

## Report



# Proceedings from the National Cancer Institute's Second International Workshop on the Biology, Prevention, and Treatment of Relapse after Hematopoietic Stem Cell Transplantation: Part II. Autologous Transplantation—Novel Agents and Immunomodulatory Strategies

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## A B S T R A C T

In the National Cancer Institute's Second International Workshop on the Biology, Prevention, and Treatment of Relapse after Hematopoietic Stem Cell Transplantation, the Scientific/Educational Session on Autologous Transplantation addressed the role of novel agents and immunomodulatory strategies in management of relapse after autologous hematopoietic stem cell transplantation (AHSCT). Concepts were illustrated through in-depth discussion of multiple myeloma, with broader discussion of areas relevant for relapse of other malignancies as well as in the setting of allogeneic transplantation. Dr. Hari provided an overview of the epidemiology of relapse after AHSCT in multiple myeloma, addressing clinical patterns, management implications, and treatment options at relapse, highlighting the implications of novel therapeutic agents in initial, maintenance, and relapse treatment. Dr. Avigan discussed current concepts in tumor vaccine design, including whole cell and antigen-specific strategies, use of an AHSCT platform to reverse tumor-associated immunosuppression and tolerance, and combining vaccines with immunomodulatory agents to promote establishment of durable antitumor immunity. Dr. Hsu reviewed the immunogenetics of natural killer (NK) cells and general NK biology, the clinical importance of autologous NK activity (eg, lymphoma and neuroblastoma), the impact of existing therapies on promotion of NK cell activity (eg, immunomodulatory drugs, monoclonal antibodies), and strategies for enhancing autologous and allogeneic NK cell effects through NK cell gene profiling.

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## INTRODUCTION

Gains in understanding the biologic effects of antitumor therapy on the immune system yield important insights into the mechanisms of tumor control and relapse after both allogeneic stem cell transplantation (AlloSCT) and autologous hematopoietic stem cell transplantation (AHSCT). In the National Cancer Institute's Second International Workshop on the Biology, Prevention, and Treatment of Relapse after Hematopoietic Stem Cell Transplantation, the Scientific/Educational Session on Autologous Transplantation: Relapse Prevention Using Novel Agents and Immunomodulatory

Strategies discussed parallels between AHSCT and AlloSCT platforms with respect to relapse biology, prevention, and treatment. Discussion of multiple myeloma (MM) relapse after autologous transplantation illustrated the evolution of disease characteristics after SCT, including the impact of the novel, immunomodulatory agents that are now standard therapies for MM before and after SCT. Immunomodulatory relapse interventions were discussed, including use of vaccine-based tumor targeting and exploitation of natural killer (NK) cell biology to achieve optimal treatment outcomes.

## I. RELAPSE AFTER AHSCT: THE MM PARADIGM

MM epitomizes many of the challenges posed by relapse after SCT, with disease being the major cause of treatment failure and rapid evolution of diagnostic and therapeutic tools yielding tectonic shifts in the clinical landscape. The following discussion of MM relapse after AHSCT provides a paradigm for considering new approaches to prevention

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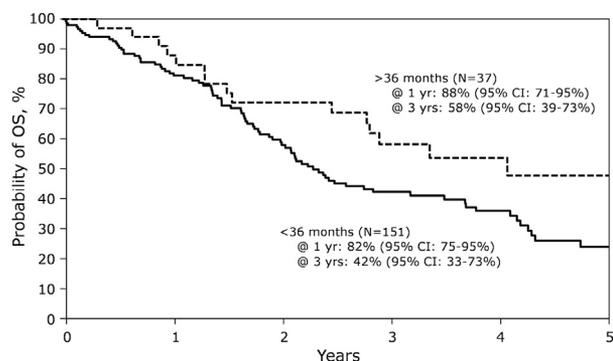
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and treatment of post-transplant relapse, approaches that could extend to AlloSCT as well.

Major advances in MM drug therapy have led to superior induction regimens, better AHST outcomes, and improved survival after relapse [1]. Many patients now receive upfront AHST with sensitive disease in complete remission (CR) or very good partial remission and achieve higher rates of post-transplantation CR, leading to superior progression-free survival (PFS). Novel agents, such as immunomodulatory drugs (IMiDs; thalidomide derivatives) and proteasome inhibitors (PIs), have also improved survival after post-transplantation progression [2]. Analysis of the Center for International Blood and Marrow Transplant Research (CIBMTR) registry data on more than 20,000 recipients of upfront AHST for MM demonstrated improved 5-year overall survival (OS) over time, including major gains in post-transplantation relapse survival [3] (Figures 1 and 2).

### Diagnosing Relapse

Defining relapse of MM post-transplantation is complicated; persistence or recurrence of a myeloma clone detected biochemically does not reliably predict clinical progression after either AHST or AlloSCT [4]. Even absent CR, a significant proportion of patients treated with AHST will achieve prolonged periods of a monoclonal gammopathy of undetermined significance (MGUS)-like state. Additionally, biochemical monitoring may miss progression after AHST; clinical relapse frequently involves a nonsecretory component and aggressive relapse often presents as extramedullary disease, with discordance between imaging (eg, magnetic resonance imaging and positron emission tomography) and



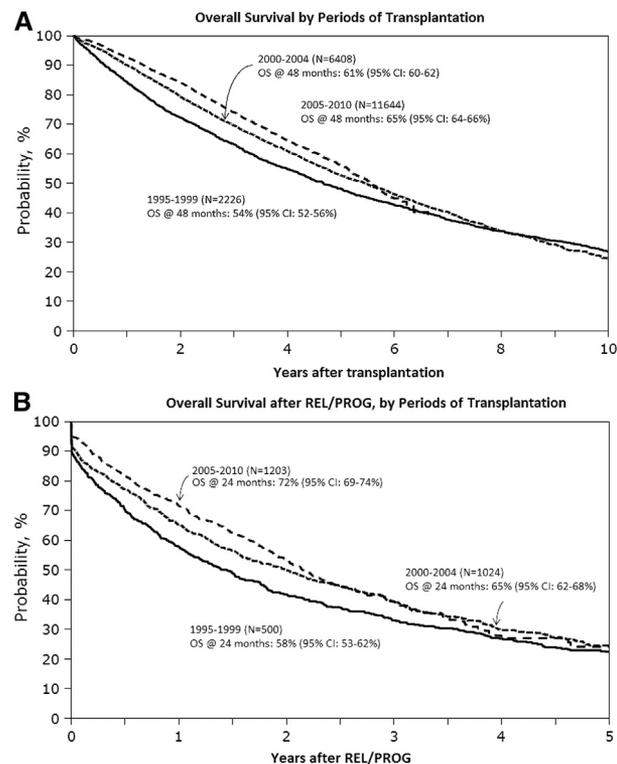
**Figure 2.** Salvage second AHST for relapsed MM. Kaplan-Meier estimates of OS after salvage second AHST for MM relapse, stratified by time to relapse after first AHST (<36 mo versus >36 mo), as reported to the CIBMTR [31].

biochemical disease parameters [5]. These distinctions have been addressed in few published studies. Although current guidelines distinguish biochemical versus clinical progression, most prior studies used biochemical criteria [6], limiting interpretation of relapse presentation, incidence, and outcome of AHST relapse by relying on traditional criteria.

### Clinical Behavior of Relapsed MM

Relapsed myeloma differs from newly diagnosed disease in clinical behavior and biologic correlates [7]. OS from first relapse is generally shorter than the progression-free interval after transplantation, although distinct clinical patterns are evident: relapse after early AHST ( $\leq 3$  prior regimens) with expected sensitivity to IMiD or PI therapy, relapse or transplantation-refractory disease resistant to prior IMiD/PI therapy (“double-refractory disease”), and primary-refractory disease. Retrospective data show outcomes of patients with AHST relapse have improved over time, coincident with the introduction of novel agents [8]. The presentation of clinical relapse is similarly variable and influenced by monitoring methods and frequency. In a Spanish registry study, two thirds of AHST recipients developed “classical” MM relapse, with increase in M-spike and organ impairment. Extramedullary disease and secondary plasma cell leukemia signified relapse in 14% and 2%, respectively, whereas 18% presented initially with isolated biochemical relapse [9].

In patients relapsing in the era preceding novel agents (1985 to 1998), Kumar et al. [8] reported a median OS of 17 months, with 84% dying by 5 years. Coincident with the introduction of IMiD and PI therapies, survival has improved for patients with first relapse after 2000, compared with those diagnosed with initial relapse before that year (23.9 versus 11.8 months, respectively) [1]. A recent international survey suggested a response rate of 58% to first-salvage treatment after AHST, with PFS and OS rates of 13 and 25 months, respectively (with diminishing response rates to successive salvage regimens) [10]. Consistent with the contribution of modern agents, the addition of bortezomib to thalidomide/dexamethasone therapy appears to improve outcomes (median OS of 3 years) [11], although the benefit does not seem to extend to those with double-refractory MM [12]. Current practice and ongoing investigations integrate novel agents into upfront MM therapy and AHST consolidation and/or maintenance; the impact of these changes on AHST outcomes and survival after relapse remains to be defined. However, the poor outcomes seen in the double-refractory setting suggest that effective therapy for relapse



**Figure 1.** CIBMTR analysis of survival trends over time after AHST for MM. (A) Kaplan-Meier estimates of OS after AHST for patients who received AHST between 1995-1999, 2000-2004, and 2005-2010. (B) OS after myeloma relapse/progression after AHST as reported to the CIBMTR for patients who relapsed between 1995-1999, 2000-2004, and 2005-2010 [3].

in patients who underwent transplantation in the current era may be difficult to achieve with available agents.

### Long-Term Survivorship

Even in the pre–novel agent era, some patients had prolonged CR or nonprogressive partial remission (MGUS-like clonal persistence). A 12-year disease-free interval from AHST has been suggested as the threshold after which clinical progression is rare [13] and is often used to define the “cure fraction” or “operationally cured” for MM therapy. In their Total Therapy 1 tandem AHST trial (1989 to 1998), Barlogie et al. [14] showed 33% and 17% OS at 10 and 15 years, respectively, with 15% and 7% event-free survival and 18% and 12% maintaining CR. CIBMTR data suggest that at 10 years, 25% to 29% of AHST recipients are alive and 10% to 15% are progression-free [15].

Long-term PFS may reflect immune-mediated disease control [16]. Superior immune reconstitution has been observed in AHST recipients with long-term disease-free survival [17,18] who demonstrate increased numbers of CD8<sup>+</sup> T cells, NK cells, dendritic cells (DCs), and normal plasma cells. Further, regulatory T cells were found to be reduced, potentially favoring the action of cytotoxic cells, recovery of B cell production, and homing of normal plasma cells into the bone marrow [19].

### Predicting Relapse Risk

With the adoption of increasingly stringent response criteria, a clear association has emerged between CR and event-free survival and OS after AHST. Because many recipients with clinical CR ultimately relapse, minimal residual disease (MRD) monitoring with increasingly sensitive PCR-based and multiparameter flow cytometry methods of detecting clonal plasma cell populations is being evaluated to identify risk of relapse [20]. PCR-based methods are highly sensitive ( $10^{-4}$  to  $10^{-6}$ ), but informative sequences cannot readily identify the myeloma clone for a substantial minority of patients. Although initial studies of PCR methods found molecular evidence of myeloma in nearly all patients with clinical CR, retrospective studies of patients who had achieved immunofixation-defined CR after AlloSCT identified molecular CR in one half [21], more molecular CR after treatment with AlloSCT than AHST [22], and that molecular CR predicted less relapse

and longer relapse-free survival [23]. Although somewhat less sensitive than PCR ( $10^{-4}$ ), multiparameter flow cytometry can distinguish clonal from normal bone marrow plasma cells in nearly all patients with MM [24]; multiparameter flow cytometry detection of MRD at day 100 after AHST has been shown to predict relapse and survival [25,26]. Although both PCR and multiparameter flow cytometry assessment of MRD demonstrate that deeper levels of remission after therapy for MM correlate with superior PFS, no level of remission that equates to a cure has been defined to date, and MRD monitoring remains available only in the research setting. Nonetheless, MRD techniques validated in the AHST setting [27] may identify patients whose relapse risk warrants additional maintenance or immune-based intervention post-transplant.

Recent advances in gene sequencing technology have opened exciting new avenues of cancer investigation with potential implications for defining relapse risk [16]. Analyses of the genomic changes that underlie disease progression demonstrate distinct patterns of clonal dynamics [27,28]. In patients with high-risk MM, for example, disease is characterized by clonal heterogeneity at diagnosis and by genomic instability, likely determinants of evolution toward increasingly aggressive disease. How to integrate and target genomic complexity therapeutically should be a high priority for future research.

### Maintenance and Consolidation Strategies to Prevent Relapse

Efforts to improve outcomes after AHST include consolidation (short-course therapy applied to eradicate residual disease) or maintenance (prolonged lower-intensity therapy applied to prevent relapse). Although improving PFS, prevention strategies have not consistently resulted in OS benefits; further, the effect of these strategies on “operational cure” rates are undetermined (Table 1). Although there appears to be little doubt that deeper remissions yield PFS benefit, many issues regarding preventive interventions remain unresolved: the optimal regimen, schedule, and duration of preventive therapy; in whom preventive therapy is unnecessary or, perhaps, harmful; the potential for emergence of resistant clones; and how preventive therapy compares with early retreatment in terms of OS and quality of life.

**Table 1**  
Maintenance/Consolidation Strategies after AHST: Recent Studies

Study	Agent	Comment	Median PFS/TTP in Superior Arm
CALGB 100104/CTN McCarthy P et al., N Engl J Med 2012;366:1770	Maintenance: lenalidomide versus placebo	Favored lenalidomide: TTP 46 mo versus 27 mo Overall mortality: 15% versus 23% 3-yr OS: 88% versus 80%	Lenalidomide TTP 46 mo
IFM Study Attal M et al., N Engl J Med 2012;366:1782	Consolidation/maintenance: lenalidomide × 2 mo → lenalidomide versus placebo	PFS favored lenalidomide: 41 mo versus 23 mo No survival benefit: >70% in both groups alive at 4 years	Lenalidomide PFS 41 mo
HOVON 65-GMMG-HD4 Sonneveld et al., J Clin Oncol 2012;30:2946	Induction/maintenance: bortezomib-based induction/maintenance versus conventional chemotherapy induction and thalidomide maintenance	Role of maintenance difficult to separate Bortezomib arm superior (PFS 35 mo versus 28 mo; OS benefit in multivariate models)	Bortezomib induction/maintenance PFS 35 mo
MRC Myeloma IX Trial Morgan G et al., Blood 2012;119:7	Maintenance: thalidomide versus none after intensive or non-intensive upfront therapy	52% stopped thalidomide PFS superior for thalidomide, but OS similar Patients with high-risk FISH had worse OS on thalidomide	Thalidomide/AHST PFS 30 mo

TTP indicates time-to-progression; CALGB, Cancer and Leukemia Group B; CTN, Blood and Marrow Transplant Clinical Trials Network; IFM, International Myeloma Foundation; HOVON 65-GMMG-HD4, Dutch-Belgian Hemato-Oncology Cooperative Group 65/German Multicenter Myeloma Group HD4; MRC, Medical Research Council; FISH, fluorescent in situ hybridization.

### Salvage Treatment of Relapse

Treatment options for MM relapse post-AHST are often limited by host and/or disease-related factors. Efficacy of bortezomib and lenalidomide in relapse has been demonstrated in phase III trials and is reflected by improved post-relapse survival since the introduction of novel agents [1]. Novel agent-based combinations (extensively reviewed [29]) appear to improve efficacy and can achieve clinically meaningful responses, as exemplified by results of the recently reported phase III comparison of thalidomide/dexamethasone with or without bortezomib for progression or relapse after AHST.

Patients relapsing within 12 to 18 months of AHST are at very high risk for rapid progression and early mortality. Even when remission can be reinduced, postremission strategies to prevent subsequent relapse have not been established. Late relapse after AHST (beyond 36 months) has a variable prognosis, perhaps influenced by the use of maintenance therapy. Patients with biochemical MM progression while receiving lower-dose maintenance have many treatment options, including use of “treatment” doses of the maintenance agent. In contrast, patients with late clinical relapse and rapid MM progression may require combination approaches to reinduce remission, with second AHST offering potentially meaningful clinical benefit.

Outcomes after relapse following current multiagent induction–AHST–consolidation–maintenance protocols have yet to be determined. From the University of Arkansas’ “total therapy” approach, comparison between successive iterations suggests an overall benefit to upfront use of the entire therapeutic arsenal, yet it may render subsequent relapse more difficult to treat [30]. Several new therapeutic targets have been identified in MM, and small-molecule inhibitors as well as novel monoclonal antibodies are in clinical development for relapse treatment (Table 2). Broadly, second-generation PI and IMiDs show promising single-agent activity, and histone deacetylase inhibitors, phosphatidylinositol 3-kinase pathway inhibitors, and cyclin-dependent kinase inhibitors are being tested in combination with novel agents.

Salvage second AHST is increasingly performed, with significantly greater clinical benefit for those with late MM relapse [31] (Figure 2) and significantly better than salvage

AlloSCT after AHST relapse [32]. In spite of a long-demonstrated graft-versus-myeloma effect, treatment-related mortality has significantly limited the therapeutic benefit of AlloSCT for MM; with the use of reduced-intensity conditioning regimens, treatment-related morbidity and mortality are modestly reduced, but increased relapse negates any survival benefit over AHST [33]. Several randomized trials evaluated the tandem AHST–AlloSCT approach to reduce the risk of relapse in the upfront transplantation setting, with discordant results [34–37]. A recent meta-analysis of the published AHST–AlloSCT versus AHST studies concluded that although CR rates are higher for AlloHCT, neither event-free survival nor OS benefit could not be consistently demonstrated [38]. Thus, whereas AlloSCT may be potentially curative, graft-versus-host disease and treatment-related mortality remain prohibitive.

## II. THE PROMISE OF CANCER VACCINES AS POST-TRANSPLANTATION IMMUNOTHERAPY

The potential promise of cellular immunotherapy in targeting hematologic malignancies is highlighted by the observation that AlloSCT is uniquely curative for a subset of patients with relapsed or high-risk disease, with disease responses to donor lymphocyte infusion suggesting that lymphocytes mediate the therapeutic effect [39–41]. However, the lack of specificity of donor lymphocytes limits response rates and results in considerable morbidity and mortality from graft-versus-host disease, driving efforts to develop a safer and more effective means of harnessing cell-mediated anti-tumor immune responses.

A promising alternative immunotherapeutic strategy is the use of cancer vaccines to educate immune effector cells in an effort to establish antitumor immunity. The design of an effective tumor vaccine depends on optimizing antigen presentation, reversing critical elements of tumor-mediated immune suppression and preventing re-establishment of tumor tolerance.

### Tumor Vaccines

Tumor cells bear unique antigens that differentiate them from normal tissues and serve as potential targets for immune effector cells. Candidate antigens for acute myeloid leukemia (AML) have included Wilms’ Tumor 1 (WT1)

**Table 2**  
Newer Agents in Trials for Relapsed MM

Agent	Therapeutic Class	Comment
<b>Drug Compounds</b>		
Carfilzomib	Irreversible PI, intravenous	Accelerated approval by FDA; lower risk of neuropathy
Ixazomib (MLN9708)	Reversible PI, oral	Phase I and II studies
Oprozomib (ONX 0912)	Irreversible PI, oral	Phase I and II studies
Marizomib (NPI-0052)	Irreversible PI, intravenous	Phase I and II studies
Pomalidomide	Novel IMiD, oral	Accelerated approval by FDA
Vorinostat	Histone deacetylase (HDAC) inhibitor, oral	Phase II and III studies, with novel agents
Panabinstat (LBH589)	HDAC inhibitor, oral	Phase II and III studies, with novel agents
Rocilinstat (ACY-1215)	HDAC6-selective inhibitor, oral	Phase I and II studies, with novel agents
AT7519	Cyclin-dependent kinase inhibitor, intravenous	Phase I/II study, with bortezomib
<b>Monoclonal Antibodies</b>		
Antibody	Target	Comment
Elotuzumab	CS1	Phase III, with lenalidomide
Daratumumab	CD38	Phase II, ongoing
SAR650984	CD38	Phase I studies ongoing, with lenalidomide
BT062	CD138	Phase I/IIa (DM-4 conjugate), with lenalidomide and dexamethasone, ongoing
Lorvotuzumab	CD56	Phase I (DM-1 conjugate), with lenalidomide and dexamethasone, ongoing
Milatuzumab	CD74	Phase I/II study (doxorubicin conjugate) ongoing

FDA indicates US Food and Drug Administration.

[42,43], proteinase 3 (PR1 peptide) [44,45], RHAMM [46], Aurora-A [47], PRAME [48], and MUC1 [49]. These antigens may be shared by the primitive stem cell population, allowing for the potential targeting of this critical source of disease relapse. In MM, a variety of tumor-associated antigens has been identified that may be selectively targeted by host immunity. These include the cancer testis antigens, MUC1, HM1.24, CYP1B1, SP17, PRAME, WT1, and heat shock protein gp96 [50–53]. Despite the presence of antigenic targets, tumor cells evade immune recognition through the presentation of antigen in the absence of costimulation and in the context of inhibitory factors that promote tolerance and immune escape [54]. In contradistinction, cancer vaccines are engineered to enhance the presentation of tumor-associated antigens resulting in antigen-specific T cell activation and expansion. A follow-up of a phase I clinical trial evaluating a WT1 peptide-based vaccine reported 3 of 8 AML patients treated with MRD had remained in remission for at least 8 years [55].

To enhance effective presentation of tumor-associated antigens *in vivo*, DC vaccine strategies have been evaluated. DCs are potent antigen-presenting cells that can be generated in large numbers *ex vivo*; tumor antigens and peptides can be loaded onto *ex vivo* generated DCs and used as a tumor vaccine [56]. Clinical studies have demonstrated successful expansion of antigen-specific T cells after administration of peptide-loaded DC vaccines, with potential implications for prevention and treatment of relapse [55,57–60].

Vaccine strategies designed to induce T cell responses directed against an individual antigen are potentially limited by tumor escape through the down-regulation of the target antigen. Alternatively, the use of whole tumor cells as a source of antigen may elicit a broader (ie, polyclonal) T cell response. One novel approach induces differentiation of DCs from myeloid leukemia blasts *ex vivo* in an effort to create a potent antigen-presenting cell that expresses a panel of autologous leukemia-derived antigens [61,62]. Potential concerns include the functional capacity of engineered antigen-presenting cells *in vivo* as well as loss of antigens characteristic of the primitive clone in the process of differentiation.

The GVAX vaccine (Dana-Farber Cancer Institute Cell Manipulation Core Facility, Boston, MA) [63] approach uses tumor cells genetically engineered to express granulocyte macrophage colony-stimulating factor resulting in recruitment of native DCs to the site of vaccination and their subsequent processing and presentation of antigens. In a phase I study, vaccination of patients with high-risk AML was well tolerated and associated with immune response and an attendant low risk of relapse [63]. We developed a vaccine model in which patient-derived AML or MM cells are fused with autologous DCs such that a broad array of tumor antigens are presented in the context of DC-mediated costimulation, resulting in the expansion of both tumor-specific helper and cytotoxic T lymphocyte (CTL) populations [64,65]. In a phase I study, vaccination of patients with DC/myeloma fusions was associated with the expansion of myeloma-specific T cells and resulted in disease stabilization in most patients with advanced disease [66].

### **Vaccination after AHST**

A major limitation to developing an effective immunologic and clinical response to vaccination involves the immunosuppressed milieu characteristic of patients with advanced malignancy. As such, AHST offers a unique

platform for examining the efficacy of tumor vaccine therapy. High-dose chemotherapy results in tumor cytoreduction as well as immunomodulatory effects of depleting regulatory T cells and disruption of tumor tolerance. Preclinical models have demonstrated transient enhanced response to tumor vaccines in the early period of immune reconstitution after high-dose chemotherapy [67]. In a phase II clinical trial, administration of GVAX in conjunction with AHST in AML patients induced antitumor immune responses in most patients and a reduction in residual disease in a subset [68].

In study of patients with MM undergoing therapy with AHST in conjunction with DC/myeloma fusions, vaccination resulted in the expansion of myeloma-specific T cells and conversion from partial to complete responses in a subset of patients [69]. Of note, the post-transplantation period was associated with significant reduction in circulating regulatory T cells. In another study, vaccination with an idio-type-based vaccine after AHST was associated with improved outcomes as compared with an historical cohort of patients treated with transplantation alone [70]. Response to vaccination in the post-transplantation period may be augmented by the administration of lymphocytes activated and expanded by CD3/CD28 costimulation *ex vivo* [71–73].

### **Immunomodulation to Enhance Response to Vaccination**

Another approach to increase vaccine potency is the adjunctive use of immunomodulatory agents, which may promote expansion of memory T cell populations critical to durable responses. Tumor-associated factors that inhibit DC maturation and activation (eg, vascular endothelial growth factor and indoleamine 2,3 dioxygenase) are potential therapeutic targets to enhance vaccine activity. Ipilimumab blocks signaling via the negative T cell regulatory checkpoint, CTLA-4, and has received US Food and Drug Administration approval as an immunotherapy for advanced-stage melanoma [74–76]. Its use is now being explored as a potential vaccine adjuvant [77]. The programmed cell death ligand 1 (PD-L1)/programmed cell death protein 1 (PD-1) pathway is a critical mediator of tolerance in the setting of chronic viral infection and malignancy, by inhibiting both DC-mediated stimulation of T cells and CTL-mediated killing of tumor targets [78,79]. PD-1 and PDL-1 directed antibodies are currently being studied as immunotherapeutic agents. Preclinical studies have demonstrated that vaccination with DC/MM fusions in conjunction with PD-1 blockade results in suppression of vaccine-induced expansion of regulatory T cells, increased CTL expansion, and enhanced myeloma-specific CTL responses [80]. Clinical studies exploring whether PD-1 blockade can facilitate establishment of durable vaccine-mediated immune responses are under way.

In summary, tumor vaccines hold promise as means of eliminating residual disease and preventing disease recurrence after AHST. Novel immunomodulatory agents and immune checkpoint blockade may have the potential to augment vaccine-induced memory T cell responses *in vivo*. Although results of preclinical and early-phase clinical studies show promise, evaluating vaccine efficacy in the setting of minimal disease is a challenge that ultimately will require evaluation in the context of randomized clinical trials with survival endpoints.

### **III. NK CELL IMMUNOGENETICS: IMPLICATIONS FOR RELAPSE RISK AND PREVENTION**

The role of innate immunity in tumor recognition and response has been recognized for decades, with the name

bestowed to NK cells based on their ability to kill tumor targets. The clinical importance of this innate surveillance system for diseased cells (ie, infected, transformed, and injured cells) was first documented in patients who lacked functional NK activity, whose recurrent viral infections and lymphoproliferative disorders led to early demise [81,82]. Since the identification of the NK cell, investigators have sought to capture their antitumor effects for the treatment of cancer. Their efforts, however, were initially slowed by deficiencies in understanding the molecular mechanisms controlling NK activity, specifically the receptor–ligand relationships dually responsible for conferring NK effector potential against nonself targets while simultaneously preventing NK reactivity to autologous cells. This report reviews the NK cell surface receptors and ligands important for NK education, highlighting the clinical situations in which specific NK subsets are most effective and emphasizing pharmacologic and genetic approaches to exploit NK antitumor effects.

### **NK Receptors and Ligands**

The NK cell is studded with an array of inhibitory and activating receptors, with signals that integrate to dictate NK response. Receptors in humans include the inhibitory and activating forms of KIR, the CD94/NKG2A heterodimer, the natural cytotoxicity receptors (NKp46, NKp44, and NKp30), NKG2D, the signaling lymphocytic activation molecule (SLAM) receptors, DNAM1, and the Fc $\gamma$  receptor IIIA (CD16). Although seemingly complex, the receptor–ligand relationships can be generally distilled into 2 types: (1) recognition of self-determinants, such as self-HLA, resulting in NK tolerance to normal autologous cells and ignorance to tumor cells with up-regulated HLA; and (2) detection of “stress-induced self molecules” normally expressed at low levels on normal cells but up-regulated on infected or transformed cells, resulting in NK activation and tumor toxicity. In humans, stress-induced molecules include the NKp30 ligand B7H6 and the NKG2D ligands MICA, MICB, and ULBP family members. Fine-tuning the response is the adaptor molecule SAP, which is critical for the activating properties of the SLAM receptor, particularly in its response to hematopoietic target cells. In addition, tumor targets can avoid NK toxicity by expressing class I molecules and the inhibitory molecule 41g-B7H3. A separate category of NK cell–tumor interaction occurs through CD16, whose binding of therapeutic monoclonal antibodies triggers antibody-dependant cell-mediated cytotoxicity (ADCC).

### **Diversity of the Functional NK Repertoire**

Thus, NK cell–tumor interactions can involve simultaneous mechanisms of NK activation and inhibition signals. The degree of the NK cell’s response, however, is highly dependent on its “education” or licensed capacity for killing, where the molecular interactions most closely linked to education are between the KIR receptors and their HLA class I ligands, HLA-C1, HLA-C2, and HLA-Bw4. Not only is interaction between the inhibitory receptor and its self-HLA ligand important for signaling tolerance to self, it is also required for the NK cell to achieve functional competence [83], where high-affinity KIR–HLA allotype combinations not only lead to higher functional capacity but also to greater potency of inhibition [84]. Expression of receptors occurs stochastically, leading to a repertoire that is diverse in number and abundance of receptors on the cell surface. Functionally translated, this phenotypic diversity results in a continuum of response to target cells. In addition, KIR and HLA regions segregate independently, leading to frequent genetic

combinations where individuals lack 1 or more class I ligands for autologous KIR (missing ligand). In these individuals, unlicensed NK cells expressing inhibitory KIR for nonself HLA represent a significant proportion of the NK repertoire and are not sensitive to class I expression on the target cell.

### **Harnessing Autologous NK Effects**

An understanding of KIR–HLA protein interactions and their perturbation in various disease states is useful in designing effective strategies to exploit autologous and allogeneic NK activity against cancer. Robust NK reconstitution has been associated with improved outcomes for AML, non-Hodgkin lymphoma, and MM after AHSCT [16,85], indicating that NK proliferation agents, such as IL-15, may enhance antitumor effects. Patients undergoing AHSCT for various solid tumors experience an improved outcome if the patient is missing 1 or more KIR ligands [86]. Among these tumors, high-risk neuroblastoma is particularly informative, because its treatment typically includes anti-GD2 monoclonal antibody therapy, where the primary mechanism of action is through NK-mediated ADCC. Unlicensed NK cells expressing KIR for nonself HLA are highly capable of ADCC with anti-GD2 antibody, whereas licensed NK cells expressing KIR for self-HLA are inhibited by induced class I expression on the tumor surface [87]. Because NK cells mediate ADCC for other monoclonal antibodies in clinical use, including the anti-CD20 antibody rituximab, it will be important to establish whether KIR–HLA genotypes predictive of missing ligand are similarly advantageous. Two novel pharmacologic approaches hold promise in this setting: monoclonal antibody activation of CD137 to enhance ADCC [88] and anti-KIR antibodies to interfere with class I-mediated inhibition of licensed NK cells [89].

New options in the treatment of MM enlist higher NK activity as mechanisms of their efficacy [90]. PIs decrease class I expression and up-regulate NKG2D ligand expression on myeloma cells, and IMiDs such as lenalidomide enhance NK function through induced T cell production of IL-2, down-regulation of PD-L1, increased natural cytotoxicity, and heightened ADCC. Promising new approaches include the anti-PD1 antibody and the anti-inhibitory KIR antibody to overcome inhibitory signaling from the myeloma target, as well as the anti-CD1 monoclonal antibody, which targets CD1-expressing myeloma cells and recruits NK cells for ADCC activity.

### **Capturing Allogeneic NK Effects**

The first clinical setting to harness the potential of the allogeneic NK cell was HLA-mismatched HSCT, where alloreactive donor NK cells stimulated by missing self demonstrated potent effects against AML [91]. Since then, it has become clear that donor NK alloreactivity can occur in the HLA-matched setting as well. Cytokine-enhanced activity of unlicensed NK cells [92] likely contributes to reduced AML relapse among patients lacking any KIR ligand [93] and not just ligands present in the donor. Donor-activating KIR are also associated with reduced relapse [94] and decreased mortality [95]. Activating KIR, however, are not all similar. KIR2DS1, the only activating KIR for which a ligand is known, is important in NK education and tolerance, and KIR2DS1-positive cells from HLA-C2 homozygous individuals have reduced function compared with KIR2DS1-positive cells from HLA-C1 individuals [96]. Translated to the clinical setting, KIR2DS1-positive donors are associated with lower relapse in AML patients, but only if the donor is not HLA-C2

homozygous [97]. The possibility of improving transplantation outcomes by incorporating KIR gene and allele typing into donor selection algorithms is now real, and prospective trials demonstrating the effectiveness of donor KIR–HLA gene profiling in donor selection will be an important step in broadening the frontiers of transplantation.

#### FUTURE DIRECTIONS AND RESEARCH PRIORITIES

A major achievement of the National Cancer Institute's Second International Workshop on the Biology, Prevention, and Treatment of Relapse after Hematopoietic Stem Cell Transplantation was the establishment of an international consortium of investigators with the objective of conducting collaborative research in the field of relapse after HSCT. This session highlighted the far-reaching effects of novel agents and immunomodulatory interventions on the clinical manifestations of relapse and the implications for possible relapse prevention and treatment trials. The discussions identified opportunities to define and therapeutically exploit areas of common as well as distinct biology between the allogeneic and autologous SCT platforms—and the potential to improve treatment outcomes in both.

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