

S-5-4

Insight Into The Graft Versus Solid Tumor Effect: Efforts To Move From Proof Of Concept To More Effective Allogeneic NK And T-Cell Based Allogeneic Immunotherapy

Richard W. Childs

NHLBI/National Institutes of Health

Allogeneic T-cells are capable of generating powerful anti-leukemic effects in a number of hematological malignancies following hematopoietic stem cell transplantation. The lack of efficacy of chemotherapeutics, radiotherapy, and cytokine-based immunotherapy for many patients with metastatic cancer has catalyzed at least in part enthusiasm for exploring allogeneic-based immunotherapy in patients with treatment-refractory solid tumors.

Although pilot trials are ongoing, graft-versus-tumor effects powerful enough to induce complete or partial remission of some metastatic solid tumors including, ovarian, breast, colon, pancreatic and renal cell carcinoma have recently been described.

Beginning in 1997, we initiated pilot trials investigating the use of nonmyeloablative allogeneic stem cell transplantation (NST) in patients with treatment refractory solid tumors including cytokine refractory renal cell carcinoma¹. All patients were conditioned with cyclophosphamide and fludarabine to facilitate donor engraftment, then were transplanted with a G-CSF mobilized blood stem cell allograft from their HLA-matched sibling. Post-transplant immunosuppression with cyclosporine was typically withdrawn early to enhance a donor immune effect against the tumor. We observed donor immune-mediated anti-tumor effects in patients with clear cell carcinoma powerful enough to induce complete or partial remission of cytokine refractory metastatic renal cell carcinoma. Twenty five of the first 68 patients treated to date have had a disease response including 6 complete responses and 19 partial responses. Four of the complete responses have proven

to be durable including the first patient transplanted with metastatic renal cell cancer who survives in remission more than 6 years after treatment. Regression of tumors was typically 1) delayed (median of 6 months) following the transplant 2) Did not occur until post-transplant immunosuppression was withdrawn 3) Occurred after a donor lymphocyte infusion in some patients 4) Were seen most commonly in patients with a history of graft-vs-host disease.

Several groups have recently observed that donor immune-mediated anti-tumor effects can be generated following allogeneic stem cell transplantation using a variety of different transplant regimens^{2,3,4}. The major morbidity associated with the procedure has been graft-vs-host disease, with most centers reporting a 10%-15% chance of procedural related mortality. Since only about 25% of patients can be expected to have an HLA matched sibling, the procedure is currently limited to a minority of patients with metastatic renal cell carcinoma. Studies evaluating the feasibility of using matched unrelated stem cell donors in this setting are planned, and if successful could extend the application of allogeneic immunotherapy to a far greater percentage of patients.

Preliminary data indicate the immune cells contributing to the graft-versus-tumor effect in RCC are donor T-cells targeting minor histocompatibility antigens and possibly antigens restricted to the tumor. At the NIH, efforts continue to identify these antigens^{5,6}. We have successfully generated donor CD8+ T-cell clones from lymphocytes obtained from responding patients that have direct cytotoxicity against the patient's RCC cells. Although some

of these T-cell clones recognize tumor alone, as determined by interferon gamma secretion, many recognize both patient tumor and autologous EBV transformed B cells. In one patient who had disease regression associated with acute GVHD, we were unable to expand tumor specific T-cell populations at the time of disease regression but could expand CD8+ T-cell clones that recognized both autologous EBV transformed B cells and patient RCC cells, strongly implicating minor histocompatibility antigens as being the target for a GVT effect in this patient. Future investigations will focus on limiting GVHD while targeting the immune response specifically to the tumor through adoptive tumor specific T-cell infusion or post-transplant vaccination strategies⁷. We have been investigating methods to use the tumor itself to stimulate RCC specific immune responses. The lack of appropriate co-stimulatory molecules such as B7.1 on most RCC cells limits this approach. Recently, several techniques to chemically (using PEG) or electrically “fuse” dendritic cells (DC’s) to tumor cells have been developed. Such tumor/DC fusions could result in the presentation of tumor antigens cross primed on dendritic cell HLA molecules. We have investigated tumor electrically fused to HLA matched sibling’s DCs for their ability to stimulate tumor-specific T cell responses. RCC tumor cells electrically fused to DCs derived from HLA-identical siblings (donors) were used to stimulate donor peripheral blood lymphocytes. Analysis of bulk cultures revealed electro-fused tumor/DCs were effective in stimulating RCC-reactive T cell populations after 2 or 3 stimulations. Efforts to improve the relatively low efficiency of fusion with this technique (typically in the range of 10%) continue in hopes these tumor/DC fusions could be used to expand RCC specific T-cell clones from naïve PBSC donors for adoptive infusion in the post-transplant setting. It is unclear what role allogeneic NK cells play in mediating GVT effects against RCC. However, it is clear that cellular inactivation through killer Ig-like receptors (KIR) may allow neoplastic cells to evade host NK-cell-mediated immunity. This may in part explain the failure of adoptively transfused

autologous NK or lymphokine activated killer cells (LAK) to mediate anti-tumor effects against most metastatic solid tumors. Alloreactive NK-cells are thought to mediate anti-leukemic effects against AML after mismatched-transplantation when KIR-ligand incompatibility existed in the direction of GVHD. Our group has recently demonstrated that solid tumor cells have similar enhanced susceptibility to allogeneic KIR-incompatible NK-cells compared to their KIR-matched autologous counterparts⁸. These data suggest immunotherapeutic strategies that use KIR-incompatible allogeneic NK-cells might have superior anti-neoplastic effects against solid tumors compared to investigational approaches using autologous NK-cells. This is currently being evaluated in an animal model.

The use of NST in patients with metastatic melanoma has produced disappointing results to date⁹. Three of the first 12 (25%) patients with metastatic melanoma transplanted had disease regression consistent with a partial response; 2 responses occurred in the immediate post transplant period, likely the consequence of a chemotherapy effect, while one response was delayed in onset (day+130) and occurred in association with chronic skin GVHD consistent with a GVT effect. Unfortunately, these disease responses have not been durable with melanoma progression occurring within 1-3 months in all responders. Although transient conditioning-related regression of metastatic melanoma may occur following NST, the likelihood of a clinically meaningful GVT effect appears low. The high risk of death from rapid disease progression discourages the present approach in patients with treatment-refractory melanoma.

The disappointing results of autologous transplantation in breast cancer have inspired studies of nonmyeloablative allogeneic transplantation in this malignancy. GVT effects, in association with GVHD have recently been described by investigators¹⁰⁻¹². In some cases, responses have occurred after donor lymphocyte infusions. Furthermore, unpublished retrospective data from the International Bone Marrow Transplant Registry suggest the risk of relapse/progression may be improved in breast cancer patients

who develop GVHD after nonmyeloablative HCT. Small cases series and anecdotal reports of GVT effects occurring after allogeneic HCT have also recently been described in patients with metastatic ovarian, adeno-carcinoma of the colon, pancreas, squamous cell lung carcinoma, neuroblastoma, and sarcomas¹³⁻¹⁶. Results from larger case series in these malignancies will likely be forthcoming. Because even low-dose chemotherapy can occasionally have surprising activity in many solid tumors, sequential radiographic imaging studies are required to help discern whether tumor regression is related to the conditioning regimen or the consequence of a GVT effect. Regimen-related mortality rates of 10% or more and low overall response rates are limiting factors that will likely keep the number of allogeneic transplants for metastatic solid tumors at relatively low numbers for the time being. Although allogeneic HCT alone is unlikely to cure most patients with metastatic tumors, reports of solid tumors regressing after nonmyeloablative stem cell transplantation have provided proof of principle of the power of allogeneic immunotherapy and laid the foundation for the development of tumor targeted allogeneic approaches and have provide optimism that allogeneic transplantation will be more effectively used in the future.