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Autologous and Allogeneic Cellular Therapies for High-Risk Pediatric Solid Tumors

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Introduction

Since the 1950s, the overall survival (OS) of children with cancer has gone from almost zero to approaching 80%. While there have been notable successes in treating solid tumors such as Wilms tumor, some childhood solid tumors, exemplified by diseases like high-risk neuroblastoma and metastatic sarcomas, have continued to elude effective therapy[1]. With the use of megatherapy techniques such as tandem transplantation, dose-escalation has been pushed to the edge of dose-limiting toxicities, and any further improvements in event-free survival (EFS) will have to be achieved through novel therapeutic approaches.

In this chapter, we will review the status of autologous and allogeneic hematopoietic stem cell transplantation (HSCT) for many pediatric solid tumor types. The vast majority of the clinical experience in transplant for pediatric solid tumors is in the autologous setting, so we will review some general principles of autologous HSCT, followed by an examination of HSCT for diseases such as Hodgkin disease, Ewing sarcoma, and neuroblastoma. Finally, we will look to the future of cell-based therapies by considering some experimental approaches to effector cell therapies.

(1) Principles of autologous HSCT

Prior to the introduction of high-dose chemotherapy (HDC) with autologous stem cell rescue (also called autologous HSCT), marrow tolerance was the limiting factor in the escalation of chemotherapy for the treatment of malignancies. With the ability to safely harvest, store and re-infuse a patient's own hematopoietic stem cells, doses of cytotoxic therapies for cancer could safely proceed beyond marrow tolerance, thereby allowing more intense treatment of certain malignancies. Two approaches to the use of HDC with stem cell rescue include: (1) myeloablative regimens, meaning that no hematopoietic recovery can occur without the stored HSCs; and (2) sub-myeloablative HDC regimens in which stem cell rescue is used to

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speed recovery, decrease toxicity and decrease the interval between courses of chemotherapy, although it is not absolutely required for engraftment[2–3]. Although the increased treatment intensity may improve disease-free survival for patients with some malignancies, this must be balanced with the increased treatment-related mortality associated with the higher doses of cytotoxic agents, as well as the potential late effects of more intense cytotoxic treatments and radiotherapeutic regimens in young children. Criteria that may help define circumstances in which HDC with stem cell rescue would be most beneficial include: (1) a tumor with good response to induction chemotherapy, but a poor 3 or 5-year EFS; and (2) a HDC regimen that can utilize multiple agents active against the disease, especially if the agents differ from those used during induction therapy. Although the use of HDC with stem cell rescue is controversial in most diseases, diseases such as Hodgkin disease and high-risk neuroblastoma (discussed below) meet the design criteria listed above and have demonstrated improved outcomes in clinical trials.

(2) Hodgkin disease

Although most pediatric patients with Hodgkin disease achieve excellent long term survival with standard chemotherapy and low dose radiation therapy, with EFS and OS of 80% and 90% respectively, a significant number of patients have refractory disease or experience relapse[4–6]. Poor prognosis in these relapsed patients is associated with chemotherapy resistant disease, short time to relapse (less than one year) and extra nodal disease at relapse as well as poor performance status in adult patients[7–8].

Adult studies comparing conventional salvage therapy with HDC with autologous stem cell rescue demonstrated benefit of the HSCT approach in relapsed disease [9–11]. Following up on a pilot study in 1991 that suggested HSCT might be a better front line therapy for high risk patients, a randomized trial was conducted comparing conventional therapy with HSCT[12–13]. Using a foundation of 4 cycles of ABVD (doxorubicin, bleomycin, vinblastine, dacarbazine), patients with high risk features (high LDH, mediastinal mass, >1 extranodal site, anemia or inguinal disease) were assigned to either 4 more cycles of ABVD or HSCT. There was no difference in EFS or OS, discouraging HSCT as front line therapy for high risk patients. Linch et al compared a standard intensified HDC regimen (BCNU, etoposide, cytarabine and melphalan [BEAM]) and autologous stem cell rescue with mini-BEAM in a randomized trial for relapsed and refractory adult patients, finding improved EFS and lower relapse rate in the intensified arm but similar OS[14]. Lastly, a large randomized study of patients aged 16–60 years with relapsed Hodgkin disease compared 4 cycles of nonmyeloablative Dexamethasone-BEAM to 2 cycles of Dexamethasone-BEAM plus a high dose BEAM with HSCT. EFS was 55% at 3 years for the HSCT group and only 34% for conventional therapy ($p=0.019$)[15]. In both trials, the lack of difference in OS may be due in part to the fact that patients who relapsed after conventional therapy went on to receive HSCT and were salvaged by that regimen.

As the incidence of Hodgkin disease places it in an age group of mostly adolescents and young adults, many studies have pooled pediatric patients (<18 years) with older patients for study. A case control series examining HSCT in children <16 years at diagnosis compared with a population >16 years found progression free survival was similar (39% vs 48%), as were most of the secondary measures and subgroup analysis between these older and younger patients[16]. This study also confirmed chemotherapy resistant disease is a poor prognostic factor in children as well as adults. A retrospective analysis of 51 children receiving autologous HSCT compared to 78 children receiving conventional salvage therapy did not find an advantage to HSCT, but may have been biased because the group proceeding to transplant had more adverse disease characteristics[17], a common issue with such retrospective analyses. Baker et al. reported on patients <21 years at time of transplant for relapsed or refractory Hodgkin disease: 5 year OS was 43% and EFS 31%, and no difference

was observed in 3 age brackets (<13 years, 13–18 years and 19–21 years)[18]. As HSCT became more common, smaller case series were published in children from Spain, Germany and Austria which again identified the presence of bulky or extranodal disease at time of HSCT as an independent poor prognostic factor in children[19–20].

The role of allogeneic HSCT has also been investigated for relapsed Hodgkin disease. While early use of myeloablative regimens and allogeneic HSCT resulted in high transplant related mortality (TRM) without much indication of benefit, some groups have reported disease regression with donor lymphocyte infusions, suggesting a graft-versus-lymphoma effect is possible[21–22]. As of this writing, there have been no randomized comparisons of allogeneic and autologous transplant for relapsed Hodgkin disease. A single center study from the Fred Hutchinson Cancer Research Center comparing 53 patients getting allogeneic HSCT to controls receiving autologous HSCT did find a significantly lower relapse rate in the allogeneic recipients (45% vs. 76%)[23]. EFS was not significantly different, and in allogeneic transplant group the competing risk of TRM was quite high (53%). The role of reduced intensity conditioning (RIC) regimens in the allogeneic transplant setting is being investigated to reduce TRM and potentially expand the graft-versus-lymphoma effect. A recent report from Europe compared 89 patients receiving a RIC allogeneic HSCT with 79 patients receiving a traditional myeloablative regimen[24]. About half of these patients had received a prior HSCT, and all were heavily pretreated. OS was superior in the RIC group (28% vs. 22%) despite a higher relapse rate (57% vs. 30%). This was likely due to the significant reduction in TRM in the RIC group (23% vs. 46%). A recurring theme in these patients is the poor prognostic indicators of chemotherapy resistant disease and presence of bulky disease at time of transplant. This study continues a trend of earlier single center reviews, and suggests that RIC allogeneic HSCT, either alone or coupled to an autologous HSCT, may have a role in the treatment of multiply relapsed patients with Hodgkin disease[21, 25–26].

While autologous HSCT is the treatment of choice for relapsed or refractory Hodgkin disease, the addition of newer agents such as monoclonal antibodies and tyrosine kinase inhibitors to conventional regimens has not been studied in a randomized fashion against HSCT. In addition, patients with chemotherapy resistant disease or bulky disease at time of HSCT still do quite poorly. RIC allogeneic HSCT should be considered for patients who relapse following autologous HSCT, and more study is needed to further identify ways to reduce TRM and increase the potential of graft versus lymphoma.

(3) Non-Hodgkin Lymphoma

Pediatric non-Hodgkin lymphoma (NHL) consists mainly of Burkitts, lymphoblastic, diffuse large B cell (DLBCL) and anaplastic large cell lymphoma. Conventional chemotherapy remains the front-line treatment of choice, with long term survival in the 60–90% range depending on histology[27–29]. Relapsed disease carries a much more dismal prognosis, and autologous HSCT has been investigated for these high risk patients. A Children's Cancer Group (CCG) study for relapsed lymphoma did not find a benefit to autologous HSCT for these patients, as EFS was not significantly changed compared to other salvage regimens[30]. A comprehensive review by Gross et al. found that some patients with relapsed NHL can be salvaged by autologous or allogeneic HSCT. As with Hodgkin disease, chemotherapy resistant disease and disease status at time of transplant significantly impact survival. As most single center experiences include multiple types of NHL to acquire enough cases for review, separating effects within each subtype is difficult. A trend towards improved salvage with allogeneic HSCT in lymphoblastic lymphoma is seen, though this is biased by the greater number of patients who underwent this procedure compared to autologous HSCT[31]. As the numbers of pediatric patients with relapsed or refractory NHL

remain small, studying the role of autologous and allogeneic HSCT against conventional therapy will remain difficult.

(4) Ewing sarcoma

Ewing sarcoma is the second most common bone tumor in children after osteosarcoma, and carries a 70% long term survival for localized disease. The backbone of this therapy includes surgical resection, anthracycline and alkylator chemotherapy (typically doxorubicin and ifosfamide) and in some cases radiation therapy. Patients with metastases, however, have a much worse outcome (4 year OS 39%) and survival after relapse is also dismal (10 year OS 10%)[32–35]. Escalation of therapy in patients with metastatic Ewing sarcoma with the core treatment agents ifosfamide, doxorubicin and cyclophosphamide or addition of ifosfamide/etoposide to a standard regimen only seemed to increase short term toxicity and secondary myelodysplasia[35].

In higher-risk Ewing sarcoma patients, autologous HSCT has been investigated for poor prognostic groups such as patients with large, unresectable tumors, patients with metastatic disease, or that subset of stage 4 patient with metastases outside of the lung, the highest risk group. A CCG study of 36 patients with Ewing sarcoma metastatic to the bone marrow at diagnosis investigated the efficacy of melphalan, etoposide and TBI followed by autologous HSCT. This led to no improvement in 2 year survival (20%) over conventional therapy[36]. These disappointing results were replicated in three other studies, using both allogeneic and autologous stem cell sources, with high rates of TRM raising further concerns[37–39]. The ongoing EuroEWING-99 trial has a study question comparing autologous HSCT with intensified standard therapy for patients with a poor local response or with lung metastases. Patients are randomized after a standardized vincristine, ifosfamide, doxorubicin and etoposide (VIDE) induction phase. The data collection is ongoing.

For those patients who have relapsed Ewing sarcoma, the outlook is grim, with a 10% 5 year OS[40]. In two single center studies, a total of 32 relapsed Ewing sarcoma patients underwent megatherapy (26 with autologous HSCT, 6 with allogeneic HSCT). Fifteen of 32 patients were reported as long term survivors, though they represent a rare subgroup that was able to achieve a second remission in this disease.[38, 41] and it is unclear if HSCT provides an advantage over intensified standard chemotherapy in these small highly selected groups. A common observation is that the outcome of megatherapy or autologous HSCT in the presence of gross residual disease is exceptionally poor (5 year OS 19%)[42]. Nevertheless, there exist been enough data to support design of studies comparing autologous HSCT to standard intensified therapy for high risk and relapsed patients, and we look forward to the results of the EuroEWING-99 trial.

(5) Rhabdomyosarcoma

Rhabdomyosarcoma is the most common sarcoma of childhood, and children with low or intermediate risk disease have excellent long term survival rates with standard chemotherapy approaches. As with neuroblastoma, high risk patients continue to do poorly despite intensification of non-myeloablative chemotherapy as in the IRS-III and IRS-IV trials (5 year OS 30% for metastatic disease, Group IV)[43–44]. In vitro and in vivo studies of relapsed rhabdomyosarcoma samples suggest sensitivity to melphalan, and based on this HSCT approaches to relapsed or high risk rhabdomyosarcoma were designed [45–46]. Of 98 HSCTs for relapsed or progressive rhabdomyosarcoma in children performed through 1994 in Europe, the OS was not different than historical controls at 20%[47]. Single institution studies with various criteria to define ‘high risk’ rhabdomyosarcoma have replicated these data, with children in remission status receiving megatherapy achieving some degree of long term OS, though it is unclear if this will be superior to intensified conventional therapy[48].

Although a randomized trial of autologous HSCT as a consolidation for high-risk patients without gross residual disease could be contemplated, there is little support for this approach in the current literature.

(6) Neuroblastoma

Neuroblastoma is the most common extra-cranial solid malignancy of childhood, and has a broad spectrum of clinical presentations and behavior. While low- and intermediate-risk neuroblastoma are mostly curable[49–50], high-risk neuroblastoma has proven refractory to conventional treatment modalities[1, 51–52]. Despite the unsatisfactory responses to conventional therapies, some improvements in outcome have been achieved through the escalation of therapeutic intensity[53]. Although even the most intense conventional therapy results in long-term EFS of less than 40%, improvements can be achieved through the addition of consolidation therapy with high-dose therapies that exceed marrow tolerance. This was originally achieved through the harvest, storage and re-infusion of autologous bone marrow capable of re-establishing tri-linear hematopoiesis[52], and later replicated using peripheral blood stem cells (PBSC)[54]. Even with this intensified consolidation therapy, outcomes still remained relatively poor. However, the ability to collect adequate PBSC in small children, along with the decreased TRM associated with their use, has allowed for even more intense consolidation therapy by enabling tandem autologous HSCT. Early studies suggest that this is a feasible approach that may improve outcomes[51, 55].

Early, non-randomized studies—Having acquired the ability to safely harvest, store and re-infuse HSC, investigators in the late 1980s and early 1990s began exploring the hypothesis that increased treatment intensity beyond marrow tolerance would improve survival in patients with high-risk neuroblastoma. Multiple early single-arm or retrospective studies suggested that autologous HSCT might indeed improve the EFS of these patients, although none of the studies were randomized and may have been influenced by selection bias[56–61]. The largest retrospective analysis was performed through the EBMT in 1997; 1,070 transplants for high-risk neuroblastoma patients were analyzed, and the 2-year survival among the group of patients who had reached a SCT procedure was 49%. Most relapses occurred within the first 18 months following transplant, and there were no survivors amongst the group of the 48 patients who relapsed and underwent a second HSCT. Notably, late relapses were found as long as 7 years from transplant[62].

Randomized trials—The promise suggested by these early studies propelled prospective evaluation of autologous HSCT for high-risk neuroblastoma. The largest of the randomized, prospective studies was a phase III trial performed by CCG. In CCG-3891, patients were randomized to a consolidation regimen consisting of autologous bone marrow transplant versus continuation chemotherapy. Following consolidation, patients were then randomized to biologic therapy with 13-*cis* retinoic acid (a maturational agent) versus no further therapy[52]. The study found that those treated with autologous HSCT had a significantly better EFS than those treated with chemotherapy alone. It was also noted that treatment with 13-*cis*-retinoic acid further improved outcome among patients without progressive disease. With an estimated 38% EFS 3.7 years from diagnosis in the best group, this study helped establish autologous HSCT followed by 6 months of oral cis-RA therapy as the new standard of care for these patients, and represented an important step forward in the treatment of high-risk neuroblastoma. Despite its important results, this study was subject to the challenges of a complex treatment plan and the 2 × 2 factorial design. Of 579 eligible patients, 379 underwent the first randomization, and 258 patients underwent the second, thereby reducing the population of patients being studied to approximately 50 patients in each of the four treatment groups. Other studies of autologous HSCT in high-risk neuroblastoma have since built on the results of CCG-3891[2, 51, 63]. Of importance,

conditioning regimens used in subsequent studies have varied widely, with the greatest difference being that some studies have used total body irradiation (TBI) in the conditioning regimen while others did not. There have been no randomized trials of the use of TBI during conditioning, and while it may improve outcomes, it also results in significant late effects in those who survive treatment. Thus, although the use of radiotherapy to the tumor bed is standard of care in neuroblastoma, the use of TBI remains controversial. Overall, these studies have led to the current core standard for neuroblastoma treatment in 2009: 5–6 cycles of induction chemotherapy, surgery, radiotherapy (at a minimum to the tumor bed) and autologous HSCT followed by oral cis-retinoic acid. To this, we may now add GD2-targeted immunotherapy based on recent data from the Children's Oncology Group (COG) ANBL0032 study presented at ASCO in 2009, which showed a superior outcome in patients who received this immunotherapy in addition to cis-retinoic acid.

Tandem transplantation—Given the evidence that dose-intensity correlates with outcome, and that HDC with autologous stem cell rescue renders a statistically significant improvement in survival, it was logical to examine sequential courses of HDC with stem cell rescue, otherwise known as tandem transplant. Tandem transplantation allows for even greater dose intensity in consolidation, with the potential to introduce different active agents at each transplant. A very early attempt to employ this technique was complicated by unacceptable TRM, primarily related to extended periods of neutropenia following the transplants[64]. Although initially discouraging, this study, like CCG-3891, was conducted using bone marrow as the stem cell source. Other stem cell sources, specifically PBSC, can provide more rapid engraftment and faster recovery times than bone marrow. The more rapid engraftment of PBSC results in decreased days with severe neutropenia and a shorter duration of mucositis, thereby resulting in a lower rate of infectious complications. With the high TRM found using bone marrow as the stem cell source for tandem transplantation, PBSC became an attractive alternative. Despite the challenges inherent in collecting PBSC from patients with high-risk neuroblastoma (young age at diagnosis, small size and blood volume), clinicians can now safely, effectively and routinely perform this procedure[65]. Following the switch from bone marrow to PBSC, several groups have re-tested the tandem transplant approach with more promising results[2, 51, 63]. The largest of these studies was conducted over 6 years at 4 cooperating institutions. The study was designed using early collection of PBSC, CD34 selection as a purging method (discussed below) and two myeloablative regimens containing distinct agents: (1) carboplatin, etoposide and cyclophosphamide, followed by (2) melphalan and TBI. TRM in this study was 6%, and included two patients who died of Epstein Barr virus lymphoproliferative disease (EBV-LPD). The issue of post-HSCT immunosuppression will be discussed below. Longer follow up of this treatment approach in a large phase II cohort has demonstrated a 3-year EFS from diagnosis of consecutively enrolled patients of 55% (most recent update shown in Fig. 1). A second multiple cycle autologous HSCT study, performed using 3 sequential HSCT procedures, found comparable results in terms of 3 year EFS (57%), although there appeared to be instability of the curve out to 4–5 years[2]. In that study, 19 of the 25 patients completed the second autologous HSCT and 17 went on to the third. Only one late TRM was observed. Based on these promising results, the current open phase III COG trial, ANBL0532, is testing single versus tandem transplant as consolidation therapy for high-risk neuroblastoma.

Processing of stem cells—In addition to increasing dose intensity, graft manipulation has been used to attempt to improve survival following autologous HSCT in neuroblastoma. Engineering of the HSC graft is possible to remove or expand desired cell populations. The most researched manipulation in the context of neuroblastoma has been purging of malignant cells prior to the infusion of the HSC product. Research in the 1990s suggested

that clonogenic tumor cells can be infused with a HSC graft, and that these cells can result in relapse of the malignancy[66]. This led to trials in neuroblastoma addressing the question of whether purging stem cell products of tumor cells could further improve post-transplant overall and disease-free survival. There are two methods to purge a HSCT product of tumor cells – either positive selection of HSC leading to the exclusion of tumor cells, or negative selection designed to specifically remove malignant cells. Positive selection of CD34 expressing cells is the primary technique available to most stem cell labs. CD34 is an antigen expressed on HSC and progenitors of all hematopoietic lineages, and positive selection of CD34 would result in the exclusion of neuroblastoma from the graft – assuming that the neuroblastoma cells themselves do not express CD34. Concerns have indeed been raised that some neuroblastoma cells may express either CD34, or surface epitopes cross-reactive with the anti-CD34 monoclonal antibodies necessary for the selection process[67–68]. Our data have not confirmed this hypothesis[69], and we, along with others, have used CD34 selection as a purging technique for PBSC products in the clinical setting[70].

Automated processes are available that are capable of selecting the CD34+ cell population away from the 99% of peripheral blood mononuclear cells that are irrelevant for engraftment, including T cells and any tumor cells that do not express CD34. Of these automated technologies, the Isolex 300i device is FDA approved, and the Miltenyi CliniMACS device is approved in Europe and may become available in the United States. In terms of negative selection, the most widely used technique in neuroblastoma has been anti-tumor monoclonal antibodies followed by a magnetic depletion step[71–72]. Although the evidence suggests that purging of bone marrow may be important, PBSC are less likely to contain tumor cells than bone marrow, and no study to date has shown that purging itself improves outcome. The COG has assessed whether graft manipulation through negative selection improves survival. COG A3973 is a recently completed, phase III, randomized comparison of purged versus unpurged PBSC given in the context of autologous HSCT for high-risk neuroblastoma. Data from this trial have yet to be published, but preliminary analyses have shown no advantage for patients receiving a purged PBSC product (S. Kreissman and W. London, unpublished data). The 2-year EFS was 51% in the unpurged group, and 47% in the purged group ($P=0.47$). The overall estimated 3-year EFS was 40%.

In 2009, the standard treatment for a patient with newly diagnosed high-risk neuroblastoma is based on the premise that maximal tolerable intensity of therapy yields maximal positive outcomes. The clinical trials outlined above have resulted in a therapeutic backbone of multi-cycle induction, PBSC collection early in induction, testing of the PBSC product for neuroblastoma contamination, as complete surgical resection as possible without organ sacrifice, autologous HSCT, and local radiotherapy, followed by biologic and immunotherapy. It is an imposing package, and in the quest to further improve outcomes, the current phase III COG ANBL0532 trial is testing single versus tandem transplant as consolidation therapy. Although maximum tolerable intensity of cytotoxic therapy has certainly been achieved, the outcomes for patients with high-risk neuroblastoma remain relatively poor, with a 5-year OS under 50%. Having reached an effective limit in terms of chemotherapeutic intensity with tandem transplant, future trials will need to focus on targeted therapies and/or immunotherapy in the hope of improving outcomes for children afflicted with this disease.

High-dose mIBG with stem cell rescue—The neuroendocrine nature of neuroblastoma makes it amenable to therapy with radiopharmaceutical agents as well. Arising from the adrenal medulla, 90–95% of neuroblastomas show characteristic uptake of catecholamines and their derivatives, making them attractive radiopharmaceutical agents. Metaiodobenzylguanidine (mIBG) is an aralkylguanidine analog of catecholamine precursors, first reported in 1979, that can be labeled with ^{123}I or ^{131}I and imaged with a

gamma camera[73]. The first report using mIBG for diagnosis and localization of neuroblastoma was in 1985[74]. Since that time, diagnostic imaging with [^{123}I -m]IBG has become the standard of care.

[^{131}I -m]IBG is a higher-energy releasing isotope that has been used for therapy of neuroblastoma since the mid-1980s. A review of the literature in 1999 showed an objective response rate to [^{131}I -m]IBG of 35% across multiple small studies, and a phase I study of its efficacy showed a response rate of 37% in children with relapsed neuroblastoma[75–76]. The dose-limiting toxicity of the [^{131}I -m]IBG is hematologic, although the marrow failure associated with high-dose [^{131}I -m]IBG can be overcome using stem cell rescue[77–78]. Building on these findings, several groups began to incorporate high-dose [^{131}I -m]IBG into the conditioning regimen for autologous HSCT. Initial, small scale pilot studies demonstrated the tolerability of high-dose [^{131}I -m]IBG combined with standard myeloablative chemotherapy[79–81]. This led to a larger, phase I study that again reported good tolerability of this combination in patients with refractory neuroblastoma, but also reported an overall survival of 58%[82]. A phase II study combining high-dose [^{131}I -m]IBG and intensive chemotherapy as consolidation is currently underway for patients with high-risk neuroblastoma. Novel modalities for using this unique radiopharmaceutical in the treatment of neuroblastoma promise further improvements in outcomes for patients with high-risk disease.

(7) Allogeneic HSCT

Allogeneic transplant for solid tumors of childhood has been studied in a very limited fashion and is rarely pursued. Some of this may be due to the neuroblastoma experience, wherein allogeneic marrow sources were not superior to purged autologous sources (see below)[83–84]. No convincing evidence of any graft versus solid tumor effect has been demonstrated in pediatric patients, although case reports continue to be suggestive. In contrast, some of the earliest reports of allogeneic graft versus solid tumor reports were in adult metastatic renal cell carcinoma[85], where 4 of 50 patients receiving myeloablative therapy and allogeneic stem cells were long term survivors. A few other reports of allogeneic activity against adult tumors exist including colon carcinoma, ovarian carcinoma and prostate carcinoma[86–88]. In review of these adult case reports and other small series, Ringden et al comment that the allogeneic effect may be a blunt application of immunotherapy that could be much more specifically applied with monoclonal antibodies without TRM[89].

This is not to discount the potential benefit of immunotherapy or cellular therapy for solid tumors, however pure myeloablation and allogeneic reconstitution does not appear to provide any specific benefit for pediatric solid tumors in the face of the considerable risk of GVHD and TRM. A recent report from Japan using a model of a murine bladder tumor, RIC and allogeneic reconstitution followed by DLI demonstrated some anti-tumor effect[90]. A recent case report from France observed that an adult patient undergoing RIC and allogeneic transplant for AML had concomitant regression of a malignant renal tumor[91].

Similarly, the potential to harness an immunotherapeutic effect has led some groups to study allogeneic HSCT for high-risk or relapsed neuroblastoma. A 2003 case report described a patient in whom residual disease noted following a haplo-identical HSCT fully resolved three years later, hinting at a potential graft-versus-neuroblastoma effect[92]. However, while the promise of a graft-versus-malignancy effect has been well-described in allogeneic transplant for liquid tumors, it has yet to be convincingly demonstrated in the setting of solid tumors[93]. Two studies published in 1994 compared allogeneic to autologous HSCT for high-risk neuroblastoma. The first compared the outcomes of 20 patients who underwent a single, HLA-matched sibling donor transplant to 36 patients who underwent autologous

transplants following identical TBI-containing conditioning regimens. Four of 20 allogeneic patients experienced TRM, compared with three of 36 autologous patients ($P = .21$). The relapse rate among allogeneic HSCT patients was 69%, compared with 46% for autologous HSCT patients ($P = .14$), and the estimated PFS rates 4 years after HSCT were 25% for allogeneic HSCT patients and 49% for autologous HSCT patients ($P = .051$)[84]. A second, case-controlled, study compared 17 allogeneic and 34 autologous cases. It found no difference in progression-free survival, 35% and 41% at 2 years respectively[94]. Although these initial results do not show any clear benefit of allogeneic vs. autologous HSCT for high-risk neuroblastoma, the advent of RIC regimens has provided the possibility that reduction of TRM will allow for the detection of a therapeutic benefit. The limited data available to date indicate that is no current role for allogeneic transplant for solid tumors in pediatric patients outside the context of well-designed clinical trials.

(8) Cellular immunotherapy

Having reached an effective limit in chemotherapeutic intensity with tandem transplant, any further improvement of survival in children with high-risk neuroblastoma will have to come from novel therapeutic approaches. The most immediate hope for an effective different treatment modality lies in immunotherapy. While several groups have published on the potential benefit of anti-neuroblastoma monoclonal antibodies[95–96], the focus of this section will be on potential cellular immunotherapies.

T cell augmentation for neuroblastoma—There were 3 cases of EBV-LPD seen among patients treated on the CHOP/DFCI tandem transplant study for neuroblastoma[97]. EBV-LPD is associated with significant immunosuppression and is usually uncommon following autologous HSCT. The Mackall group has suggested that T cell depletion that is produced by CD34 selection (as used in that study) may not increase immunosuppression[98–99]. However, the CHOP/DFCI experience would suggest that the combination of the use of a CD34 selected PBSC product and tandem transplant including TBI may be significantly more immunosuppressive than conventional autologous HSCT using unmanipulated PBSC[70, 97].

Regardless of the regimen used, the issue of immunosuppression induced by autologous HSCT is extremely important when considering alternative approaches to treating high-risk neuroblastoma. Although there is some suggestion that tandem HSCT may improve outcome in these patients (above), it is indisputable that we have reached the limit of dose escalation. An alternative approach is required. T cell-based therapies, possibly paired with a cancer vaccine, represent a major area to explore novel treatments[100–101]. However, the limitations are clear: T cells, which may have anti-tumor efficacy, are not suited to treating bulk disease and almost certainly best deployed at the point of minimal residual disease. This is the point reached after chemotherapy, surgery, radiation and HSCT. Immunotherapy and/or tumor vaccines should be deployed as quickly as possible after completion of conventional therapy, but this is also a point where numbers of T cells and effector function are minimal to absent. One solution to this problem is to provide T cells to the patient in an attempt to speed immunological recovery. This also has the potential to harness a profoundly lymphopenic environment supportive of homeostatic expansion. Unfortunately, the passenger T cells provided with a PBSC product, although large in number, do not provide this solution, as recovery of cellular immunity after standard autologous HSCT takes many months.

We have recently tested an alternative approach in studies at the University of Pennsylvania and Children's Hospital of Philadelphia. The cell product utilized in all of these studies is ex-vivo activated and expanded autologous T cells, using an artificial "antigen presenting

cell” of anti-CD3 and anti-CD28 activating antibodies coupled to beads[102]. The GMP cell manufacturing process produces a highly activated polyclonal T cell population, with a T cell repertoire representative of the full repertoire of the cells input into the culture[103–104]. We have referred to the infusion of these activated T cells into lymphodepleted patients as T cell augmentation (TCA). We have completed a phase I trial of TCA in adult and pediatric patients with high-risk lymphoma following autologous CD34-selected PBSCT[105], demonstrating promising normalization of lymphocyte counts. In many cases, an absolute lymphocytosis was observed following TCA, suggesting that homeostatic T cell proliferation was induced.

In ongoing studies, we have tested TCA in patients with high-risk neuroblastoma (unpublished data, S. Grupp). In a series of studies, we are assessing the impact of TCA on immune reconstitution in these profoundly immunodeficient patients. These patients are an interesting group to study TCA, as the need for HSCT is known at diagnosis and T cells may thus be collected prior to exposure to any immunosuppressive chemotherapy. Some of our preliminary results are presented in Fig. 2. Patients getting a CD34-selected PBSC product have slow recovery of CD4+ T cells, which is significantly and strikingly improved after TCA given on d+12 after PBSC infusion. Interestingly, CD4 recovery is even more rapid when the infusion time is moved to d+2, with supranormal lymphocyte and T cell counts apparent as soon as 10 days after TCA. Among patients receiving d+2 TCA, we have observed lymphocyte counts on d+12 post-HSCT as high as 10,000/ μ L. Four of these patients experienced an engraftment syndrome clinically indistinguishable from autologous GVHD, with fever, a rash characteristic of GVHD and, in the two cases where skin biopsies were performed, biopsies consistent with GVHD as well. In the current study, we are assessing the impact of TCA on response to two vaccines, Prevnar conjugate vaccine and influenza vaccine. Preliminary analysis of the patients receiving Prevnar on d+12 post-SCT shows protective anti-pneumococcal antibody titers to multiple serotypes as early as d+30 (S. Grupp, unpublished data), which supports the hypothesis that TCA could be used to support an anti-cancer immunization strategy early after HSCT and achievement of MRD. Similar results in patients with myeloma receiving TCA and Prevnar vaccination have recently been published by Carl June and co-workers[106].

A possible target for a therapeutic cancer vaccine could be the cancer antigen survivin. Survivin is expressed in neuroblastoma, with expression correlating with adverse outcome[107–108]. In our studies, we have observed high expression of survivin in all tested tumor biopsies from high-risk neuroblastoma patients[109]. More importantly, we have found that most patients who are HLA-A2+ and are thus assessable for T cells recognizing survivin by tetramer staining have such T cells. These T cells can be expanded and will kill both allogeneic and autologous neuroblastoma in the appropriate HLA context. When whole neuroblastoma RNA is transfected into antigen-presenting cells and these cells are used to expand T cells with neuroblastoma specificity, the immunodominant epitope in the effector T cell response is survivin[109].

Chimeric immunoreceptors—Although anti-neuroblastoma monoclonal antibodies have had promising results, antibody-mediated antitumor activity may be dependent on functional adaptive immunity. Patients with neuroblastoma have been heavily treated with cytotoxic chemotherapy and radiation, with resulting immunosuppression, lymphopenia and dysfunctional T-cells. While these challenges may be partly overcome by the TCA strategy described above, direct tumor targeting of neuroblastoma by T-cells is additionally hampered by a paucity of tumor-specific antigens, and by the requirement for antigen processing and MHC-restricted antigen presentation. These immune-evasion strategies may be confronted through the generation of a chimeric immunoreceptor (CIR). The CIR is an engineered T-cell receptor (TCR) comprised of an antibody-like extracellular domain fused

to an intracellular, functional TCR domain. The CIR was first described by Eshhar in 1993[110], and has been developed and extended over the last 15 years. The first report of CIR-modified T-cells specific for neuroblastoma was published in 2001[111], and research since that time has led to an early-phase clinical trial published in 2007[112]. To safely re-direct T-cells against a tumor, the CIR must target a tumor-specific antigen that is minimally expressed on normal tissues.

In the Park trial the authors targeted the L1 cell-adhesion molecule (L1-CAM). L1-CAM is expressed on neuroblastoma cells and to a lesser extent on normal adrenal medulla and sympathetic ganglia. The trial was designed to test the feasibility harvesting, genetic modification, expansion and re-infusion of autologous T-cells, as well as safety. The feasibility was successfully demonstrated, and there were no neural toxicities associated with the infusions. While outcome was not a primary measure of the trial, there was at least one patient with a complete response. An important 2008 study from the Brenner group used the CIR approach, engineering Epstein-Barr virus-specific cytotoxic T lymphocytes (EBV-CTLs) to express a CIR recognizing GD2 and using these cells in an 11 patient clinical trial. The use of EBV-CTLs was chosen to address the issue of persistence in the recipient, as conventionally activated T cells used in clinical trials have often demonstrated poor persistence and expansion. The study demonstrated much better persistence of the EBV-CTLs compared to activated T cells. Four patients demonstrated responses[113]. These trials, as well as others examining the use of CIR-modified T-cells in other malignancies, have demonstrated the feasibility of using genetic modification to re-direct autologous T-cells against malignancies. As technologies improve, and the experience with CIRs increases, harnessing a patient's own immune system in the battle against high-risk pediatric cancers will likely become a promising new therapeutic frontier.

Conclusion

In designing future therapies, consideration will also have to include the long-term consequences of those therapies. As an example, although almost 1 in 2 patients with high-risk neuroblastoma may become long-term survivors with intensive multimodal therapy, their quality of life must also be considered. A recent case series examining 23 survivors of high-risk neuroblastoma treated with autologous HSCT demonstrated that these patients can experience long-term complications including growth (100%) and pubertal failure (83%), hearing impairment (73%), orthopedic complications (63%), renal impairment (47%) and thyroid abnormalities (36%)[114]. This serves as a reminder that although the imperative to cure children with this terrible disease is strong, those designing therapeutic interventions must recall the motto 'cure is not enough'.

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Figure 1.

Kaplan–Meier curves showing OS and EFS for consecutively treated patients undergoing tandem autologous HSCT for high-risk neuroblastoma. The patients were conditioned with carboplatin/etoposide/cyclophosphamide for the first autologous HSCT procedure, followed by melphalan/TBI for the second. (a) OS from diagnosis, (b) EFS from diagnosis and (c) EFS from diagnosis stratified by MYCN (nMYC) amplification. Modified from Fish and Grupp, Bone Marrow Transplantation (2008) 41, 159–165.

Figure 2.

Effect of TCA on peripheral blood CD4 cell recovery after immunoablative and myeloablative tandem HSCT for neuroblastoma. Patients were given autologous, costimulated, activated T cell infusions on the indicated day after the second HSCT. In 4 patients, an engraftment syndrome (Eng Synd) was observed, consisting of a pruritic rash with or without fever. This correlated with higher CD4 counts after TCA. Similar results were seen in the CD8 count (data not shown).