Published in final edited form as:

Immunol Allergy Clin North Am. 2010 May ; 30(2): 179–194. doi:10.1016/j.iac.2010.02.001.

Hematopoietic Cell Transplantation for Wiskott-Aldrich Syndrome: Advances in Biology and Future Directions for Treatment

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Wiskott-Aldrich Syndrome: Clinical and laboratory features

The Wiskott-Aldrich syndrome (WAS) is an X-linked disorder characterized by a triad of diagnostic clinical elements: immunodeficiency, eczema and hemorrhage due to thrombocytopenia with small-sized platelets. Manifestations of immunodeficiency in patients with WAS include recurrent and severe infections, autoimmunity, and malignancies. WAS was originally described in 1936 [1], but the X-linked pattern of inheritance was defined only 18 years later [2]. The gene responsible for disease, WAS, was cloned in 1994 [3] and encodes a 502-amino acid protein (WAS protein, WASp) selectively expressed in hematopoietic cells, where it acts as a key regulator of the actin cytoskeleton (reviewed in [4]). WAS mutations that abrogate or significantly impair expression and/or function of WASp are responsible for not only for WAS but also for its milder form, X-linked thrombocytopenia (XLT) [5]. The latter, characterized by hemorrhages due to thrombocytopenia associated with no or minor infections and eczema, is allelic to WAS [5]. The platelet count may significantly fluctuate, and hemorrhagic manifestations may be particularly mild, in patients with intermittent X-linked thrombocytopenia [6]. In contrast, some missense mutations in the Cdc42-binding domain of WAS result in constitutive activation of the protein, causing X-linked neutropenia (XLN) [7–9], with neither thrombocytopenia nor signs of T-cell immunodeficiency. The phenotype of XLN is very different from that observed in WAS/XLT, and is characterized by increased apoptosis and defects of mitosis and cytokinesis [10] that may lead to myelodysplasia. The variability of clinical manifestations associated with null and hypomorphic WAS mutations has led to the development of a scoring system to grade the severity of the disease (Table 1).

Thrombocytopenia with small-sized platelets is the most consistent feature of the disease. Hemorrhages occur in >80% of the patients [11,12] and commonly include petechiae, epistaxis and bloody diarrhea. Severe bleeding episodes (intestinal or intracranial hemorrhages) are also common (20–30%) and cause death in 4–10% of the patients [11,12].

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Bacterial (otitis media, skin abscesses, pneumonia, sepsis, meningitis) and viral (especially due to herpes simplex and cytomegalovirus) infections are common, and are particularly severe in patients with WAS [11]. Several immunological abnormalities contribute to the increased susceptibility to infections. Patients with WAS are unable to mount antibody responses to carbohydrate antigens [13], and their response to protein antigens is also often impaired; in contrast, response to T-dependent antigens is typically normal in patients with XLT [14]. The inability to mount antibody responses to carbohydrate antigens and the increased susceptibility to invasive infections caused by blood-borne pathogens correlate with severe abnormalities of the marginal zone of the spleen [15], and similar findings have been reported in *Was*^{-/-} mice [16,17]. Immunoglobulin abnormalities (low IgM, high IgA and high IgE serum levels) are observed with similar frequency in patients with WAS and with XLT ([11], Notarangelo unpublished observation). However, defects of cell-mediated immunity are more common and severe among WAS patients. In particular, T lymphocytes from patients with WAS fail to respond immobilized anti-CD3 monoclonal antibody, show reduced proliferation to mitogens and antigens and are impaired in their ability to secrete interleukin-2 and other Th1 cytokines upon in vitro activation [18–20]. In addition, patients with WAS show progressive T and B cell lymphopenia, but reduction of naive circulating T lymphocytes is apparent already early in life [21]. Defective cytolytic activity of natural killer cells may also contribute to increased frequency of viral infections [22], and is more severe in patients with WAS than with XLT [23]. Finally, monocytes and dendritic cells (DCs) from patients with WAS show severe abnormalities of the actin cytoskeleton and impaired directional migration [24]. Defective interaction between DCs and T lymphocytes may cause impaired T-cell priming, as also shown in the murine model of the disease [25,26]. Eczema is common (80%) in patients with WAS [12], but less so (41%) in patients with XLT [11]. In addition to increased IgE serum levels [11] and skewed Th2 cytokine profile [19], defective migration of Langerhans cells has also been implicated in the pathophysiology [27,28].

The incidence of autoimmune manifestations is markedly increased among patients with WAS, and ranges from 22% to 72% in various series [11,12,29,30]. Autoimmune hemolytic anemia (AIHA), vasculitis, arthritis, nephropathy and inflammatory bowel disease are particularly common. In addition, idiopathic thrombocytopenic purpura (ITP) has been frequently observed in patients who develop a relapse of thrombocytopenia following splenectomy, and may even contribute to the pathophysiology of thrombocytopenia in unsplenectomized WAS patients [31]. Although the scoring system (Table 1) dictates that patients with XLT do not have autoimmune manifestations at diagnosis, these may develop over time, and IgA nephropathy is particularly common (19%) [11]. Occurrence of autoimmunity in WAS has prognostic implications and has been associated with reduced survival and higher risk of developing malignancies [12,29]. Multiple mechanisms may account for autoimmunity in WAS [30,32]. WASP-deficient natural regulatory T (nTreg) cells are severely impaired in their suppressive function [33–35]. Furthermore, patients with WAS and *Was*^{-/-} mice have a reduced number and impaired function of invariant natural killer T (NKT) cells [36,37], another population of cells with important immunoregulatory properties. Finally, impairment in the ability of DCs to migrate in response to chemoattractants and to interact with T lymphocytes, and chronic immune activation resulting from inefficient pathogen clearance may also play a role in the autoimmunity of WAS [30].

Tumors have been reported in 13% [12] and 22% [11] of the patients in two series. They may occur in childhood, but are more frequent during adolescence or early adulthood, and are mainly represented by leukemias, myelodysplasia and lymphomas. WAS patients are particularly susceptible to lymphoproliferative disease due to Epstein-Barr virus (EBV) [38]. It has been speculated that the increased occurrence of tumors in patients with WAS reflects the immunodeficiency of the disease, however the observation that tumors are almost uniquely restricted to hematopoietic cells (i.e., the same cell types to which expression of WASp is

restricted) indicates that WASp-dependent, hematopoietic cell-intrinsic abnormalities may play an important role in tumorigenesis.

Initial reports had indicated that median survival in patients with WAS is 20 years of age [12]. Death is mostly caused by hemorrhage, malignancy and severe infection. Conservative management includes prompt and aggressive treatment of infections, immunoglobulin replacement therapy in patients with antibody deficiency, immunosuppressive drugs for autoimmune manifestations, surveillance for tumors, and anti-CD20 monoclonal antibody in patients with EBV lymphoproliferative disease. In the past, elective splenectomy has been used to reverse the thrombocytopenia, resulting in significant increase of the platelet count in up to 85% of the patients. However, a significant fraction of patients (15%) develop chronic ITP after splenectomy. Furthermore, splenectomy in WAS leads to increased risk of invasive pyogenic infections even in patients who receive antimicrobial prophylaxis. For this reason, many no longer considered splenectomy to be part of the routine therapeutic plan [39].

In spite of advances in diagnosis and clinical care, patients with severe disease (in particular, those who fail to express WASp) continue to have poor survival. In one recent study, the 20-year probability of overall survival was 0% for patients who fail to express WASp vs. 92.3% among those who express reduced levels of normal-sized protein [11]. These data emphasize the importance of considering hematopoietic cell transplantation (HCT) in the treatment of WAS, especially for patients with more severe forms of the disease.

Molecular basis of WAS and genotype-phenotype correlation analysis

Variability of the clinical and laboratory features among patients with WAS mutations has prompted genotype-phenotype correlation analysis. Polyclonal and monoclonal antibodies to WASp have been developed and used successfully for diagnostic and prognostic purposes [40–42]. Mutation analysis at the WAS locus has shown that the vast majority of XLT patients carry missense mutations in exons 1 and 2 of the gene [43]. This corresponds to a region at the N-terminus of WASp that interacts with the WASP-interacting protein (WIP) [44], which stabilizes WASp [45]. Accordingly, patients with XLT who carry missense mutations in exons 1 and 2 of the WAS gene typically have reduced amounts of normal-sized WASp [11,41,43]. Occasionally, an XLT phenotype is also observed in patients who carry splice-site mutations, allowing for residual expression of full-sized transcript [43]. In contrast, a more severe WAS phenotype is generally associated with nonsense and frameshift mutations [43]. Mutation analysis alone is of limited value in predicting the clinical phenotype, however; patients with WAS may carry also missense mutations (especially in regions other than exons 1 and 2) and on the other hand some missense mutations in exon 2 are associated with a severe clinical phenotype. Analysis of WASp expression in lymphocytes has been used with great success in predicting the clinical phenotype. In a study of 50 patients with WAS mutations, positivity for WASp expression correlated with reduced incidence of severe infections, lower risk of mortality from intracranial hemorrhage and prolonged survival [11]. However, it is important to note that patients with XLT may progress to WAS with age, and may develop autoimmune complications and malignancies, albeit with reduced frequency and later in life than patients with WAS.

Finally, somatic mutations, many of which restore WASp expression, have been frequently observed in patients with WAS [46]. The higher frequency of revertants among T lymphocytes (especially CD8⁺ T cells) indicates that WASp expression confers a stronger selective proliferation and/or differentiation advantage among such cells. These data are in keeping with *in vivo* observations in *Was*^{+/-} mice, in which a striking predominance of WASp-expressing cells has been observed among peripheral T cells (especially CD8⁺ and Treg lymphocytes), as well as among NKT lymphocytes and marginal zone B cells, but not among myeloid cells

[16,17]. There is currently, however, no conclusive evidence that emergence of revertant clones in patients with WAS is associated with clinical improvement. Altogether, these observations suggest that expansion of certain WASp-expressing cells can be expected in patients developing mixed chimerism, a tendency that may have important clinical implications for HCT and gene therapy.

Hematopoietic cell transplantation in WAS: A Historical Perspective

The formal proof that HCT could be used to cure WAS revealed a requirement for both immunosuppression and myelosuppression that still underlies the standard approach to curative therapy today. Successful induction of high level donor lymphocyte chimerism and development of isohemagglutinin antibodies 6 weeks after immunosuppression with 200 mg/kg of cyclophosphamide and HCT from an HLA-matched sibling was reported in 1968 [47]. The patient remained thrombocytopenic and did not convert blood type to donor, and eventually died at 36 years of age secondary to complications of graft-versus-host disease (GvHD) following a second transplant preceded by myeloablative conditioning [48]. Correction of hematopoiesis in patients with WAS requires myelosuppression in addition to immunosuppression, as demonstrated in 1978 by Parkman et. al. They reported two patients who achieved normalization of hematological and immunological abnormalities after HCT with the use of anti-human thymocyte serum and total body irradiation (one patient also received procarbazine) [49]. Within a few years, two reports showed that myeloablative doses of busulfan and immunosuppression with cyclophosphamide also corrected the disease [50, 51]. This regimen has been the standard preparative backbone for HCT for WAS, and indeed for most non-malignant disease. Thus the main principles of conditioning for WAS were established nearly 30 years ago.

Initially, HCT for WAS was mostly restricted to patients for whom an HLA-identical sibling was available. The demonstration that *in vitro* T-cell depleted grafts from HLA-mismatched family donors (parents) could successfully reconstitute immunity in patients with severe combined immune deficiency (SCID) [52,53] led investigators to explore a similar approach for WAS. Results, however, have been disappointing. An early report from Memorial Sloan-Kettering showed only 1 of 6 patients surviving; two patients had graft rejection despite TBI based conditioning and EBV positive lymphoma, while 3 developed GVHD [54]. Summary data from the pooled European experience show 45% survival of 43 patients undergoing parental transplant compared to 81% survival of 32 patients undergoing sibling matched transplant [55]. Unlike patients with SCID, who lack T cell function entirely, patients with WAS, even when heavily immunosuppressed, apparently resist engraftment in the T cell depleted setting. Given these poor outcomes and difficulties in particular with post-transplant EBV-driven lymphoproliferative disease, T replete unrelated donor bone marrow transplants were performed with greater frequency, and are indeed the majority performed today (Figure 1).

Current survival after myeloablative HCT

The rarity of WAS and variety of donor sources used (matched sibling, matched and mismatched unrelated adult HSC, haploidentical related, and matched and mismatched cord blood) necessitate cooperative registry studies to analyze even straightforward outcomes such as survival. The European registry reported outcomes of 444 patients with non-SCID immunodeficiency enrolled from 1968 to 1999, of whom 103 patients had WAS with 62% overall 3 year survival [55]. Analysis of the full non-SCID cohort clearly demonstrated that matched sibling donors fared best with 3 year survival of 71% versus 59% and 42% for matched/mismatched unrelated donor and mismatched related, respectively [55]. This result was mirrored in the smaller WAS cohort with 81% of 32 matched sibling donor recipients

surviving versus 45% of 43 mismatched related recipients surviving [55]. A study of the International Bone Marrow Transplant Registry surveying outcomes of patients from a similar timeframe, overlapping with the European cohort, confirmed that matched sibling recipients had a superior 5 year survival (87% of 55 patients) compared to mismatched related recipients (52% of 48 patients) [56]. These data were also independently confirmed in other smaller reports [54,57,58]. Thus unlike the good to excellent outcomes of mismatched related transplants reported for SCID [55,59], poor outcomes following this approach for WAS have led many centers to favor unrelated donors for patients who lack a family match.

The use of closely matched unrelated donors for WAS rose dramatically during the 90's; of 67 patients included in the survey of the International Bone Marrow Transplant Registry, 35 or 53% were transplanted from 1990–1993, and 29 or 43% from 1994–1996. This trend has continued in Europe as shown in Figure 1, depicting both the percentage and absolute numbers of unrelated donor transplants reported to the SCETIDE registry prior to 1995, from 1995–1999 and 2000–2005. For these time periods 12, 12 and 35 patients underwent unrelated donor transplants, respectively, and because the number of mismatched related transplants decreased, the percentage of unrelated donor transplants from 2000–2005 was 71% (Gennery et al, manuscript submitted). This increase in unrelated donor transplants likely reflects both the increasing availability of alternative donors through expansion of the bone marrow donor international registry, and evidence from the CIBMTR study which demonstrated that the survival of 52 boys with WAS undergoing unrelated donor BMT at <5 years old was nearly identical to that of 55 boys undergoing matched sibling BMT (87% for matched sibling, RR of death for unrelated donor < 5 years 1.34). Furthermore, advances in high-resolution typing of HLA alleles has resulted in progressive improvement of donor-recipient matching, and hence optimal selection of unrelated donors. An international survey of 73 centers caring for WAS patients in 2002 revealed that not all centers, particularly smaller ones, offered early HCT for WAS patients, but that 77.7% of larger centers, offered unrelated donor HCT routinely [60].

Experience with cord blood transplantation is accumulating, though very little summary data are available. The first reports of outcome after cord blood transplant for various disorders including WAS were published by Thomson et. al. (1 patient of 30) and Knutsen et. al. (1 patient of 8) in 2000 [61,62]. At least 29 other cases are known from the literature, many reported in aggregate with other diagnoses [57,63–72] and one interesting report details the case of a boy treated with 2 separate unrelated cord blood units infused 8 days apart [73]. The largest report from Japan described 57 patients with WAS, of whom 15 underwent cord blood transplant with 80% 5 year survival (12 of 15), similar to both unrelated donor recipients (80%, 17 of 21) and matched sibling donor recipients (82%, 9 of 11) from the same cohort [57]. Overall survival in these smaller series appear very good, in line with preliminary data from the Center for International Blood and Marrow Transplant Research (CIBMTR) showing 75% 3 year survival in 65 patients transplanted with cord blood under 5 years of age [74].

These studies suffer from limitations due to the long period required to collect sufficient numbers. They fail to capture the effect of incremental changes in clinical care and improved high resolution HLA typing that have impacted on transplant outcome. Thus, in the current era, except for historically poor outcome of haploidentical transplantation, comparative data are not yet mature to draw firm conclusions about the relative merits of donor match or stem cell source.

Immune function and hematopoietic correction after HCT

Complete donor chimerism cures the life threatening manifestations of WAS, including hemorrhage, infection, autoimmunity and malignancy, and can be achieved using

myeloablative doses of busulfan in combination with cyclophosphamide or fludarabine, to facilitate robust and stable donor cell chimerism [75]. However, mixed chimerism has been often reported following HCT for WAS, even when a myeloablative conditioning regimen is used [76,77]. Furthermore, several groups have made use of reduced intensity conditioning regimens (RIC) in the attempt to reduce drug-related toxicity in WAS patients who had significant pre-transplant complications [39,66,78,79]. Typically, the use of RIC for WAS results in mixed/split chimerism, where a variable proportion of lymphoid (especially, T lymphocytes) cells are of donor origin, whereas myeloid cells remain mostly of recipient origin. Preferential engraftment and/or expansion of donor T lymphocytes has also been seen in studies of naturally occurring gene revertants [46], human carriers [41], and heterozygous *Was*^{+/-} mice [17], overall supporting the notion that the selective advantage conferred by normal WASp protein is lineage specific. In particular, it is well known that T cells from carriers of WAS mutations display a nonrandom pattern of X-chromosome inactivation, independent of the severity of the WAS gene mutation, whereas myeloid cells (and to a lesser degree, also B lymphocytes) from carriers of XLT contain a fraction of WASp^{dim} cells [41,80]. Similarly, a stronger selective advantage for WASp⁺ cells within T lymphocytes than in other blood lineages has been also observed in *Was*^{+/-} mice [17]. Finally, accumulation of WASp⁺ T cells has been frequently reported in WAS patients, due to somatic second-site mutations or true reversion events that restore WASp expression; in contrast, few examples are known of gene reversion in B or NK lymphocytes, and no cases of reversion in myeloid cells have been ever reported in WAS ([81] and reviewed in [46]).

Overall, these observations suggest that WASp expressing cells should have a selective advantage in WAS patients developing mixed chimerism after HCT. Indeed, this advantage in transplanted WAS patients is clearest in the T lineage, and is associated with significant clinical improvement, but not full correction of the disease. As stated above, initial attempts at matched related HCT for WAS resulted in donor T cells alone, with cure of T dependent clinical manifestations such as eczema [49]. The percentage of WASp-expressing lymphocytes in 6 patients with mixed chimerism reported by Yamaguchi et. al. was clearly higher at every time point measured than the percentage in monocytes, and donor chimerism was higher within CD8 than within CD4 T cells [77]. After myeloablative transplant, out of 21 patients at a single institution who had evaluation of T, B and monocyte WASp expression, 6 patients had less than 100% donor chimerism, and in 5 of 6, T lineage chimerism was higher than monocyte chimerism. Two patients who had autologous reconstitution in the myeloid lineage, with relapse of thrombocytopenia, nevertheless retained 40–43% of T cells expressing WASp [58].

Severe abnormalities of nTreg and NKT cells, two populations with important immunoregulatory properties, have been observed in patients with WAS and *Was*^{-/-} mice and likely contribute to the autoimmunity of the disease [33–37]. On the other hand, a striking selective advantage for WASp expressing nTreg and NKT cells has been demonstrated in a competitive setting both in humans and mice [33,37]. Therefore, it follows that engraftment of donor-derived nTreg and NKT cells may result in amelioration or even disappearance of autoimmunity in WAS patients who develop mixed chimerism after HCT. However, data from a large European study show that autoimmune manifestations are a common complication after HCT for WAS, and are particularly frequent in patients who develop mixed chimerism [76]. Ascertaining the relationship between cell type specific chimerism and autoimmunity is necessarily difficult given the overlap between autoimmune and alloimmune phenomena post-HCT, and requires further study.

Based on the current literature, we thus surmise that loss of myeloid chimerism need not be accompanied by loss of T chimerism, and that a minimal threshold of myeloid chimerism must be maintained to prevent recurrence of clinically significant thrombocytopenia. It remains

unclear what degree of donor chimerism is sufficient to prevent long-term complications of WAS, particularly autoimmunity. The kinetics and stability of donor chimerism in those with mixed or split chimerism, is also unclear. These questions are the subject of multi-institutional study of 203 WAS patients post-transplant currently under analysis (Moratto et al, manuscript in preparation).

Selection of patients for HCT

Because of the heterogeneity of clinical phenotype in patients with WAS and variable outcome post-HCT depending on factors such as age and degree of HLA match, deciding which patients should be transplanted is not entirely straightforward. Certain subsets of WAS patients have excellent survival after HCT. However, the requirement for full myeloablation to achieve the greatest likelihood of full donor chimerism and freedom from autologous reconstitution necessarily puts some patients at risk of early transplant-related mortality. There is only one comparative study of outcomes after supportive care versus transplantation, published in 1993 [82]. This retrospective review of 62 patients showed that 18 of 31 splenectomized patients were alive, versus 14 of 19 undergoing HCT (12/12, 1/4 and 1/3 following matched sibling, parental and unrelated donor transplants respectively). In the absence of more modern comparative studies that capture the impact of either therapy on long-term complications, the decision to take on the acute and chronic toxicities of transplantation at present should be made in context of known predictors of poor outcome with supportive care alone.

The combination of clinical score and WASp expression allows for informative genotype-phenotype correlation that strongly supports early transplantation for certain patients. The presence of autoimmunity, particularly autoimmune hemolytic anemia or thrombocytopenia (manifested as failure to maintain platelet counts over 20,000 after splenectomy) is associated with a 2–3 fold risk of poor prognosis [29]. Patients achieving a clinical score of 5 due to autoimmunity (or malignancy) are at high risk and should be transplanted. Data to support transplantation based on WASp expression status come from Imai et. al. This retrospective comparison of 27 WASp positive and 23 WASp negative patients in Japan showed that absence of WASp expression correlated best with measures of infection, particularly opportunistic infection; bacterial infection was 4 times more likely in WASp negative patients compared to WASp positive patients, while fungal and *Pneumocystis* infection was only seen in WASp negative patients [11]. The retrospective nature of this study of course is subject to ascertainment bias, but nevertheless showed that the 10 year probability of survival was significantly lower in WASp negative patients (17 of 23 or 76% versus 26 of 27 or 92.3%), even including 11 of 12 patients who survived after HCT [11]. These data suggest that patients with a clinical score of 3 (recurrent bacterial infections) or 4 (severe infection, including opportunistic infection) are likely to be WASp negative, and that patients with scores of 4 or 5 regardless of WASp expression status should be transplanted with the best possible donor at an early age. Because survival after sibling matched or well matched unrelated donor HCT in patients under 5 years of age appears to be equivalent in large summary data [56], a young WASp negative patient with a well matched donor, with a score of 1 or 2, should also be strongly considered for early HCT prior to the age of 5.

Predictors that guide the decision to transplant XLT patients, who generally are WASp positive, with normal or reduced levels of full-length protein, and who generally do not have clinical features meriting a score of 3 or above, are distinctly lacking. While the vast majority of such patients appear to be protected from malignancy, anecdotal reports of family members suffering from lymphoma exist [11]. Some patients do develop autoimmunity, and in fact the presence or absence of WASp fails to segregate with autoimmune disease. Indeed, vasculitis, arthritis, autoimmune hemolytic anemia and IgA nephropathy are all reported in WASp positive patients [11]. Biomarkers to determine which XLT patients will go on to have

autoimmunity do not yet exist. Routinely available tests of immunologic function are also surprisingly lacking in sensitivity and specificity, as many patients with WAS and even lacking WASp protein have normal lymphocyte numbers, proliferation to mitogens, and total immunoglobulin levels, and a few may even demonstrate specific antibody responses to carbohydrate antigens [12]. HCT for XLT patients with clinical scores of 1–2, with WASp expression, should thus generally be limited to those with a matched sibling donor, and alternative donor transplantation considered only for those with severe transfusion-dependent thrombocytopenia or intracranial hemorrhage.

For those patients with high clinical scores, relative contraindications to HCT include older age at transplant or pre-existing organ damage, such as lung disease, particularly if only mismatched donors are available. The propensity of WAS patients to develop mixed chimerism even after fully myeloablative busulfan and cyclophosphamide predicts that reduced intensity conditioning may result in recipient-dominated mixed chimerism or autologous reconstitution. For this category of patients, somatic gene therapy, which has been successfully employed in mouse models and in human cells (reviewed in [83]), can be considered. In light of the relative resistance to replacement of the niche by WASp normal HSC, however, full myeloablation might not be avoidable and will be used in some of the trials currently planned (personal communication Adrian Thrasher, Marina Cavazzana-Calvo).

Conclusions and Future Directions

Since the first transplant to treat WAS in 1968, there has been enormous progress in our understanding of WAS, namely the identification of the causative gene, characterization of the protein and its function, the discovery that XLT is also caused by mutations in WASp, and demonstration of a strong – yet incomplete -- genotype-phenotype correlation. Likewise, studies of knockout mice, human carriers, natural revertants, and patients post HCT have revealed intriguing biology about cell type specific functions of WASp. In the post-HCT setting, development and survival of particular lineages, especially the T lineage, is favored.

Important progress has been made with regard to survival. Allogeneic HCT experience has matured and outcomes have improved as advanced pediatric intensive care, parenteral nutrition, and an expanded antimicrobial armamentarium have all become widely available. Allogeneic HCT for WAS has evolved from a specialized experimental technique reserved for a few patients, to a standard curative therapy to be considered immediately at the time of diagnosis. That well matched unrelated donor HCT in young patients results in similar survival to those receiving sibling grafts, has encouraged the use of HCT to the near exclusion of previously popular supportive measures, such as splenectomy. Indeed because well reconstituted WAS patients post-HCT who were previously splenectomized are nevertheless at risk of severe infections, including fatal sepsis [58,76], we would recommend that splenectomy be reserved only for patients who are unlikely to ever be transplanted.

In turn, there may be fewer and fewer patients for whom transplantation is relatively contraindicated; historically negative predictive factors may no longer portend poor outcome. The availability of sensitive testing for EBV and growing experience with anti-CD20 antibody has revolutionized prevention and treatment of EBV lymphoproliferative disease, such that the once uniformly dismal outcome of haploidentical HCT may have improved significantly [84]. The growing use of cord blood transplantation has expanded donor availability, although the effects of this new donor source and of improvements in HLA matching on outcomes remain to be measured. Whether the previously reported favorable effect of transplanting at a young age on survival will persist in light of these advances also must be reassessed in future studies.

Management of XLT remains controversial. While several groups consider HCT from matched related donors an option for XLT patients who are severely thrombocytopenic, this strategy is

not unanimously accepted, and use of alternative donors is even more controversial in this setting. Progress in this area is hampered by the lack of studies of long-term outcome in large cohorts of untransplanted XLT patients. Defining biomarkers that predict which XLT patients are at risk for late complications, such as lymphoma or refractory autoimmunity, may in turn identify those for whom the merits of early transplantation outweigh the upfront and late toxicities of allogeneic HCT.

The current short and long-term toxicities of HCT remain the main stumbling block for our ability to cure every patient with WAS and XLT, and much remains to be done. Indeed the substance of the treatment itself has changed little. The standard conditioning strategy of busulfan and cyclophosphamide is the same as in 1981. While this approach leads to full donor engraftment in most cases, a moderate proportion of patients develop mixed chimerism. This is even more common when reduced intensity regimens are used, in the attempt to diminish drug-related toxicity. A number of questions remain. What is the minimum level of donor chimerism in the HSC or myeloid compartment required to maintain safe platelet levels post-HCT? How stable is donor chimerism over the long-term and can we predict who will maintain or lose chimerism? Is mixed chimerism in the T compartment, particularly in nTreg or NKT cell lineages, sufficient to control autoimmunity? What degree of donor chimerism is sufficient to prevent malignancy? A multi-institutional retrospective study exploring these questions is nearly complete (Moratto, manuscript in preparation) but ultimately, rigorous prospective long-term studies of cell type specific chimerism and clinical outcome are required to answer these questions. Similarly, the efficacy of any novel conditioning approaches used to improve on the toxicity profile of busulfan and cyclophosphamide must be interpreted in relation to the desired level of chimerism to be achieved in each compartment. Continued multi-institutional and international collaboration to study this rare but fascinating disorder will be needed to answer these questions.

Acknowledgments

This work was supported by the Manton Foundation (to LDN).

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Figure 1.

Hematopoietic cell transplants for Wiskott-Aldrich syndrome in the SCETIDE Registry

The absolute number (left) and percentages (right) of HCT performed for WAS using different donor types (genotypically identical matched sibling, white; phenotypically identical matched family member, speckled; unrelated donor, black; mismatched haploidentical family member, grey striped) as reported to the SCETIDE Registry for the indicated time periods is shown.

(Data courtesy of Andrew Gennery et. al. on behalf of the SCETIDE Registry.)

Table 1

Scoring system to grade the severity of clinical manifestations in patients with Wiskott-Aldrich syndrome and X-linked thrombocytopenia

Score	iXLT		XLT		WAS		
	<1	1	2	3	4	5	
Thrombocytopenia	-/+	+	+	+	+	+	+
Small platelets	+	+	+	+	+	+	+
Eczema	-	-	(+)	+	++	(+)/+/++	
Immunodeficiency	-	-/(++)	(+)	+	+	(+)/+	
Infections	-	-	(+)	+	+/++	(+)/+/++	
Autoimmunity and/or malignancy	-	-	-	-	-	+	

iXLT, intermittent X-linked thrombocytopenia

Scoring system: -, absent; (+), mild; +, present; ++, present and severe

Patients who develop a score 5 are defined as 5A if they present autoimmunity, and 5M if they develop malignancies.

Patients may change their score during life-time. In particular, patients with XLT may progress to WAS and may occasionally reach a score of 5.
(Modified from: Ochs et al., 2009).