. Several of the genetic mutations implicated in SCID also have been found to be present in patients with PTCD, thus termed "leaky SCID". New genetic mutations also have been characterized, while the genetic causes in many patients remain unrecognized [32,33,34]. The more common of these rare disorders are listed in Table 2.

Until gene therapy is perfected, allogeneic marrow transplantation remains the only curative therapy available for these disorders. In general, conventional regimens have included both immunoablative and myeloablative agents to overcome

Syndrome	Mutation	Clinical features	Laboratory features
Omenn's	<i>RAG1 or RAG2</i>	HSM Rash Adenopathy	Eosinophilia Lymphocytosis
ZAP70	<i>ZAP70</i>		CD8+ lymphopenia High IgE levels
HIGM	CD40L or CD40	X-linked Pyogenic infection Sclerosing cholangitis	High IgM & low IgG levels
Common Variable Immunodeficiency	Unknown	Autoimmunity Allergy	Low IgG
Cartilage Hair Hypoplasia	endoribonuclease RMRP	Dwarfism Hypoplastic hair growth	
Wiskott Aldrich	<i>WASP</i>	Eczema	Thrombocytopenia Small platelet size
IPEX	<i>FOXP3</i>	X-linked Diabetes Diarrhea Rash	
XLP	<i>SH2D1A</i>	X-linked EBV- lymphoproliferative disease	Hypogammaglobulinemia
Bare Lymphocyte syndrome (Class II HLA deficiency)	<i>RFXANK, RFX5, RFSAP, CIITA</i>	Pulmonary infection Diarrhea Hepatitis	Hypogammaglobulinemia CD4 lymphopenia Absent antigen-specific T cell responses
Class I HLA deficiency	<i>TAP1 or TAP2</i>		CD8 lymphopenia
DiGeorge syndrome	10p13 or 22q11.2	Hypocalcemia Cardiac anomalies	CD3 lymphopenia Elevated IgE

**Table 2.** Primary T cell immunodeficiency syndromes commonly referred for HCT

Abbreviations:

HIGM, Hyper IgM;

HLA, Human leukocyte antigen;

HSM, hepatosplenomegaly;

IPEX, Immunodeficiency-polyendocrinopathy-enteropathy X-linked;

XLP, X-linked lymphoproliferative

residual immune barriers to engraftment and to ensure multilineage chimerism. The combination of busulfan (1 mg/kg x 16 doses) and cyclophosphamide (200 mg/kg total dose), with or without anti-thymocyte globulin is the most commonly used regimen. Our experience indicates that busulfan is metabolized faster in young patients, therefore in order to optimize engraftment it is prudent to adjust the daily doses to achieve steady state concentration (C<sub>ss</sub>) of >200 ng/ml in recipients of HLA-identical sibling grafts and >400 ng/ml in recipients of HLA-matched unrelated grafts [35].

In 2003 the EBMT reported a retrospective analysis of outcome for patients with primary immunodeficiency disease other than SCID [12,36]. A higher incidence of engraftment was reported in patients who received HLA-identical sibling grafts (99%), compared to those who received haploidentical or unrelated donor grafts (75–80%). In addition, survival at 3 years was better for patients who received HLA-identical sibling grafts (71%) compared to haploidentical grafts [42% (p=.0006)]. Survival after HLA-matched unrelated donor grafts was 59%, which was not statistically significantly different from the HLA-identical sibling cohort. The main cause of death following HCT was infection.

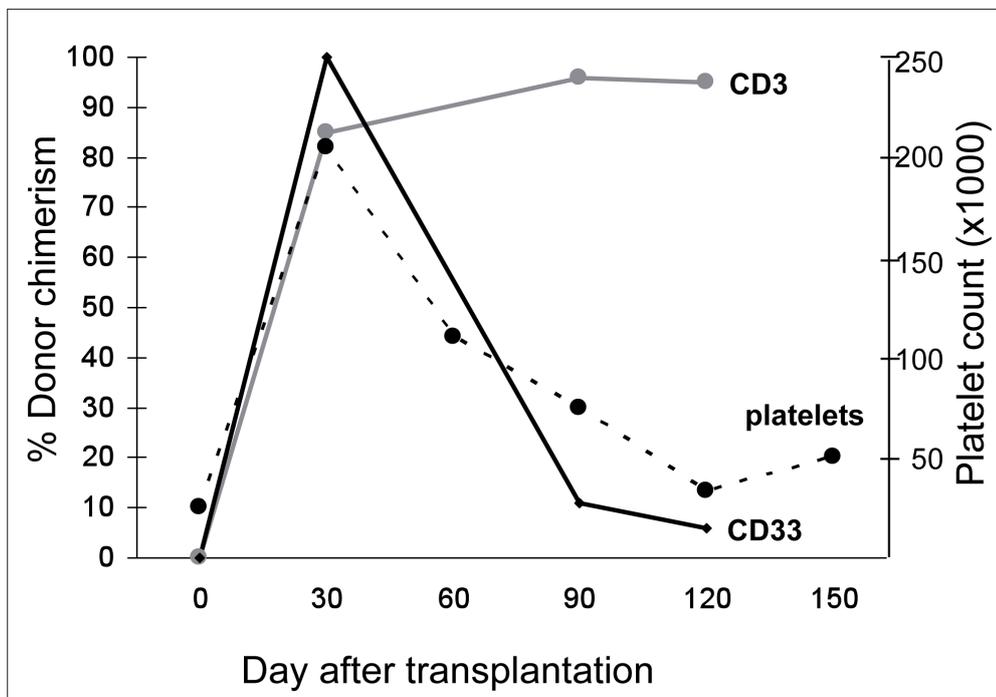
Worse outcomes were seen in patients with PTCO compared to other types of immune deficiencies, regardless of donor. Overall survival was 63% and 35% after HLA-identical sibling grafts or mismatched grafts, respectively [12,36]. Unfortunately, and in contrast to the results for SCID, the EBMT study found no improvement in survival of the non-SCID cohorts over the two decades included in the analysis, thus highlighting important differences and problems associated with PTCO compared to SCID. Although life-threatening infections may be less common early in life, children with PTCO often develop organ damage from chronic infections, particularly lung disease, prior to HCT. Given that a conditioning regimen is necessary to ensure engraftment, the associated risks for organ toxicity, hemorrhage, and infection are compounded by these co-morbidities. Several studies have associated inferior survival with presence of co-morbidities, including opportunistic infections, Epstein-Barr virus lymphoproliferation, and pulmonary dysfunction [8,23]. Among boys with X-linked hyper-IgM (XHIM) excessive mortality has been associated with the presence of pulmonary infection or liver disease at time of HCT [37].

In theory, split chimerism (donor T/host B chimerism) or partial donor T cell chimerism should be sufficient to ameliorate the immune dysfunction in most types of PTCO. Accordingly, reducing the intensity of conditioning may be a strategy to reduce regimen-related toxicity without sacrificing immune reconstitution. Specific factors associated with the molecular defect must be taken into consideration, as partial chimerism may not be sufficient for some defects. DiGeorge syndrome and the Bare Lymphocyte syndromes (BLS) pose particular challenges, because the T cell deficiency results from inadequate thymopoiesis, caused by absence of the thymus or absence of HLA molecules on the thymic epithelial cells, respectively. The molecular defects that cause BLS block the transcription of HLA genes; therefore antigen-presenting cells also are defective. Hence, donor chimerism must

be established in B cell and dendritic cell lineages. In both disorders, absence of thymic function may prevent formation of naïve donor T cells; therefore full chimerism provides the most likely chance that a broad range of donor antigen-specific memory cells may be established long term.

Molecular deficits that allow T cell proliferation, but preclude intercellular interactions, such as X-linked hyper-IgM (XHIM) syndrome or Immune Dysregulation-Polyendocrinopathy-Enteropathy X-linked (IPEX) syndrome, generally require conditioning in order to prevent T cell mediated graft rejection; however, partial T cell chimerism appears to restore immune function as well as full donor chimerism [38,39]. The autoimmune manifestations of the IPEX syndrome are caused by absence of CD4+CD25+ FOXP3+ regulatory T cells (Tregs), important for sustaining self-tolerance. Normal numbers of FOXP3 expressing CD4+CD25+ cells have been observed in boys with mixed donor/host chimerism, and several case reports indicate that mixed chimerism is sufficient to ameliorate many of the manifestations of IPEX, including the enteropathy, anemia, failure to thrive, and the susceptibility to infections. Curiously, we and others have observed improvement in diabetes, although in theory autoimmune-mediated islet cell destruction happens well before HCT is carried out [38]. The most common cause of the XHIM syndrome is a mutation in the CD40 ligand gene, which is expressed by T cells and provides a critical signal for B cells to switch antibody production from IgM to IgG subtypes. Theoretically, the introduction of some normal CD40 ligand-expressing T cells should be sufficient to reverse the disease, even if B cells of host origin remain. Amelioration of disease symptoms with mixed chimerism has been observed for some boys treated for XHIM, although no detailed analysis of B cell function was reported [40,41].

*Wiskott-Aldrich Syndrome (WAS)*. HCT offers significantly improved survival chances for patients with WAS, without which about 50% will die from infection, autoimmune disease, or lymphoproliferative disease by the third decade of life [42]. The WAS mutation affects lymphoid and hematopoietic compartments, both of which are corrected by HCT. Accordingly most patients are conditioned with both immunosuppression and high dose chemotherapy to facilitate multilineage donor cell engraftment. Figure 1 shows cell counts in a patient who was too ill with opportunistic infections to receive high dose conditioning and became a split chimera after reduced intensity conditioning and unrelated marrow transplantation. Following transplantation the T cell immune defect was corrected, but thrombocytopenia persisted as did host CD33+ cells. After the infections resolved, a second graft was given with a high intensity (directed at myeloid cells), but without immunoablative preparative regimen, resulting in amelioration of thrombocytopenia as the CD33+ compartment converted to donor type. The importance of achieving full donor chimerism was shown recently in a retrospective analysis of results in 96 patients, which showed an almost 2-fold reduction in mortality among patients with full chimerism compared to those with mixed or split chimerism [43]. Mixed chimerism also is associated with a significantly higher risk for developing autoimmune manifestations after HCT.



**Figure 1.** Correction of thrombocytopenia in Wiskott-Aldrich correlates with donor chimerism of CD33+ cell subset

At 30 days after hematopoietic cell transplant, a high proportion of both CD3 (gray line) and CD33 (black line) subsets mark as donor origin. Over time, the proportion of donor CD3 cells remains stable, while the proportion of donor CD33 cells decreases. The platelet count (black dashed line) reaches a normal level when the CD33 donor chimerism is high, and falls proportionately with the fall in donor CD33 chimerism.

HCT using HLA-identical sibling marrow grafts is highly successful in treating WAS, with approximately 88% event-free survival [43]. The combination of busulfan (1 mg/kg x 16 doses) and cyclophosphamide (200 mg/kg total dose), with or without anti-thymocyte globulin is the most commonly used conditioning regimen. Busulfan is metabolized faster in younger patients, therefore it is prudent to monitor levels and target the dose to achieve a C<sub>ss</sub> of greater than 200 ng/ml to assure engraftment [35]. An EBMT analysis determined that patient age, disease severity, and splenectomy, among other factors, did not affect outcome [43].

Results of alternative donor HCT for treatment of WAS have improved over time, particularly for recipients of unrelated marrow grafts. Most recent studies have shown approximately 70%–78% long-term survival [43,44]. In a 2001 study facilitated by the International Bone Marrow Transplant Registry (IBMTR), age of 5 years or older was associated with an increased risk of mortality after HCT. Survival for patients younger than 5 years was about the same as for recipients of HLA-identical sibling grafts. In addition, several studies found comparable survival rates among recipients of HLA-matched related and unrelated grafts [44,45]. The most commonly used regimen was a combination of busulfan, cyclophosphamide, and ATG. In our experience, targeting the dose of busulfan to achieve a C<sub>SS</sub> above 400 ng/ml facilitates engraftment [35].

Encouraging results have also been reported for umbilical cord blood transplants, but most information comes from case reports or part of smaller series of patients with immune deficiencies. Among 15 patients reported by Ozsahin, et al, event-free survival was approximately 70%, similar to recipients

of unrelated marrow grafts [44]. Most patients in these reports were conditioned with the combination of busulfan, cyclophosphamide, and antithymocyte globulin, which the Japanese group found to be an important factor for improved survival when compared to other regimens [44]. Reconstitution of immunity after cord blood approximates that observed with unrelated marrow grafts.

There is more information about haploidentical transplants for WAS, although the historical results are less encouraging. The EBMT study showed a 4–5-fold increase in mortality after haploidentical compared to HLA-identical sibling grafts [43]. Others have reported similar results [36,44,46]. These reports show a high incidence of graft failure and poor immune

reconstitution following T-depleted haploidentical marrow grafts. The Tübingen team provided a promising case report of “mega-dose” purified CD34+ haploidentical grafts used to overcome the barrier to engraftment without engendering GVHD [47].

### Inherited immune defects not primarily affecting T cells

A wide range of rare immune deficiency syndromes result from defects in B lymphocytes, NK cells, or nonlymphocytic subsets including neutrophils (Table 3).

The individual immune defects predispose the patients to specific infections, for example with *Aspergillus* in patients with chronic granulomatous disease (CGD) or *Staphylococcus aureus* in leukocyte adhesion deficiency (LAD)-I, and are associated with distinctive co-morbidities, such as infiltrative pulmonary or central nervous system disease in hemophagocytic lymphohistiocytosis (HLH) or recalcitrant colitis in CGD. As above, the decision to treat with HCT must take into consideration the prognosis of the disorder with available supportive care, the presence of co-morbidities that might increase the risk of mortality following HCT, and the availability of a suitable donor. Unfortunately, because these diseases are rare, meaningful prospective studies reporting on large series of patients with a single disease entity are not available. Within the European retrospective study, survival for specific subsets, 48 patients with phagocytic disorders, and 90 patients with hemophagocytic disorders, was reported to be approximately 70% and 68%, respectively, for recipients of HLA-identical sibling grafts. The small number of patients with phagocytic cell disorders had a similar outcome after

Syndrome	Mutation	Clinical features	Laboratory features
Histiocytic disorders			
FHL	<i>PRF1</i> <i>UNC13D</i> <i>STX11</i>	Fever Hepatosplenomegaly Lymphadenopathy Rash Pulmonary infiltrates Neurologic deficits	Pancytopenia Hypertriglyceridemia Hypofibrinogenemia Elevated liver function tests
Multifocal LCH	Unknown	Bone lesions Endocrinopathy Rash Hepatosplenomegaly	Anemia
Myeloid disorders			
Chronic Granulomatous Disease	<i>p91PHOX</i> <i>CYBA</i> <i>NCF1</i>	Pneumonia Cellulitis	Catalase-positive bacteria Aspergillus
Chediak-Higashi Syndrome	<i>CHSI</i>	Pyogenic infections Gingivitis Oculocutaneous albinism Hemorrhage	Neutropenia Giant intracellular granules
Leukocyte Adhesion defect	$\beta$ -2 integrin	Bacterial infections Stomatitis	Leukocytosis
Kostmann's neutropenia	GCSF-R	Bacteremia/sepsis	Neutropenia
Shwachman-Diamond Syndrome	Unknown	Pancreatic enzyme deficiency	Neutropenia

**Table 3.** Histiocytic and myeloid immune deficiency syndromes commonly referred for HCT

Abbreviations:

FHL, Familial hemophagocytic lymphohistiocytosis;

LCH, Langerhans cell histiocytosis

transplantation of unrelated marrow, in contrast to patients with hemophagocytic disorders in whom survival was only 28% after unrelated grafts.

Studies that include larger numbers of patients with single diagnoses are limited to a few disorders such as CGD and HLH. The prognosis for CGD patients has improved considerably with more aggressive use of gamma-interferon and antibiotic prophylaxis. HCT is indicated for patients with recurrent life-threatening infections or organ dysfunction caused by refractory granulomatous disease [48]. A series of patients transplanted with HLA-identical sibling marrow (n=10) or unrelated marrow or cord blood (n=10) was reported recently from the United Kingdom [49]. Most patients were conditioned with busulfan and cyclophosphamide, and alemtuzumab was given to recipients of unrelated grafts. Survival was reported to be 90% with a median follow-up of 61 months; one patient in each group died from disseminated infection. Neutrophil oxidative burst was normalized in patients with mixed as well as full donor chimerism. Del Giudice et al published a review of case reports and small series of patients with CGD that included several long-term survivors with stable mixed chimerism, consistent with the notion that a small proportion of normal cells improves disease symptoms [50].

Without HCT, the prognosis for patients with HLH is poor even with intensive supportive care and anti-inflammatory regimens that include etoposide, cyclosporine, and prednisone [51,52]. The outcome is considerably improved when HCT

is performed after control of the initial inflammatory state is gained and infiltrative disease has resolved. The HLH 94 protocol reported 50–60% overall survival for the 113 children entered into the study; overall survival was 65% for the subgroup of 65 children given HCT. The study results suggested that intrathecal chemotherapy plays an important role in gaining disease control [52]. A retrospective analysis of 91 patients in the CIBMTR registry given unrelated marrow grafts found 53% overall survival [53]. Most patients were conditioned with busulfan, cyclophosphamide, and etoposide, with or without ATG. Survival was worse among the small subgroup with active disease at time of HCT, as well as among recipients of HLA-mismatched grafts. Disease manifestations resolved among patients with mixed as well as full donor chimerism. Early mortality (before day 100) was 32%, however, suggesting that a reduced intensity conditioning regimen might be of benefit.

### Development of reduced toxicity regimens

Conditioning regimens that do not employ agents at doses resulting in long-lasting marrow aplasia are referred to as reduced intensity conditioning (RIC) regimens. Until recently, those regimens have been used routinely for only two conditions: severe aplastic anemia and SCID. Regimens for aplastic anemia have included immunosuppressive agents alone to overcome the allo-immune rejection responses, since these patients are thought to have „unoccupied“ marrow space. These reduced intensity regimens have resulted in a markedly

lower incidence of both early and late complications [54,55]. SCID patients have no immune system capable of rejecting the graft, and therefore do not require conditioning except in the instances discussed above [11].

As the power of the graft vs. leukemia (GVL) effect became evident in the late 1970s and early 1980s, subsequent studies found that donor lymphocyte infusions (DLI) could be used to treat leukemic relapse after HCT [56,57]. The success of DLI set the stage for the introduction of reduced intensity conditioning for HCT, based on the hypothesis that the graft itself created the needed space through a subclinical GVH reaction directed toward recipient hematopoietic cells. Based on insights from animal models and armed with new potent immunosuppressive agents such as 2-CDA and fludarabine, investigators in Texas, Israel, Seattle, Boston, and Washington, DC pioneered less toxic regimens that facilitated partial or full chimerism in most patients [58,59,60,61,62,63,64].

These studies demonstrated that intensive immunosuppression alone following stem cell infusion was sufficient to establish full or partial donor chimerism and that conditioning was not required for creation of marrow space. The extent of donor cell engraftment following low intensity regimens depends on multiple factors, including the degree of intensity of the preparative regimen, the source of hematopoietic cells (marrow vs. peripheral blood stem cells), the degree of HLA-matching, and the extent of T cell depletion. Most low intensity protocols use PBSC to facilitate engraftment and enhance GVL reactions, as the product may contain 10-fold greater

numbers of T cells and 4-fold greater number of hematopoietic stem cells compared to marrow [65].

There are several reasons for the further development of low intensity regimens for the establishment of mixed chimerism in patients with Non-SCID primary immune deficiency disorders. First, the potential risks of high dose conditioning regimens include early treatment related mortality and late effects, such as infertility, hormonal dysfunction, growth failure, and secondary malignancies. These risks may deter patients and families from seeking treatment before co-morbidities arise. Second, as discussed earlier, the risk for regimen-related mortality increases significantly among patients with disseminated infection, pulmonary disease or other organ dysfunction. Third, reversal of disease symptoms with partial chimerism, which may be achievable with low intensity conditioning, has been demonstrated in a number of studies [36,66].

The main challenge in translating the success of protocols using RIC to patients with primary immune deficiency is the reliance on PBSC grafts, which may be difficult (or impossible) to collect from pediatric donors; also it confers a high risk of GVHD. Some progress has been made using marrow or CD34+ selected PBSC. Some results with HLA-matched related or unrelated marrow using RIC are summarized in Table 4.

A high rate of engraftment of marrow grafts has been reported after the combination of fludarabine and melphalan plus, an in vivo T-depleting agent, such as ATG or Campath®, and appears to be associated with low mortality rates [38,67,68,70]. The combination of cyclophosphamide, fludarabine, and

Author	Diagnosis	Regimen	Donor	Rejection (No.)	Survival (No.)	FU (mo)
<b>Reduced Intensity Conditioning</b>						
(Rao et al. 2005 [67])	27 PID/ 6 SCID	Flu/ Mel/Campath	BM	0	31	
(Amrolia et al. 2000 [68])	6 ID/ 2 SCID	Flu/ Mel/ ATG		0	7	6-18
(Rao et al. 2007 [38])	4 IPEX	Flu/ Mel/Campath	BM	0	4	6-25
(Cohen et al. 2007 [69])	7 PID/ EBV-LPD	Flu/ Mel/Campath	BM or PBSC	0	7	0
<b>Minimal Toxicity (non-myeloablative) Conditioning</b>						
(Horwitz et al. 2001 [70])	10 CGD	Flu/ Cy/ ATG Post-HCT DLI	CD34+ PBSC	1	7	16-26
(Burroughs et al. 2007 [71])	10 PID+SC-M	Flu / 2 Gy TBI	PBSC	1	7	9-96

**Table 4.** Intensity conditioning regimens and transplant outcome in immunodeficiency syndromes

Abbreviations:

ATG, anti-thymocyte globulin;

BM, bone marrow;

CB, cord blood;

CGD, chronic granulomatous disease;

DLI, donor lymphocyte infusion;

EBV-LPD, Epstein Barr virus lymphoproliferative disease;

Flu, fludarabine;

HCT, hematopoietic cell transplant;

IPEX, Immunodeficiency-polyendocrinopathy-enteropathy X-linked;

Mel, melphalan;

MSD, matched sibling donor;

PBSC, peripheral blood stem cells;

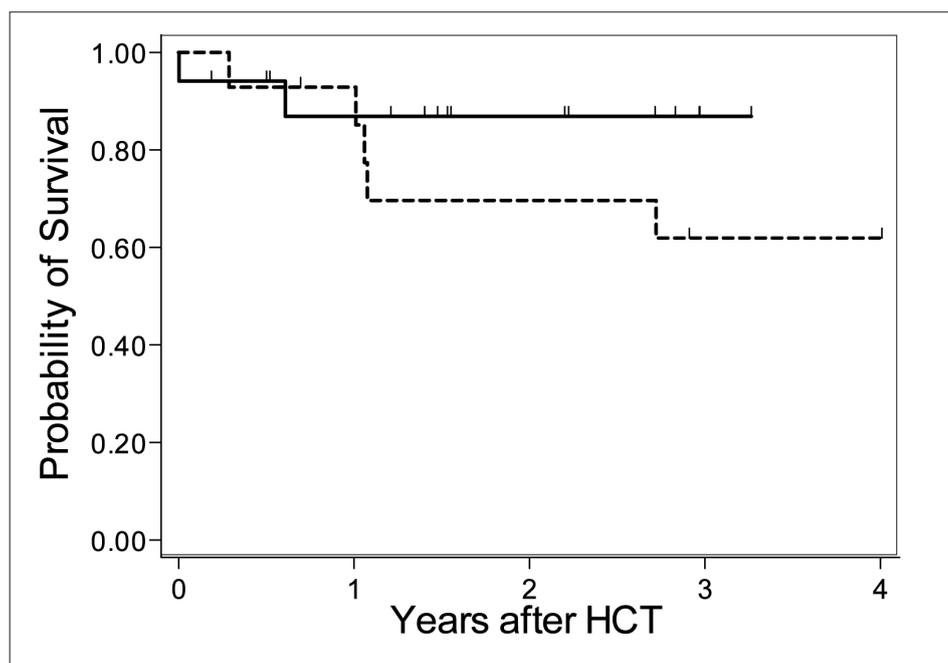
PID, primary immune deficiency (nonSCID);

SCID, severe combined immune deficiency;

SC-M, severe co-morbidities;

TBI, total body irradiation

ATG has been studied as a low-intensity regimen to facilitate engraftment of CD34+ selected PBSC [70]. In the latter study, the benefit of in vivo and ex vivo T-depletion for reducing GVHD was at least partially abrogated by the use of DLI to improve the level of donor cell chimerism. The Seattle group has studied the combination of fludarabine and low-dose TBI in patients who would be expected to have very poor survival following conventional conditioning for HCT, such as those with disseminated opportunistic infections, mechanical ventilation, or other organ damage. No regimen-related mortality was observed in the first cohort given 2 Gy TBI, however chronic GVHD was observed in 70% of patients, presumably related to the use of PBSC grafts [71]. The substitution of marrow for PBSC in the subsequent cohort appears promising, and early mortality has not been increased despite increasing the dose of TBI to 4 Gy (Figure 2).



**Figure 2.** Survival of patients with severe infections or pulmonary disease after reduced intensity conditioning HCT is improved with bone marrow as the sole source of allogeneic hematopoietic cells. The first cohort of patients (dashed line) was conditioned with fludarabine and 2 Gy total body irradiation and most patients received peripheral blood stem cell grafts. The second cohort (solid line) was conditioned with fludarabine and 4 Gy total body irradiation and was given marrow grafts.

Taken together, these studies suggest that low-intensity regimens offer the potential for achieving donor cell engraftment with less morbidity than standard regimens, an important consideration for patients who currently may consider the risks of conventional transplants unacceptably high.

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## Трансплантация гемопоэтических стволовых клеток для лечения первичных иммунодефицитов

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### Резюме

Трансплантация гемопоэтических клеток (ТГСК) является средством лечения первичных синдромов иммунодефицита (ПСИД), представляющих собой группу заболеваний, первично нарушающих один из ростков, например, лимфоидный или миелоидный. В целом, применение различных кондиционирующих режимов ТГСК зависит от типа ПСИД. Некоторые синдромы, вызывающие глубокий иммунодефицит, могут и не требовать кондиционирования. Возможно, однако, что существует иммунный барьер даже в случаях тяжелого комбинированного иммунодефицита (ТКИД), особенно в ситуации с расхождением по HLA или при гаплоидентичной ТГСК. Например, донорский В-клеточный химеризм менее вероятен при дефиците  $\gamma$ -цепей (X-сцепленный ТКИД), поскольку клетки больного занимают нишу В-клеток, нежели при синдромах без В-клеток (например при дефиците аденозиндезаминазы). Иммунный дефект может быть исправлен путем частичного восстановления нормальных иммунных клеток, другими словами – может и не потребоваться полный донорский химеризм поврежденной клеточной субпопуляции. Эта концепция может служить дальнейшим доводом в пользу ограничения интенсивности кондиционирующего режима.

ТКИД включает в себя широкий круг врожденных дефектов, которые в каждом случае приводят к глубокому иммунодефициту как Т-, так и В-клеточной функции. Индивидуальные генетические дефекты ведут к развитию разнообразных фенотипов, и, поскольку цель ТГСК состоит в восстановлении как Т-, так и В-клеточных функций, то фенотип ТКИД должен приниматься в расчет, наряду со степенью различий донора и реципиента. Другие биологические факторы, ассоциированные с фенотипом ТКИД могут влиять на барьер приживления, такие, как НК-клетки больного, которые могут выжимать после интенсивных кондиционирующих режимов.

Одной из проблем в анализе исходов ТГСК у больных ТКИД является относительная редкость этого заболевания, что требует больших многоцентровых программ. Недавние исследования показали, что наиболее важным фактором лучшего приживления после HLA-идентичной пересадки от сибса является более юный возраст в момент ТГСК. Факторами, существенно связанными с лучшим выживанием, после гаплоидентичных трансплантаций были: В+ фенотип больных ТКИД, защищенная (асептическая) среда обитания, и отсутствие легочных инфекций до ТГСК.

Внедрение неонатального скрининга и диагностика *in utero* позволили рано выявлять ТКИД и, тем самым, способствуют лечению в раннем возрасте.

Синдромы с первичным Т-клеточным иммунодефицитом (ПТКИД) могут быть дифференцированы от ТКИД по снижению, но не полному отсутствию Т-клеточной функции, или же по отсутствию Т-клеточной активности при наличии функций В-лимфоцитов или НК-клеток. Аллогенная пересадка костного мозга остается единственным исцеляющим методом лечения, доступным для таких заболеваний. Независимо от донорских факторов, у больных с ПТКИД наблюдаются худшие клинические исходы, по сравнению с другими типами ИДС. Хотя опасные для жизни инфекции могут быть в раннем возрасте менее частыми, у детей с ПТКИД часто развивается органная патология из-за хронических инфекций, в особенности болезни легких до проведения ТГСК.

ТГСК при синдроме Вискотт-Олдрича дает больным значительные шансы на выживание. Показано, что достижение полного донорского химеризма является здесь благоприятным фактором. В целом, однако, многочисленные исследования при ИДС показывают, что кондиционирование низкой интенсивности создает условия для приживления донорских клеток при меньшей заболеваемости по сравнению со стандартными режимами, что является важным фактором для больных, у которых может быть неприемлемо высоким риск от проведения обычных трансплантаций.

**Ключевые слова:** первичные иммунодефициты, тяжелый комбинированный иммунодефицит (ТКИД), первичные Т-клеточные дефициты, трансплантация гемопоэтических стволовых клеток (ТГСК), кондиционирующие режимы, клинические исходы