Post-transplant immunotherapy designed to prevent cancer recurrence


Abstract: Most stem cell transplants are performed to treat neoplasms. Yet many patients survive the transplant but die from cancer recurrence post-transplant. The incorporation of post-transplant immunotherapy offers the potential for enhanced antitumor efficacy.

The use of HSC transplantation as treatment for hematopoietic neoplasms is based on the hypothesis that provision of sufficiently high doses of ablative chemotherapy or radiotherapy can eradicate residual neoplastic cells. However, the ablative therapy also destroys the host’s hematopoietic cells. The purpose of provision of HSC following ablative therapy is the ‘rescue’ of the hematopoietic system. This process presumes that irreversible toxicity to host tissues by the ablative treatment will only influence the hematopoietic system, while other organ systems critical for life will be able to recover spontaneously. It also presumes that the ablative treatment to the hematopoietic system is also effective at eradicating all clonogenic neoplastic cells.

Now, 30 years following the first successful application of allogeneic bone marrow transplantation (1,2), clinical experience has shown that these assumptions are far from uniformly true. Major dose-dependent toxicities to other organ systems are associated with ablative BMT regimens and can cause dose-dependent, at times irreversible, toxicities to multiple non-hematopoietic tissues. Furthermore, a substantial fraction of patients receiving BMT for neoplastic disease survive the hematopoietic reconstitution only to have subsequent relapse of their original neoplasm.

During these same 30 years, substantial progress in basic research has provided insights regarding the mechanisms of the immune system, and provided the necessary biotechnology to apply immunotherapy to the treatment of malignancy. This work includes prospective use of immunotherapeutic principles following BMT as well as investigations of anti-tumor immune reactions that occur ‘spontaneously’ as part of standard clinical allogeneic BMT treatments.

The purpose of this manuscript is to summarize aspects of our current understanding and new approaches for the prospective use of immunotherapeutic principles in the treatment of neoplasms, in association with allogeneic or autologous SCT. The emphasis here will be on immunotherapeutic applications in the context of childhood cancer treatment, with a focus on mechanisms potentially useful in the autologous SCT setting.

Allogeneic BMT and the GvL phenomenon

The initial clinical observation that a graft versus host reaction could impact and dramatically inhibit leukemia relapse following BMT (3) has led to substantial interest in the ‘graft-versus-leukemia’ effect associated with BMT. The GvL phenomenon has been clearly identified in clinical studies of large numbers of BMT recipients (4,5). Detailed assessments of data from the

Abbreviations: BMT, bone marrow transplant; ADCC, antibody-dependent cellular cytotoxicity; ANLL, acute non-lymphocytic leukemia; BMT, bone marrow transplant; GvHD, graft-versus-host disease; GvL, graft versus leukemia; HLA, human leukocyte antigen; HSC, hematopoietic stem cells; IL2, interleukin-2; NK, natural killer; SCID, severe combined immune deficiency; SCT, stem cell transplantation; EBV, Epstein-Barr virus; GM-CSF, granulocyte-macrophage colony-stimulating factor.
International Bone Marrow Transplant Registry documented that an anti-leukemic effect was associated with GvHD itself. Furthermore, even in the absence of clinically detectable GvHD, the recipients of allogeneic HLA-identical sibling marrow had less leukemic relapse than recipients of syngeneic (identical twin) marrow, or T-cell-depleted HLA-identical sibling marrow (5). These observations proved that there was an allore cognition component mediated by T cells in the GvL effect.

Much effort has been focused on distinguishing the GvHD from the GvL components of this GvL effect. This effort is being pursued by attempting to identify the targets that may be of importance in immune recognition associated with the GvL effect.

Several molecular targets and mechanisms (Table 1) might account for preferential destruction of leukemia cells compared with non-leukemic cells following allogeneic SCT.

1. Leukemia cells might be recognized by alloreactive (allograft) recognition directed against virtually all host tissues; however, the leukemia cells might be more susceptible to the mechanisms of lysis mediated by the immune cells.

2. Leukemia cells may express certain leukemia-specific antigens (such as exogenous oncopgenic viral antigens like those associated with EBV (6), or with oncogenic fusion proteins associated with tumor-selective translocations (7).

3. Minor histocompatibility antigens might be preferentially expressed or recognized on leukemia cells (8).

4. Minor antigens expressed on leukemic and non-leukemic cells might be presented better on leukemic cells due to increased expression of histocompatibility molecules or co-stimulatory molecules involved in antigen presentation (9).

5. Tissue-specific antigens, such as differentiation antigens, might be expressed only by the tumor cells and by normal cells of that histological type and not by other normal tissues. As such, a strong ‘tissue-specific’ alloreaction might destroy all leukemia cells and all host hematopoietic cells. Destruction of the normal hematopoietic cells would be of no consequence provided that allogeneic stem cells are being provided and fully engrafted.

Table 1. Potential mechanisms of GvL

- Leukemic cells may be more susceptible to lytic mechanisms
- Leukemic cells may express leukemia-specific antigens
- Leukemia cells may over-express certain minor antigens
- Leukemia cells may present antigens more effectively
- Leukemia cells may express tissue-specific antigens
- Leukemia cells may be destroyed by the ‘innate’ immune system

6. Finally, the allore cognition process might activate release of multiple cytokines, including IL2, which act on cells of the ‘innate’ immune system, such as NK cells, potentially activating effector mechanisms that are preferentially destructive to the malignant cells.

It remains uncertain which of these mechanisms is of greatest clinical importance. Analyses of BMT recipients for early leukemia (5) suggest that approximately one-third of leukemia patients who are alive following allogeneic HLA-identical sibling BMT have survived their leukemia because of the role of the GvL reaction mediated by their sibling’s immune cells. As more than 10 000 leukemia patients are now alive following allogeneic BMT (10), it appears that more than 3000 leukemia patients are alive due to their ‘GvL’ reaction. This number of cancer survivors probably exceeds the total of all other patients alive following cancer treatment as a result of direct prospective immunotherapy.

Multiple approaches are being pursued to prospectively utilize the GvL reaction to help prevent leukemia recurrence following SCT. Much enthusiasm is currently being placed into the process of donor lymphocyte infusions (11–13). Despite the potent clinical anti-tumor effect identified for the GvL phenomenon, it will be complicated to translate these observations into effective anti-tumor treatments for patients that do not have HLA-identical siblings, when considering the use of alternative sources of stem cells (peripheral blood or placental blood), as well as alternative donors (HLA non-identical relatives or HLA matched (or partially matched) unrelated donors).

If it were possible to effectively utilize anti-tumor immune mechanisms without requiring allogeneic SCT, this would circumvent the complexities associated with GvHD and the multiple variables involved in distinct donor-host mismatches and immune interactions.

Potential for induction of anti-tumor immunotherapy following autologous SCT

With the exception of recognition of foreign alloantigens, the same potential targets (Table 2) may be relevant for tumor recognition by autologous immune mechanisms as those relevant to the alloreactive GvL effect. However, cancer itself is associated with immune dysfunction. Thus, immune strategies that might activate potent immune responses in healthy individuals might not be effective in patients with neoplasms. This may be only more complicated by the
ablative therapy associated with the pre-SCT 'conditioning' used for both allogeneic and autologous HSCT. One approach to boost anti-tumor immunity is the provision of immunostimulatory growth factors such as interferons or interleukins. IL2 has been studied extensively as a stimulator of T cells, B cells, NK cells and monocytes, acting through the trimolecular IL2 receptor (14). The NK activation induced by IL2 is associated with an anti-tumor effect in animal models as well as in cancer patients, particularly those with renal cell cancer or melanoma (15,16).

As multiple immunotherapy models in tumor-bearing mice document better anti-tumor efficacy when tested in the minimal residual disease setting, testing of immunostimulation for patients has moved to an analogous setting. Work led by Fefer (17) has shown an IL2 regimen that can be provided following autologous SCT is well tolerated, activates NK function in vivo, and may be associated with anti-tumor effects against myeloid leukemia and lymphoid malignancies. However, anti-tumor efficacy in the minimal residual disease setting can best be demonstrated through clinically controlled randomized trials. This issue is currently being tested by the Children's Cancer Group in the setting of ANLL.

IL2 has been given to ANLL patients following completion of intensive chemotherapy (just short of ABMT-level ablative therapy) and was found to be well tolerated (18). This same pilot trial documented potent activation of immune function in all treated patients, as determined by measurement of serum soluble IL2 receptor levels. This same regimen is currently being tested in a randomized controlled trial for children with newly diagnosed ANLL as part of Children’s Cancer Group study 2961. This randomized clinical trial will determine whether the same IL2 regimen published previously (17,18) will prevent leukemic recurrence following attainment of remission by potent chemotherapy, and also determine whether parameters of immune activation (such as soluble IL2 receptor levels) correlate with anti-tumor effects.

While the anti-tumor mechanism of action for IL2 remains to be further characterized, NK cells appear to be playing a role. However, the mechanism of NK recognition does not necessar-ily provide a tumor-specific destructive signal. Augmenting the ability of NK cells to selectively destroy tumor cells might enhance anti-tumor efficacy. This goal is currently being pursued by passive provision of tumor-selective monoclonal antibodies.

Augmenting anti-tumor selectivity by passive administration of tumor-reactive antibody

The NK cells activated in vivo by IL2 are potent at mediating ADCC in vitro against tumor cells that have been pre-treated with tumor-reactive antibody of appropriate isotype and configuration for provision of ADCC (19). The GD2 disialoganglioside is a target molecule of particular importance for antibody treatment of pediatric malignancies. This molecule is strongly expressed on neuroblastoma, and several murine antibodies have been generated against it. The 14.G2A murine antibody is potent at recognizing neuroblastoma cells and mediating ADCC. It has been used in several clinical trials for patients with neuroblastoma as well as melanoma. Recently, the Children’s Cancer Group has completed a trial combining IL2 with this 14.G2A antibody (20).

This phase I study treated 33 patients with refractory neuroblastoma (or other GD2+ tumors) with IL2 to activate NK cells and 14.G2A murine antibody to help focus the anti-tumor effects of the NK cells to mediate in vivo ADCC. Lymphocytes from patients receiving this therapy were potent at mediating in vitro destruction of neuroblastoma target cells when the 14.G2A antibody was added in vitro (21). More importantly, ADCC was mediated by these patient’s lymphocytes when serum from these same patients was added to the assay, following in vivo provision of the 14.G2A antibody (21). These results proved that conditions had been achieved in vivo that allow the circulating antibody to bind to tumor cells and mediate ADCC with the patient’s own effectors. Despite the refractory nature of neuroblastoma for most patients treated in this study, two patients did show measurable anti-tumor effects while the vast majority showed no clear evidence of anti-tumor efficacy based on clinical criteria. Nevertheless, further testing in the minimal residual disease setting may be more appropriate.

However, the use of murine monoclonal antibodies is associated with induction of a human anti-mouse antibody response (20). A chimeric form of this same antibody has been generated and has been tested in a similar
regimen together with IL2 for patients with melanoma (22). Human anti-chimeric antibody responses were seen in some patients, but not during the first cycles of treatment for most patients.

In order to move this approach into the minimal residual disease setting, the Children's Cancer Group has tested the chimeric 14.18 antibody together with GM-CSF following autologous bone marrow transplant for neuroblastoma. This recently completed protocol has determined the dose of chimeric 14.18 antibody that can be combined safely following ABMT with GM-CSF. The regimen was tolerated acceptably and 22 patients have been evaluated (23). The Children's Cancer Group is now testing a modification of this regimen that includes GM-CSF during the first and third course of ch14.18 treatment and IL2 during the second course in order to activate both NK cells and monocytes/granulocytes to mediate ADCC following ABMT. This pilot study (24) is currently underway. The next step in this process will require the randomized testing of this approach. The Children's Cancer Group, the Pediatric Oncology Group and the National Cancer Institute are working together to draft an intergroup protocol that will test a similar immunotherapy regimen following ABMT for patients with neuroblastoma in a randomized controlled trial. This type of randomized trial should definitively test whether this combined GM-CSF, IL2 and ch14.18 regimen can provide protection from relapse following ABMT for neuroblastoma. Concurrent laboratory analyses performed on these patients may help to identify which immunologic mechanisms are associated with anti-tumor effects and whether parameters of minimal residual disease detection correlate with disease-free survival.

Molecularly engineered reagents

Just as localizing effector cells to the sites of metastases through antibodies recognizing malignant cells can add tumor specificity, it may be beneficial to further localize immune cell activation at these same sites by localizing effector cell stimulation to the sites of micrometastases. This is the rationale behind the creation of 'immunocytokines'. These immunocytokines are fusion proteins consisting of tumor-reactive antibodies linked chemically or genetically to immune activating molecules. One molecule that has gone through extensive preclinical testing is the ch14.18-IL2 fusion protein. This fusion protein has an IL2 (human) molecule linked genetically to the carboxy-terminus of each heavy chain of the ch14.18 immunoglobulin. It retains specificity and ability to bind to the same tumors that are recognized by the ch14.18 antibody. Furthermore, it delivers IL2 to the surface of the tumor cell, thereby providing immune activation (25).

In murine models, animals with established but small microscopic metastases show clearance of all detectable metastatic disease following treatment with this fusion protein. Remarkably, this same reaction can occur against a syngeneic neuroblastoma in immunodeficient mice (either SCID mice or T-cell-depleted animals) (26). This murine model documents that it is NK cells rather than T cells that are potent in mediating this fusion protein-facilitated tumor eradication. This is particularly important for potential application to treatment of human patients after ablative chemoradiotherapy and autologous stem cell infusions. Such patients are known to have deficient T-cell function but do have IL2-responsive NK cells.

The humanized form of this reagent has been created, and clinical testing of this molecule in patients with refractory melanoma has begun. Subsequent testing for patients with refractory neuroblastoma through a Children's Cancer Group/Pediatric Oncology Group intergroup study should begin shortly (27). Depending upon the results of the intergroup study utilizing the ch14.18 antibody plus cytokines given as separate molecules, it seems likely that subsequent clinical testing of this fusion protein would be best performed in the minimal residual disease setting. This would likely be best achieved in neuroblastoma patients following ablative chemotherapy and autologous stem cell infusion (28).

Summary

The combined use of surgery, chemotherapy and radiotherapy is able to induce clinical remissions for the vast majority of all patients with cancer, especially in the case of pediatric malignancies. However, for too many of these patients, cryptic, clinically undetectable, microscopic metastases present at the time of achieving remission are resistant to the previously used treatment and grow relentlessly, ultimately leading to relapse and death. Currently, one of the most effective means for achieving a state of remission and minimizing (at times eradicating) all malignant cells,
involves the use of 'supra-lethal' cytotoxic therapy (chemotherapy as well as radiation therapy) in the clinical modality known as stem cell transplantation. However, even here, all too many patients with malignancies that are in remission following SCT succumb to recurrent disease. What appears to be needed are mechanisms to eradicate the remaining microscopic metastatic cells following SCT using treatment approaches that are not themselves myelotoxic and which involve destructive mechanisms that are effective against chemotherapy-resistant tumor cells. Manipulations utilizing immune reactivity, recognition and destruction of tumor cells in vivo, known collectively as 'immunotherapy', may provide this potential. Thousands of patients are currently alive due to the GvL reaction. They document the capabilities of anti-tumor immune reactions following SCT. The challenge that clearly remains is how to take advantage of these immune mechanisms in an effective manner, and hopefully do so without requiring allogeneic stem cell reconstitution. Current preclinical and clinical trials testing novel mechanisms for accomplishing this may help direct future work towards this goal.

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