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Radiosensitive Severe Combined Immunodeficiency Disease

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Synopsis

Inherited defects in components of the non-homologous end joining DNA repair mechanism produce a T-B-NK⁺ severe combined immunodeficiency disease (SCID) characterized by heightened sensitivity to ionizing radiation. Patients with the radiosensitive form of SCID may also have increased short- and long-term sensitivity to the alkylator-based chemotherapy regimens traditionally utilized for conditioning prior to allogeneic hematopoietic cell transplantation (HCT). Known etiologies of radiosensitive SCID include deficiencies of Artemis, DNA Ligase IV, DNA-dependent protein kinase catalytic subunit (DNA-PKcs), and Cernunnos-XLF, all of which have been treated with HCT. Because of their sensitivity to certain forms of chemotherapy, the approach to donor selection and type of conditioning regimen utilized for a radiosensitive SCID patient requires careful consideration. Significantly more research needs to be done in order to determine the long-term outcomes of radiosensitive SCID patients following HCT, as well as to discover novel non-toxic approaches to HCT that might benefit those with intrinsic radio- and chemo-sensitivity, as well as potentially all patients undergoing an HCT.

Keywords

severe combined immunodeficiency disease; radiosensitive; hematopoietic cell transplant; Artemis; DNA Ligase IV

Introduction

Severe Combined Immunodeficiency Disease (SCID) has classically been divided into those patients with residual B cells (T-B⁺ phenotype) and those whose defects produce an absence of both T cells and B cells (T-B⁻ phenotype). The T-B⁻ phenotype accounts for approximately 30% of SCID patients and is associated with worse outcomes following hematopoietic cell transplantation (HCT) in most [1–4], but not all [5] studies. A variety of genetic mutations have now been linked to the T-B⁻ phenotype, most of which result in defects in the protein

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machinery required for the V(D)J recombination events critical for producing the diverse repertoire of the T- and B-cell immune system.

The first step in V(D)J recombination involves creation of double-stranded DNA (dsDNA) breaks and subsequent hairpin formation by an enzymatic complex produced by the Recombination Activating genes (RAG) 1 and 2 (Figure 1). Defects in RAG also produce a T-B- form of SCID, but without radiosensitivity [6]. However, once the dsDNA breaks are created by the RAG complex, proper repair must take place in order to avoid a differentiation arrest, which in B cells occurs at the transition from cytoplasmic Ig μ negative to Ig μ positive pre-B cells, and in T cells occurs at the transition from pro-T to double negative pro-T cells [7,8].

Non-Homologous End Joining

Eukaryotic cells possess two mechanisms by which dsDNA breaks are repaired: homologous recombination (HR) and non-homologous end joining (NHEJ). Defects in genes that produce components of the homologous recombination pathway result in diseases such as Ataxia Telangiectasia [9], Seckel Syndrome [10], Nijmegen Breakage Syndrome [11], and Fanconi Anemia [11], which are characterized by physical abnormalities with either immunodeficiency and/or predisposition to cancer development.

The NHEJ pathway is especially critical in the repair of the dsDNA breaks created by the RAG process during the V(D)J recombination in T- and B- lymphocytes (Figure 2). After a dsDNA break is created, the first protein that binds to the ends of the dsDNA breaks is a heterodimer known as Ku 80/86 [12]. Ku then recruits a complex made up of two proteins: Artemis (also known as DNA cross-link repair enzyme 1C, or DCLRE1C) and DNA-dependent protein kinase catalytic subunit (DNA-PKcs). This complex performs two functions via its nuclease activity: first, it opens the DNA hairpins created by the RAG complex; and second, it acts to trim the ends to variable extents, thereby contributing to functional diversity [13]. Finally, the two ends of DNA are ligated together, a task carried out by a complex of two proteins: DNA Ligase IV and X-ray cross-complementation group 4 protein (XRCC4) [14,15]. Another factor, known as Cernunnos-XLF, accumulates at the site of dsDNA breaks and appears to stimulate the DNA Ligase IV: XRCC4 complex [16].

Agents Responsible for Double Stranded DNA Breaks

Although defects in NHEJ are classically considered to produce “radiosensitive” forms of SCID, in fact, a wide variety of agents other than ionizing radiation produce dsDNA breaks via reactive oxygen species. These breaks would normally be repaired through the same NHEJ mechanism as radiation-induced damage. Cell lines from Artemis-deficient patients are moderately sensitive to mitomycin C, an alkylating agent that causes DNA crosslinks of guanine nucleotides by attaching an alkyl group [17]. Crosslinking makes it impossible for DNA strands to successfully uncoil and separate during the normal DNA replication process, so that affected cells are unable to divide properly. The stalled replication fork is normally repaired by excision of the damaged area, and subsequent DNA re-joining via the components of either the homologous recombination or NHEJ pathways.

This property of alkylating agents forms the basis of their utility as anti-cancer chemotherapeutic agents. These chemotherapy medications were then adopted for their utility in the conditioning process to prepare a patient (including potentially a patient with SCID) to undergo an allogeneic HCT, since they can target the immune system, in order to prevent graft rejection, and/or host hematopoietic cells, thereby opening niches in the bone marrow microenvironment for donor cells to attach and grow. Classical alkylating agents include those in the nitrogen mustard family (e.g., cyclophosphamide and melphalan), as well as busulfan and thiopeta [18].

Another essential component of the allogeneic HCT process is control over the alloreactive donor T cells ability to cause graft-versus-host disease (GVHD). Traditionally, this has been accomplished through the use of calcineurin inhibitors, which block downstream signaling from the IL-2 receptor. Interestingly, the calcineurin inhibitor cyclosporine can produce dsDNA breaks, at least in cells deficient in DNA Ligase IV [19].

Diagnostic Tests for Radiation Sensitivity

Even before specific mutations in the various components of the NHEJ pathway were identified, a subset of patients with T-B- SCID were known to have an increased sensitivity to ionizing radiation [20]. Initial experiments were carried out by creating granulocyte-macrophage colony-forming units (CFU-GM) from patient bone marrow samples. The CFU-GM were exposed to increasing doses of irradiation (0.5 – 3 Gray) and survival relative to non-irradiated cells were compared to bone marrow from healthy age-matched controls [20,21].

The current approach to assessing radiation sensitivity involves creating primary skin fibroblasts from patients with SCID. Fibroblasts in exponential growth phase are then exposed to increasing doses of radiation (1 – 6 Gray) and then re-grown for 10–14 days. Survival relative to non-irradiated cells and healthy controls is then calculated. [7,22–24]. The difficulties with this approach are the acquisition of cell samples and the time it takes to generate the fibroblasts from a skin biopsy in culture (6–8 weeks) when there is a sense of urgency about doing an HCT to correct the underlying disease.

Severe Combined Immunodeficiency Syndromes

While HR can repair a wide variety of DNA damage, only NHEJ appears to be capable of repairing the hairpins formed during V(D)J recombination in T- and B-cell development. Therefore, defects in any of the proteins involved in NHEJ are not “cross-repaired” by HR, and are thus incompatible with normal maturation of both T- and B-cells. However, since NK cells do not undergo V(D)J recombination, and are thus present in normal numbers, patients with defects in NHEJ have a phenotype of T-B-NK+ SCID. The presence of normal NK cells in these patients is clinically very important, since NK cells differentiate self from foreign cells based on interactions of killer immunoglobulin-like receptor (KIR) ligands with HLA class I antigens. Because of this, immunocompetent NK cells in radiosensitive SCID patients pose a significant barrier to engraftment of HLA-mismatched donor stem cells [25,26].

Mutations in Artemis, DNA Ligase IV, DNA-PKcs, and Cernunnos-XLF have all been reported to cause SCID in humans. So far to date, human mutations in Ku or XRCC4 have not been described, though a mouse model of Ku deficiency does exist [27]. Defects in XRCC4 appear to be embryonically lethal in mice [28].

Artemis Deficiency

The Artemis protein, in complex with DNA-PKcs, performs the crucial NHEJ function of opening the DNA hairpins created by the RAG complex, and then acts to nucleolytically trim the ends to variable extents. Mutations in the Artemis gene represent the most common cause of radiosensitive SCID.

For almost three decades, it has been known that Native Americans from the Navajo and Apache tribes (Athabascan-speakers) in the southwestern United States had an extremely high incidence of T-B-NK+ SCID (SCIDA), with an estimated 52 cases per 100,000 live births [29]. The mutation in Athabascan-speaking Native American infants with SCID was localized to chromosome 10p [30], and was eventually found to be a null mutation in the Artemis gene [31], which had previously been identified as defective in other patients with radiosensitive T-

B- SCID [32]. In addition to Athabascan-speaking Native Americans, Artemis mutations have been described in infants of European, Turkish, and Japanese descent [32,33].

Only one paper to date has specifically focused on outcomes following HCT for Athabascan-related SCID [34]. However, this paper was limited by the fact that it was published prior to the discovery of the mutation in the Artemis gene, and subsequent analysis has discovered that two of the patients with T-B-NK+ SCID included in this report actually had mutations in RAG-1, rather than Artemis [24]. This paper still represents the largest series of Artemis-deficient patients reported, and it was the first to highlight the role of alkylating agents on growth and development of secondary teeth.

Since the discovery of the Artemis gene, several other papers have begun to specifically identify small numbers of Artemis-deficient patients who have undergone HCT (summarized in Table 1) [5,7,33,35–37]. Although the numbers are too small for any definitive conclusions, in general most patients with Artemis deficiency fail to develop B cells following non-conditioned HCT and require life-long immunoglobulin replacement [34,38]. However, some patients with Artemis deficiency do survive the conditioning process and have evidence of B cell recovery, though information regarding long-term follow-up of these patients is limited.

As to be expected, patients with Artemis deficiency appear to have increased sensitivity to therapeutic doses of ionizing radiation. Both SCIDA patients that received total body irradiation (700 cGy) died from toxic complications [34]. Whether Artemis-deficient patients have increased late effects from non-therapeutic doses of radiation in the form of radiographs is not known. Furthermore, patients with Artemis deficiency that receive alkylator-based conditioning regimens may have growth delay and failure of permanent tooth development [34]. Amongst SCID patients alive 2 years post-HCT, those with Artemis deficiency had the poorest long-term event-free survival, highest rates of infection, GVHD and/or autoimmunity, and need for nutritional support [38].

Interestingly, hypomorphic mutations in Artemis have also been described in four patients, resulting in a partial immunodeficiency and a predisposition to EBV-associated lymphomas [39]. Fibroblasts from these patients did show sensitivity to ionizing radiation.

DNA Ligase IV Deficiency

DNA Ligase IV is a crucial enzyme in the final DNA re-joining step of NHEJ. Null mutations in DNA Ligase IV are embryonically lethal in mice [40]. Hypomorphic mutations in DNA Ligase IV were first described in four patients as part of a syndrome associated with microcephaly, unusual facial features, growth retardation, and developmental delay, resembling Seckel Syndrome, but with immunodeficiency [41]. Cell lines from these patients demonstrated pronounced radiosensitivity.

Two similar Moroccan siblings were reported to undergo haploidentical HCT from their mother [42]. The first patient was conditioned with busulfan, cyclophosphamide (doses not specified), and anti-thymocyte globulin (ATG). She developed an EBV-associated post-transplant lymphoproliferative disease (PTLD) and died [19]. The second patient was conditioned with busulfan (8 mg/kg total dose), cyclophosphamide (200 mg/kg total dose), and ATG. She developed severe sinusoidal obstruction syndrome (SOS) and died [19]. Two German siblings have been described with similar features [43]. The first patient died of aspergillosis during the preparative regimen for HCT. The second patient was conditioned with thiotepea (15 mg/kg total dose), fludarabine (5.72 mg/kg total dose), and ATG and underwent matched unrelated donor BMT [19]. She developed microangiopathic hemolytic anemia but was surviving at 8 months post-HCT. The patient reportedly had full donor chimerism and improving T-cell

reconstitution (B-cell reconstitution was not reported), but significant neurodevelopmental delay.

The degree of immunodeficiency seen in Ligase IV Syndrome is variable. A 14 year-old Japanese girl with a Ligase IV mutation and clinical features consistent with the syndrome showed evidence of a combined immunodeficiency and expired from an EBV-associated lymphoma [44]. A 10 year-old German girl had only low immunoglobulin levels with recurrent otitis and respiratory tract infections, but developed progressive bone marrow aplasia [45]. She was conditioned with cyclophosphamide (40 mg/kg total dose), fludarabine (120 mg/m² total dose), and ATG and underwent a matched sibling BMT. She achieved full donor chimerism and immune reconstitution, including response to vaccinations. Since, achievement of 100% donor chimerism is not commonly seen following non-myeloablative HCT for Artemis deficiency [34], it is possible that the pre-HCT marrow hypoplasia seen in this patient caused sufficient open niches for donor HSC engraftment to occur, which has been reported in X-linked SCID [46].

Interestingly, it appears that mutations in Ligase IV can also produce SCID without developmental defects and syndromic features [47]. The Turkish patient in this report appeared to have a “leaky” mutation that allowed the development of low levels of circulating B cells. The V(D)J junctions in this patient demonstrated excessive nucleotide deletions, presumably caused by prolonged exposure to the exonuclease activity of the Artemis:DNA-PKcs complex during the delayed ligation process. She presented in the 2nd year of life, later than most patients with T-B-NK+ SCID, with severe respiratory infections, perineal candidiasis, chronic diarrhea, and fever. This patient was conditioned with busulfan (approximately 8 mg/kg total dose) and cyclophosphamide (approximately 160 mg/kg total dose) and underwent a matched sibling umbilical cord blood transplant. She developed probable SOS and died.

While only a few patients have been reported, it appears that patients with deficiency of DNA Ligase IV, either in the syndromic form or as an isolated cause of T-B-NK+ SCID, have excessive toxicity and mortality following conventional myeloablative HCT with busulfan-based conditioning comparable to what has been seen in Artemis deficient patients [34]. Interestingly, *in vitro* exposure of DNA Ligase IV-deficient cell lines has not shown an increased sensitivity to busulfan, or the non-alkylators fludarabine and methotrexate, at least at the concentrations utilized [19]. However, it appears that cyclosporine alone, but especially in combination with busulfan and fludarabine, did cause increased dsDNA breaks. A similar effect was not seen in Artemis-deficient cells.

Finally, not all mutations in DNA Ligase IV appear to cause clinical immunodeficiency, as some patients have been discovered only after exhibiting excessive toxicity following therapy for cancer. A 14-year-old normal-appearing Turkish boy with T-cell acute lymphoblastic leukemia (ALL) had severe toxicity following standard doses of chemotherapy and died of encephalopathy following prophylactic cranial irradiation [48]. Later, his cells were found to harbor a mutation in DNA Ligase IV [49]. A 4-year-old German-Canadian boy with clinical features of DNA Ligase IV Syndrome but without associated immunodeficiency was also identified after presenting with T-cell ALL [50]. Following routine chemotherapy he developed prolonged neutropenia and died of presumed sepsis. These unfortunate outcomes highlight the probable heightened sensitivity of patients with mutations in DNA Ligase IV to both radiation and some chemotherapeutic agents.

DNA-PKcs Deficiency

DNA-dependent protein kinase catalytic subunit (DNA-PKcs) is the partner of Artemis in DNA hairpin-opening complex. Defects in the DNA-PKcs gene are known to produce SCID in mice [51], Arabian horses [52], as well as Jack Russell terriers [53]. *In vitro* studies have

demonstrated that cells deficient in DNA-PKcs are not only radiosensitive, but have increased DS DNA breaks following treatment with other alkylating chemotherapeutic agents, such as nitrogen mustard [54], melphalan [54,55], chlorambucil [55], and adriamycin [55]. However, DNA-PKcs cells do not show increased sensitivity to the anti-metabolite 5-FU [55], or to the topoisomerase II inhibitor etoposide [56], neither of which are alkylators. So far, null mutations in DNA-PKcs have not been described in humans, suggesting that this protein may play a significant role in development of cells other than lymphocytes and null mutations may be embryonically lethal.

Recently the first human patient with a DNA-PKcs missense mutation (L3062R) was reported [8]. Interestingly, the mutation did not affect the kinase activity or the DNA end-binding capacity, but appeared to insufficiently activate Artemis function. Not surprisingly, this Turkish patient presented with a radiosensitive form of T-B-NK+ SCID. She did not have microcephaly or other physical stigmata, though did present with a large aphthous ulcer, reminiscent of that described in Athabascan-speaking patients with Artemis deficiency [34, 57]. She underwent a non-conditioned HCT from her HLA-identical cousin, and interestingly had full recovery of B-cell numbers, though the functionality of the B cells was not reported. This would be an unusual finding, since B cell recovery is not generally seen following non-conditioned transplantation for Artemis deficiency [34].

Cernunnos-XLF Deficiency

Cernunnos-XLF is another factor that accumulates at the site of dsDNA breaks and appears to function by stimulating the DNA Ligase IV:XRCC4 complex [16]. Five patients from four families (French, Turkish, and Italian) comprised the first report of mutations in the Cernunnos-XLF gene [58]. All patients presented with severe infections and a T-B-NK+ SCID phenotype. Interestingly, they also demonstrated microcephaly, growth retardation, and some had bird-like facies and/or bony malformations. One patient also had bone marrow aplasia. As expected, skin fibroblasts demonstrated an increased sensitivity to ionizing radiation, comparable to that seen in Artemis-deficient cells. The first two patients died of infection, while the other three were being supported by antibiotic and immunoglobulin prophylaxis.

The first reported HCT for a patient with Cernunnos-XLF deficiency utilized a reduced-intensity regimen of fludarabine (120 mg/m² total dose), cyclophosphamide (1200 mg/m² total dose), and ATG with peripheral blood stem cells from a 7/8 matched unrelated donor [59]. The patient developed grade 2 skin acute GVHD, as well as an EBV-associated PTLT requiring treatment with 16 doses of rituximab. At two-year follow-up, the patient had 100% donor engraftment and normal T cell numbers, but was still receiving replacement immunoglobulin for low B cell numbers, possibly as a consequence of the prolonged Rituximab therapy.

Similar to what was noted above in one patient with DNA Ligase IV deficiency, achievement of 100% donor chimerism is not commonly seen following non-myeloablative HCT for Artemis deficiency [34]. One explanation is that the pre-HCT marrow hypoplasia seen in this patient may have caused sufficient open niches for donor HSC engraftment to occur. An alternate explanation is that the occurrence of acute GVHD caused by alloreactive T cells mediated host HSCs destruction, thereby opening niches for the donor HSC engraftment, which has been reported in adenosine deaminase-deficient SCID [60].

Omenn Syndrome—Omenn syndrome, a SCID phenotype associated with erythroderma, hepatosplenomegaly, lymphadenopathy, alopecia and elevated numbers of activated T cells with a restricted T-cell receptor repertoire, is classically seen in T-B- SCID patients with hypomorphic defects in the RAG genes [61]. More recently, however, it has been appreciated that a wide variety of genetic defects can contribute to the development of Omenn Syndrome,

including a compound heterozygous Artemis mutation with partial activity [23] and a patient with heterozygous mutations in the DNA ligase IV gene [62].

The presence of Omenn syndrome in a patient with an underlying radiosensitive form of SCID could prove to be problematic, since the activated autologous T-cell clones in addition to the normally functioning NK cells serve as a barrier to donor cell engraftment [61]. While pharmacologic suppression of these T cells with corticosteroids and/or cyclosporine has helped to facilitate engraftment of bone marrow from HLA-identical donors, transplantation from alternative donors without myeloablative conditioning has been associated with poor overall survival [61]. Therefore, in general, patients with Omenn syndrome undergoing alternative donor HCT are given myeloablative conditioning [61], which in a patient with underlying radiosensitivity may be associated with long-term complications.

The previously described Omenn patient with a defect in Artemis underwent myeloablative conditioning (exact details not reported) and an HLA-haploidentical transplant from his mother, with achievement of complete donor chimerism and normal immune function, however, long-term follow-up and details regarding possible late effects of the conditioning were lacking [23]. Of note, primary skin fibroblasts from this patient had identical radiation sensitivity to those patients with complete Artemis deficiency [23], indicating that a partial defect in Artemis may be sufficient to produce a potentially clinically relevant degree of radiosensitivity. The patient with Omenn syndrome due to defects in the DNA Ligase IV gene received a conditioning regimen of busulfan (16 mg/kg total dose) and cyclophosphamide (200 mg/kg total dose) and had significant short-term complications following HLA-matched unrelated donor BMT but survived. She also developed numerous late effects, including microcephaly, developmental delay, and short stature [62], however, many of these problems are similar to the manifestations in other reported patients with DNA Ligase IV Syndrome, irrespective of HCT [41]. Therefore, additional patients with Omenn syndrome secondary to defects in the NHEJ complex will need to be described, with careful attention to late effects, before it is clear how these individuals respond to myeloablative conditioning.

Approaches to HCT

Currently, testing of cells from SCID patients for radiosensitivity is only available on a research basis. Therefore, we recommend that all newly diagnosed T-B-NK+ SCID patients be evaluated for the presence of a mutation in either of the RAG genes. Sequencing of the RAG genes is commercially available. In the absence of a RAG mutation, genotyping for an Artemis mutation (also commercially available) should be done and a radiosensitive form of SCID should be assumed. Until more detailed long-term analysis of sufficient numbers of radiosensitive SCID patients has been performed, the most cautious approach to HCT would involve complete avoidance of therapeutic irradiation and utilization of the least amount (if any) of alkylator-based chemotherapy as possible in order to obtain donor cell engraftment.

Donor Selection

Radiosensitive SCID patients with HLA-matched siblings should proceed directly to bone marrow transplant without the use of pre-transplant conditioning. Eradication of transplacentally-acquired maternally-engrafted T-cells prior to matched sibling HCT is not necessary for subsequent donor T-cell engraftment [34,37], possibly because the maternal T-cells generally have a limited T-cell receptor diversity and are functionally anergic [63,64]. However, if maternal GVHD is present, lympholytic agents may need to be utilized as part of the anti-GVHD therapy. Success has been reported with both ATG [34] and fludarabine [46], neither of which would be predicted to produce dsDNA breaks, and therefore should not have significant toxicity for a radiosensitive SCID patient.

Cyclosporin is generally administered for four to six months following matched-sibling HCT for GVHD prophylaxis. Many centers will also administer a second agent, such as short-course methotrexate or mycophenolate mofetil. While concerns exist regarding the safety of cyclosporin for patients with DNA Ligase IV deficiency, until the use calcineurin-inhibitor-free GVHD prophylaxis regimens are better understood, it still likely needs to be utilized. An alternate possibility for patients with Ligase IV deficiency would be partial *ex vivo* T-cell depletion, however this approach has not yet been reported in great detail [65], and too vigorous removal of donor T-cells could adversely affect engraftment.

In the absence of an HLA-matched sibling, utilization of an unrelated donor (volunteer adult or umbilical cord blood) versus a haploidentical donor is dependent on a multitude of factors, including: 1) pre-existing infections at the time of presentation; 2) the relative rarity of certain ethnic groups within the unrelated donor database; and 3) local expertise with *ex vivo* T-cell depletion techniques [66]. One advantage of T-cell depleted haploidentical HCT, especially for patients with DNA Ligase IV deficiency, is that post-HCT administration of GVHD prophylactic medications is not needed.

If the decision is made to proceed with a haploidentical donor, the presence of transplacentally-acquired maternally-engrafted T-cells must be ascertained, as these cells can interfere with engraftment of stem cells from a paternal donor [67]. The presence of pre-HCT maternally engrafted cells is associated with an increased likelihood of post-HCT T-cell engraftment when a maternal donor is utilized, presumably because the tolerance to maternal cells by residual host immune cells indicate permissiveness to maternal donor stem cells [68]. However, in the absence of maternal engraftment, engraftment following haploidentical HCT is less successful, presumably due to the presence of major histocompatibility barriers recognized by normally-functioning host NK cells. A prospective trial designed to test the hypothesis that the administration of megadoses (approximately 20×10^6 CD34+ cells/kg recipient body weight) of haploidentical donor stem cells would improve B-cell reconstitution following non-conditioned HCT was not successful in overcoming NK mediated graft resistance and resulted in only a 43% engraftment rate in patients without preexisting maternal engraftment [68].

Another significant problem with haploidentical HCT has been that T-cell immunologic recovery can be slow, allowing progression of underlying infections. However, the use of megadoses of donor stem cells may result in faster T-cell recovery [68]. In addition, the use of non-specific haploidentical donor lymphocyte infusions has been shown to be safe and potentially associated with accelerated T-cell recovery and clearance of infections [69]. Furthermore, several groups are also showing success at creating pathogen-specific T-cells to be used to either treat or prevent infection following haploidentical HCT [70–72]. Finally, if poor T-cell function persists following haploidentical HCT, non-conditioned stem cell boosts have also been useful for augmenting immunity [38,73], especially if administered less than 1-year post-HCT [74].

The decision to utilize an unrelated donor is associated with different problems. The search process for an unrelated donor can be lengthy, especially for patients with underrepresented ethnic backgrounds. This delay might not be tolerated if the patient presents with a serious infection. The time to transplant might be shortened if umbilical cord blood (UCB) is used, given its increased permissiveness of HLA-mismatches and more rapid acquisition time. In addition, since virtually all radiosensitive SCID patients will be very young, the limited cell doses in UCB grafts is rarely a significant issue. However, utilization of an unrelated donor generally requires the use of pre-transplant conditioning, as well as post-HCT pharmacologic immunosuppression for GVHD prophylaxis.

Conditioning

The use of pre-HCT conditioning is controversial, even for patients with non-radiosensitive forms of SCID [66], given the concerns that myeloablative doses of chemotherapy can be associated with not only short-term toxicity (such as SOS), but also infertility, hormonal deficiencies and short stature, and potentially malignancy and neurocognitive delay [75,76]. These concerns are heightened in the setting of a radiosensitive SCID patient, but formal long-term analysis of sufficient numbers of such patients is lacking. Recent data suggest that cognitive and behavioral function in SCID patients post-HCT is identical between those that received conditioning therapy and those that did not, however, it should be noted that no children with radiosensitive SCID were specifically documented in this report and the overall number of patients was relatively small [77]. Similarly, although there were no cases of true secondary malignancy (excluding EBV-mediated lymphoproliferation) post-HCT in 117 patients with immunodeficiency, it is unknown whether any of these patients had radiosensitive SCID [78]. A more recent cohort of 90 patients with SCID (including 12 with Artemis deficiency) surviving more than two years post-HCT only demonstrated one case of secondary myelodysplasia in a patient with reticular dysgenesis [38].

It has been reported that among patients with T-B- SCID, only those that received myeloablative conditioning with busulfan achieved normal B-cell function post-HCT [1]. Similarly, another report suggests that T-B- SCID patients have improved survival if conditioning is utilized [3]. Presumably the use of myeloablative chemotherapy creates space for hematopoietic stem cell engraftment in the niches of the bone marrow. These early reports are limited, however, by the lack of molecular diagnosis of patients, so that patients with non-radiosensitive RAG deficiency are lumped together with patients with Artemis deficiency.

Furthermore, the dogma that myeloablative conditioning is absolutely required for bone marrow stem cell engraftment and subsequent B-cell recovery has been challenged by rare reports of patients with T-B-NK+ SCID who develop B-cell recovery following non-ablative HCT [34,45,68,79]. An interesting report of full B-cell recovery following matched sibling HCT for Artemis-deficient SCID further demonstrates our incomplete understanding of the factors involved in B-cell recovery [37]. In this report, the patient had first undergone a non-conditioned BMT from her HLA-identical brother, with T-cell recovery, but absent B-cells. She then underwent a second BMT from the same donor with low-dose busulfan (4 mg/kg total dose), which was well tolerated. The patient subsequently developed functional B cells of donor origin with vaccine responses, but without IgA production. However, her NK cells and myeloid cells remained of host origin, suggesting that only a selective B-cell precursor graft was achieved.

Currently, almost all reported unrelated donor HCTs for treatment of SCID utilize some form of pre-HCT conditioning. Theoretically, a radiosensitive SCID patient undergoing HCT from a perfectly-matched unrelated donor might be expected to show evidence of T-cell recovery in a fashion similar to that seen in matched sibling HCTs, since the lack of HLA class I differences would minimize NK cell alloreactivity. However, to the best of our knowledge, this has not yet been reported. Although some studies indicate that full doses of busulfan (16 mg/kg or more) can be administered to patients with radiosensitive forms of SCID, the reported toxic mortality is not insignificant and the follow-up is too limited for full understanding of the possible late effects [5,34,36]. If busulfan is to be used, then pharmacokinetic targeting is highly recommended in order to avoid the excessive toxicity (mucositis and SOS) associated with high levels. A concentration steady state (C_{ss}) of approximately 600 ng/mL should be sufficient for donor HSC engraftment [80] and for children with radiosensitivity an even lower C_{ss} might suffice, though data are lacking. Another option is to utilize a reduced intensity regimen of melphalan (140 mg/m²), fludarabine (150 mg/m²), and alemtuzumab or ATG

[81,82], however, melphalan is also an alkylator and long-term follow-up after receiving this agent is incomplete.

For a T-cell depleted haploidentical HCT in a patient with SCID, myeloablative-type conditioning, similar to that used for unrelated donors, has been utilized [2,5,83]. However, there are also successful reports utilizing either no conditioning or solely immunoablative conditioning [2,34,65,83]. For a haploidentical HCT, the major barrier to engraftment in a T-B-NK+ radiosensitive SCID patient is the presence of the immunocompetent NK cells, which differentiate self from foreign cells based on interactions of KIRs with HLA class I antigens [25,26]. With the use of megadose CD34+ cell grafts, 43% of non-maternally engrafted patients with NK+ forms of SCID will show evidence of T-cell engraftment in the absence of conditioning [68]. T-cell engraftment was seen in all eight patients that had evidence of transplacental maternal T-cell engraftment at the time of diagnosis, thus conditioning is not needed for these patients [68]. Therefore, we generally recommend a non-conditioned HCT as the initial approach. If the patient does not show evidence of T-cell recovery in a timely fashion following a non-conditioned HCT, then a repeat HCT with conditioning must be considered. If conditioning is required, at first glance, fludarabine would seem to be a good choice, since it generally has less short-term toxicity than cyclophosphamide; however, unlike cyclophosphamide [84], fludarabine does not inhibit NK cells [85]. Serotherapy, in the form of ATG or alemtuzumab, does appear to inhibit at least some populations of NK cells and, since serotherapy should cause no long-term toxicity in radiosensitive SCID patients, it should likely be part of any haploidentical conditioning regimen [86].

Future Directions

Now that defects in four of the genes involved in the NHEJ pathway are known to cause SCID, and as diagnostic tests of radiosensitivity become more available, we will soon be able to characterize each patient's genotype prior to HCT. This will significantly improve our management of these patients, particularly in terms of what conditioning agents (or doses) are best avoided.

The rarity of these various disorders makes the performance of prospective hypothesis-testing in clinical trials exceedingly difficult. Clinical trials will only be possible through large multicenter collaborations. Until those become available, murine models can help in developing novel approaches to conditioning although not all of these models truly mimic the human disease. For example, several mouse models of Artemis deficiency have been created. The first model was limited by "leakiness" that allowed the production of some T cells [87]. A more recent model more accurately mimics the typical human phenotype [88]. Similar mouse models exist for DNA Ligase IV [89], DNA PKcs [51], and Cernunnos-XLF [90] deficiencies. These models should prove invaluable for pre-clinical testing of new approaches to therapy for patients with radiosensitive forms of SCID.

One priority of pre-clinical testing is to determine the toxicity and the ability of currently available agents to eradicate NK cells and/or open hematopoietic niches. Optimally these would be agents that do not produce dsDNA breaks. For example, fludarabine [19], azathioprine [84], and 5-fluorouracil [55], produce their effects through NHEJ-repair-independent mechanisms and are thus presumably relatively safe to use in patients with radiosensitive forms of SCID. However, none of these agents have significant effects on inhibiting NK cells or in creating space in the bone marrow. But by extension, newer anti-metabolites, such as the significantly myelosuppressive agent clofarabine, might soon have utility in creating space for donor HSCs to engraft without significant risk for long-term effects.

An alternate approach to the creation of HSC niches would be to avoid the use of classical chemotherapy-based conditioning regimens altogether. This could potentially be done through

the administration of agents which cause HSC to leave the marrow space, such as the CXCR4-inhibitor Plerixafor (AMD3100). There are preliminary data in mice that Plerixafor opens niches for donor stem cells to engraft [91]. Alternately, monoclonal antibodies to antigens expressed on HSCs, such as c-kit, could be used to eliminate HSC [92]. Finally, donor T cells might be used to target host HSC. This has been reported to occur spontaneously in a few patients with either ADA- or IL2R γ chain-deficient SCID [46,60]. In a mouse model, pretreatment of host-sensitized naïve donor T cells with photochemical therapy using psoralen and UVA light prevents proliferation but preserves their cytotoxic alloreactivity to the point where GVHD is minimized but donor HSC engraftment is facilitated [93].

Ultimately, gene therapy may prove to be a safe and effective approach to correcting the immunodeficiency associated with these radiosensitive disorders. To date, this has been studied in animal models of Artemis deficiency [94,95]. Results indicate that both T- and B-cell reconstitution can occur, but the technique is still limited by the fact that it requires the use of either busulfan or total body irradiation.

Conclusions

Inherited defects are known to occur in four components of the NHEJ DNA repair mechanism and produce a T-B-NK $^{+}$ severe combined immunodeficiency disease (SCID) characterized by heightened sensitivity to radiation and alkylating agents. These include deficiencies of Artemis, DNA Ligase IV, DNA-PKcs, and Cernunnos-XLF, but only Artemis deficiency has been found in more than a small number of patients. In addition to its role in V(D)J recombination, NHEJ is involved in repair of dsDNA breaks following not only ionizing radiation, but also possibly many forms of alkylator-based chemotherapy. Since the follow-up of patients with radiosensitive SCID that have undergone HCT is relatively limited, the exact short- and long-term toxicities of myeloablative conditioning are unclear. Any patient with T-B-NK $^{+}$ SCID without a defect in RAG should be presumed to have a radiosensitive form of SCID, and confirmatory radiation sensitivity testing should be done if possible. However, the results of this testing can take a significant time to return and HCT should not be delayed. Testing for Artemis mutations is commercially available and should be done on all suspected radiosensitive SCID patients. Once the optimal donor has been identified, patients should proceed with the minimal amount of pre-HCT conditioning needed in order to overcome engraftment barriers, utilizing agents thought to not cause a significant degree of dsDNA breaks (such as ATG or alemtuzumab) whenever possible. HLA matched sibling donors and maternal donors, when there is transplacental maternal engraftment, do not need pre-HCT conditioning therapy, although the likelihood of B- cell reconstitution is much lower. Radiosensitive SCID patients should be followed very closely post-HCT for potential late effects and, optimally, entered into collaborative group registries or trials in order to capture important data regarding HCT for these rare patients. More research needs to be done in order discover novel non-toxic approaches to HCT that might benefit not only those with radiosensitive SCID, but someday potentially all patients.

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Figure 1.

V(D)J Recombination: Initial process and hairpin formation.

RSS = Recombination Signal Sequences; RAG = Recombinase Activating Gene Protein
 (Adapted with permission from: Cowan, MJ. T cell negative, B cell negative, NK cell positive severe combined immunodeficiency disease. In: UpToDate, Basow, DS (Ed), UpToDate, Waltham, MA 2009. Copyright © 2009 UpToDate, Inc. For more information visit www.uptodate.com.)

Figure 2.

The Non-Homologous End Joining Pathway

DNA PKcs = DNA-dependent protein kinase catalytic subunit; XRCC4 = X-ray cross-complementation group 4 protein

(Adapted with permission from: Cowan, MJ. T cell negative, B cell negative, NK cell positive severe combined immunodeficiency disease. In: UpToDate, Basow, DS (Ed), UpToDate, Waltham, MA 2009. Copyright © 2009 UpToDate, Inc. For more information visit www.uptodate.com.)

Table 1

Published Reports of Outcomes Following HCT for Artemis-Deficient SCID

Reference	Year	Number	Donor	Conditioning	Outcomes
O'Marcaigh, et al.	2001	14	Varied	Varied	10 Alive: 3 with B-cell recovery 4 Dead: Infection, AIHA
Noordzij, et al.	2003	4	NR	NR	2 Alive: 1 with B-cell recovery 2 Dead: Infection; MOF
Kobayashi, et al.	2003	4	NR	NR	2 Alive: T&B cell recovery 2 Dead: CMV, BO
van der Burg, et al.	2006	1	Sibling	None	Alive: No B-cell recovery
Friedrich, et al.	2007	2	Repeat	BU4	Alive: T&B cell recovery
Grunebaum, et al.	2006	2	Haplo	None	2 Alive: 1 with donor B cells
Slatter, et al.	2008	5	MUD	BU20/CY200	1 Alive; 1 Dead (CMV)
		1	MUD	BU8/CY200	2 Alive; 3 Dead (Interstitial Pneumonitis, AC)
		1	Haplo	BU8/CY200	Alive: Retransplanted
		2	Haplo	BU16/CY200	Alive: B-cell recovery
					Alive: B-cell recovery

NR Not Reported

MUD Match Unrelated Donor

Haplo Haploidentical Donor

BU Busulfan (# is mg/kg)

CY200 Cyclophosphamide (200 mg/kg)

MOF Multi-Organ Failure

AIHA Autoimmune Hemolytic Anemia

AC Autoimmune Cytopenia

CMV Cytomegalovirus

BO Bronchiolitis Obliterans