

Scientific Abstract

An anti-leukemic effect of allogeneic lymphocytes was described nearly 40 years ago (1). The existence of a graft-versus-leukemia (GVL) effect was shown by studies in which leukemic mice treated with irradiation and allogeneic bone marrow were more likely to be cured of leukemia than mice treated with irradiation and syngeneic marrow. Numerous studies over the years have further documented the existence of this effect and delineated its mechanisms in animal models (2,3).

Data accumulated in the late 1970s and 1980s strongly suggested the existence of this graft versus leukemia effect in human bone marrow transplant (BMT) (4-6). Substantially higher relapse rates were observed in recipients of syngeneic grafts as opposed to allogeneic grafts. Among recipients of allografts, relapse rates were higher if graft vs host disease (GVHD) did not occur or if grafts were T-cell depleted. *Horowitz et al.*, in an analysis of 2,254 patients with AML in first remission, ALL in first remission, or CML in chronic phase for the International Bone Marrow Transplant Registry (IBMTR), gave strong support to this concept (7). These investigators studied four groups in detail: recipients of non-T-cell depleted allografts without GVHD, recipients of non-T-cell depleted allografts with GVHD, recipients of T-cell depleted allografts, and recipients of syngeneic transplants. The relative risk of relapse in AML patients was higher in syngeneic transplant recipients than in allogeneic transplant recipients (relative risk 2.58, $p=.008$). Among allograft recipients, GVHD was associated with a significantly lower relative risk of relapse in patients with ALL (relative risk 0.38 for patients with acute and chronic GVHD, $p=.02$), AML (relative risk 0.34 for patients with acute and chronic GVHD, $p=.008$) and CML (relative risk 0.24 for patients with acute and chronic GVHD, $p=.03$). CML patients who received T-cell depleted allografts had a higher risk of relapse (relative risk 5.14, $p=.0001$). T-cell depletion did not result in increased relapse rates in patients with ALL or AML. Further data analysis suggested different anti-leukemia mechanisms: (1) An anti-leukemia effect of GVHD (decreased risk of relapse in non-T-cell depleted allograft recipients with GVHD as compared with recipients of non-T-cell depleted allografts without GVHD); (2) An anti-leukemic effect of allogeneic grafts independent of GVHD (increased risk of relapse in AML patients who received syngeneic transplant compared to recipients of allografts without GVHD); (3) An anti-leukemia effect independent of GVHD that is altered by T-cell depletion (CML recipients of T-cell depleted transplants with or without GVHD had higher probabilities of relapse than recipients of non-T-cell depleted allografts without GVHD). Thus these data provided strong but indirect evidence of a powerful GVL effect in humans. Additionally, higher relapse rates have been noted in patients with non-Hodgkin's lymphoma or Hodgkin's disease receiving similar chemo/radiotherapy regimens after autologous transplant (45%) as compared to allogeneic transplant (15%) suggesting a potential antitumor effect associated with infusion of allogeneic marrow (8,9).

Further evidence of a human GVL effect was provided by the observation of remission after discontinuation of immunosuppression and development of GVHD in patients with post-allograft relapse. Patients with relapsed lymphoma, CML and AML after an

allograft were described who developed complete remission after discontinuation of immunosuppression and subsequent development of GVHD (10-12).

Morbidity and mortality related to GVHD, however, limit the approach of mononuclear cell infusion therapy for the treatment of leukemia which has relapsed following BMT. Theoretically, eradication of effector cells mediating GVHD after mononuclear cell infusion may reduce the morbidity and mortality related to GVHD.

One approach used to eradicate effector cells after they have produced their desired effect is to introduce a suicide gene such as herpes simplex virus thymidine kinase (HSV-TK). This gene confers sensitivity to ganciclovir (GCV) which is a nucleoside analogue that is monophosphorylated by HSV-TK and further phosphorylated to GCV triphosphate which inhibits DNA polymerase and results in cellular apoptosis. This suicide approach is not limited to specific cell types. Several studies have explored transfection of HSV-TK into a variety of malignant cell lines (U251 glioblastoma, A172 glioblastoma, A375 melanoma, A549 lung carcinoma, SK-BR-3 breast adenocarcinoma, Hct15 colon adenocarcinoma, 786-0 renal cell carcinoma, CT26 adenocarcinoma cells, K3T3 sarcoma cells and Ly18 lymphoma cells) (13-16). *Moolten* was the first to describe the eradication of herpes simplex virus infected tumor cells *in vitro* by treatment with ganciclovir. This observation has been extended to treatment of tumor cells transduced with the HSV-TK gene in murine models (16). Studies with HSV-TK gene performed in murine and rat brain tumor models by *Culver et al.* suggest efficacy which has led to the initiation of several trials (17-18). In one animal study, 23 of 30 rats that had HSV-TK gene inserted directly into cerebral glioma had complete tumor regression after treatment with ganciclovir; by comparison, 25/25 non-transduced rats treated with ganciclovir had progression of cancer. In another study, brain tumor cells (4×10^4 gliosarcoma cells) were injected into the rat's frontal lobe area. The expected lethality in this model is 100% within 4 weeks. HSV-TK producer cells or saline were injected directly into the tumor 5 days after inoculation and ganciclovir treatment (150mg/kg/bid) was initiated 10 days after tumor inoculation. Complete macroscopic elimination of tumor occurred in all 14 rats that had injection of the HSV-TK producer cells and in none of the rats receiving saline. Microscopic analysis revealed no residual tumor in 11 of 14 rats (18-19). Interestingly, despite the observation of apoptosis in nearly 100% of the malignant cells only 10% of tumor cells had actually been successfully transduced with HSV-TK. This is termed the "bystander" effect (20). It is postulated that phosphorylated ganciclovir may pass to adjacent cells through intracellular gap junctions, as studies with radiolabeled ganciclovir have shown that phosphorylated labeled ganciclovir can pass from HSV-TK positive cells to HSV-TK negative cells (20).

References

1. Barnes D, Louti J, Neal F. Treatment of murine leukemia with x-rays and homologous bone marrow. *Br Med J* 1956;2:626-630.
2. Truitt RL, LeFever AV, Shih CC-Y, Jeske JM, Martin TM (1990). Graft-vs-leukemia effect. In: Graft-vs-Host Disease. Immunology, Pathophysiology, and Treatment, Burakoff SJ, Deeg HJ, Ferrara J, Atkinson K, eds. (New York: Marcel Dekker, Inc.), pp. 177-204.