

Present results and perspectives of allogeneic non-myeloablative hematopoietic stem cell transplantation for treatment of human solid tumors

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Several clinical observations have confirmed that a donor immune-mediated anti-malignancy effect, called graft-versus-leukemia or graft-versus-tumor, occurs following allogeneic hematopoietic stem cell transplantation. While the potential antitumor effect mediated by donor lymphocytes has been established in many hematological malignancies, its efficacy in inducing clinically meaningful responses in solid tumors has been largely unexplored despite evidence of its potential benefit in experimental animal models. Only in recent years has the investigational application of non-myeloablative stem cell transplantation in patients with refractory non-hematological cancers proved that a graft-versus-tumor effect can be generated in patients with metastatic renal cell cancer and possibly with other solid tumors. In the present article we review the biological basis, development and early clinical results of this novel immunotherapeutic approach for solid tumors.

Key words: allogeneic hematopoietic stem cell transplantation, graft-versus-host disease, graft-versus-tumor, solid tumors

Introduction

In the last two decades, various immunological strategies have been assessed to stimulate antitumor immunity in cancer patients, including vaccination with peptide- or tumor lysate-pulsed dendritic cells or cytokine-mediated immunotherapy [1–3]. While to date these approaches have resulted in limited clinical benefits, they have established proof-of-concept of the efficacy of immunotherapy for cancer, laying the foundation for the development of novel immunotherapeutic strategies. In this field, there is a growing perception that the graft-versus-malignancy reaction that follows allogeneic hematopoietic stem cell transplantation (HSCT) is arguably the most potent form of cancer immunotherapy currently in clinical use [4].

Graft-versus-leukemia effect

One of the most important advances in the field of HSCT for leukemia has been the observation that even the most intense conditioning regimen does not reliably eliminate leukemia clones and that patients experiencing acute or chronic graft-versus-host disease (GVHD) have a lower relapse rate than comparable patients without such complications [5–7]. This led to the speculation that the alloresponse of donor lymphocytes against malignant cells (graft-versus-leukemia, GVL) has a major therapeutic role in the setting of allo-HSCT [8].

The most striking and direct evidence supporting the GVL effect is the observation that patients with chronic myeloid leukemia (CML) and other hematological malignancies relapsing after allogeneic transplantation can be re-induced into complete remission by a donor lymphocyte infusion (DLI) [9–11]. The GVL effect appears mediated by donor T lymphocytes and/or natural killer (NK) cells recognizing host antigens presented in the context of the major histocompatibility complex (MHC) class I and II molecules of leukemic cells [12]; it has been suggested that the tissue distribution/restriction pattern of the target antigen will ultimately determine whether the GVT response is accompanied by GVHD [13].

An antitumor effect mediated by the donor immune system is well recognized also in advanced lymphoid malignancies [14]. The graft-versus-lymphoma effect appears to be particularly important for a reduced incidence of relapse after allogeneic HSCT [14, 15].

Based on the hypothesis that an immune-mediated antitumor effect following allogeneic HSCT might be sufficient in promoting cure of some hematological malignancies, several authors began to explore the use of less-intensive preparative regimens capable of providing sufficient immunosuppression to achieve sustained engraftment of donor hematopoietic cells without ablating host hematopoiesis. This novel approach, named non-myeloablative stem cell transplantation (NST), offers the theoretical advantage of low treatment-related mortality (TRM) and morbidity and can be utilized also in patients not eligible for conventional myeloablative conditioning regimens because of advanced age or comorbid diseases. The post-transplantation infusion of donor lymphocytes is usually part of NST protocols; DLIs are given to convert mixed

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to full donor chimerism and to promote the immune antitumor effect in non-responding patients [16].

NST has proved to be effective in the treatment of various hematological malignancies [14, 17–19] and is now under investigation also in solid tumors as detailed below.

Graft-versus-(solid) tumor effect

Several investigators have documented immune-mediated anti-tumor effects in experimental animal models of allogeneic transplantation. A GVT effect analogous to that seen in the GVL response was first demonstrated by Moskovitch and Slavin [20] against spontaneous lymphosarcoma in New Zealand black mouse hybrids following induction of host-versus-graft transplantation tolerance. Subsequently the same group documented the generation of GVT effect in a murine mammary carcinoma model of BALB/c mice, using naive MHC-mismatched splenocytes [21, 22]. In this model, survival of tumor-bearing hosts was significantly prolonged after transplantation of splenocytes with different grades of HLA disparities. In particular, 21 of 22 secondary recipients of tumor-bearing mice grafted with allogeneic spleen cells did not develop lung metastases, in contrast to syngeneic recipients. This finding provided evidence that minimal residual disease can be effectively eradicated by allogeneic HSCT and that GVT is more effective in the presence of a low tumor burden. The GVT effect can be enhanced by pre-immunization of donor lymphocytes, as shown in a murine mammary carcinoma experimental system, in which a higher rate of survival was observed among mice receiving lymphocytes primed with tumor cells, as compared with mice treated with unmanipulated donor cells [23]. Taken together these observations offer clinicians promising perspectives, since the higher efficacy was not coupled with an equivalent risk of developing acute GVHD, suggesting that it is possible to generate lymphocytes specific for neoplastic cells.

For several reasons, an allogeneic-based immunotherapy for metastatic solid tumors could theoretically have efficacy superior to approaches designed to enhance autologous immunity [4]. First, a healthy donor immune system has yet to develop tolerance against tumor antigens and, once tolerance comes out, regular boosts of immune cells (i.e. DLI) with antitumor potential can subsequently be added. Secondly, donor T cells can be capable of recognizing a wide pool of antigens including minor histocompatibility antigens. In addition, some characteristics of epithelial tumor cells, such as their origin from normal tissues that are a target for GVHD and the high degree of MHC class I expression, may favor the generation of a GVT effect.

Allogeneic HSCT for treatment of solid tumors

Despite laboratory evidence of a GVT effect and of a possible role of allogeneic HSCT against solid tumors, this approach has been investigated in humans only recently after the demonstration that sustained engraftment of donor hematopoietic stem cells can be accomplished with the use of NST. In fact, because of the associated morbidity and mortality, conventional myeloablative high-dose chemotherapy and HSCT is restricted to young, medically fit patients, thus excluding the vast majority of cancer patients from

this therapy. In addition, high doses of cytotoxic drugs are not required as patients with chemo-refractory diseases are treated.

Renal cell carcinoma

Early clinical trials aimed at exploring GVT following NST were focused on metastatic renal cell carcinoma (RCC) as this tumor is known to be immunogenic and sensitive to immune-based therapies [24]. RCC may be susceptible to a GVT effect as T lymphocytes have been shown to be an important component of the antitumor immunological response. In addition to the known effects of cytokines in this disease, it has been reported that clonally expanded cytotoxic T lymphocytes (CTLs) are present in primary and metastatic RCC specimens and demonstrate HLA-restricted cytotoxicity against RCC cell lines [25]. These antigen-specific CTLs have also been shown to persist *in vivo* [26]. To optimize the possibility of generating a GVT effect *in vivo*, rapid and complete engraftment of the donor immune system is required [27]. This goal was achieved in pioneering studies by Childs and coworkers with the use of: (i) a fludarabine–cyclophosphamide highly immunosuppressive conditioning regimens along with a rapid withdrawal of the post-transplantation immunosuppression; (ii) peripheral blood mobilized stem cells which contain high T-cell doses; and (iii) the possible post-transplantation DLI to convert mixed to full donor T-cell chimerism [27].

Ten of the first 19 patients [28] and more recently 19 of 42 patients [29] who received NST at the National Institute of Health had a measurable response, and four patients enjoyed complete, long-lasting responses. Importantly, disease regression typically occurred after ciclosporin had been withdrawn and chimerism had transitioned from mixed to predominantly donor T cell. Acute GVHD occurred concomitantly with tumor regression in several patients. However, acute GVHD was not always required for the generation of a GVT effect as one patient had a complete response without GVHD and several others had tumor regression months after acute GVHD had resolved. This clinical observation, along with *in vitro* studies on CTL populations of donor origin [4], supports the working hypothesis that distinct T-cell populations, capable of recognizing tumor-restricted antigens or molecules shared by both the tumor and the normal tissues, may be involved in producing tumor response following NST. Interestingly, it was observed that only clear-cell carcinoma and not other histologies appears to be a target of a GVT effect. Moreover, patients who had failed to respond to interferon- α before NST had disease response when they were retreated with interferon- α after transplantation.

Other reports have provided further evidence that RCC may be susceptible to a GVT effect [30, 31]. In particular, Rini et al. [31] observed partial responses in four of 12 patients treated with a fludarabine–cyclophosphamide regimen similar to that used by Childs et al. Importantly, the antitumor effect was observed only after 100% donor T-cell chimerism and 6 months after transplantation in all four patients [27].

Overall the reported experiences [28, 30–32] in RCC suggest that younger, otherwise healthy patients with low-volume, slow-growing disease are the best candidates for allogeneic transplantation.

Solid tumors other than RCC

A small number of reports of GVT effects in humans following HSCT from HLA-matched family donors provide evidence that allogeneic antitumor effects can be induced against solid tumors other than RCC as summarized in Table 1. In a pivotal study, Eibl et al. [33] firstly described a GVT effect in a woman with refractory metastatic breast cancer (BC) treated with allogeneic HSCT. The patient experienced regression of liver metastasis associated with the onset of acute GVHD. In addition, the authors demonstrated, in the post-transplantation phase, an expansion of CTLs reactive against patient hematopoietic minor histocompatibility antigens which were capable of lysing partially HLA-matched BC cell lines. More recently, further evidence of a clinically meaningful GVT effect against BC has been reported by Ueno et al. [34], Bregni et al. [30] and Carella et al. [35].

Single-case reports and small series of patients with cancer of various histologies treated with allogeneic HSCT consist of carcinoma of the ovary [36, 37], lung [38], colorectum [39] and prostate [37], as well as sarcoma [32]. In most cases responses were accompanied by the occurrence of acute GVHD. Table 1 summarizes the results of published reports in which different solid tumors appeared to be susceptible to allogeneic immune attack following allogeneic HSCT.

Although a GVT response has been shown *in vitro* in two cases of metastatic melanoma [40], preliminary results of NST in this disease are discouraging as median survival following NST was 100 days in 25 patients reported by Childs et al. [41]. The likelihood of a clinically meaningful GVT effect appears low, as only five patients had a transient conditioning-related tumor regression. This unfavorable experience highlights the need for alternative immune-based strategies in treatment-refractory melanoma [42].

Limitation of NST for solid tumors and present clinical approaches

While stimulating results have been reported mostly in RCC following allogeneic transplantation, it is important to consider these responses in the context of the clinical therapeutic index of the procedure taking into account the risks of transplantation-related morbidity and mortality. Although in NST the regimen-related toxicities appear to be fewer than in conventional high-dose myeloablative transplants, the risk of TRM is still in the range of 10–20%, possibly higher in patients with poor performance status (PS), within 100 days from graft. Given the potential for life-threatening complications, early trials of allogeneic HSCT for solid malignancies involved patients with progressive metastatic disease, often with large tumor masses and a poor PS, in whom prior treatments had failed. Such patients are more frail and the risk of transplantation-related complications and mortality is higher [32]. In addition, the efficacy of immunotherapy with allogeneic lymphocytes requires the full engraftment of the donor lymphocytes [28]. For these reasons and because of the frequent requirement for a long post-transplantation immune suppression, a major limitation of NST in solid tumors is the delay in the anti-tumor response due to the time required for the recovery of cell-

mediated immunity and for the generation of T-cell response. Therefore, patients with poor PS and/or rapidly progressing metastatic disease are not likely to live long enough for the generation of a GVT effect and should not be treated with NST. Ongoing studies of NST are in fact selecting patients at an early stage of metastatic disease with a lower tumor burden. Intensified chemotherapeutic treatments in selected patients with chemosensitive diseases [35] or surgical debulking [28] are currently utilized to reduce the tumor mass before NST.

Study in RCC [28, 30, 31] used substantial doses of alkylating agents in their preparative regimens placing all patients at risk of bleeding and infection. The Seattle group have also seen dramatic responses of RCC after NST using low-dose, i.e. 30 mg/m² × 3, fludarabine plus low-dose, i.e. 2 Gy, total-body irradiation [43]. This lower-dose regimen does not cause pancytopenia and can be managed on an outpatient basis. Whether response rates will be different with the less-toxic Seattle approach is under investigation. However, the optimal non-myeloablative preparative regimen for patients with solid tumors undergoing allografting is likely to depend on several factors, including the aggressiveness of the patient's malignancy and the immunocompetence of the recipient. Immune-compromised patients, such as those previously treated with intensified chemoradiotherapy, could require less intensive immunosuppression to achieve engraftment than a fully immunocompetent recipient [44]. In contrast, in patients with highly proliferative malignancies, a more aggressive conditioning regimen, along with a rapid tapering of post-transplantation immune suppression, may favor the generation of a GVT effect more rapidly. Ongoing studies will hopefully clarify these points and provide information on the optimal post-transplantation immunosuppression and on the incidence of long-term complications of NST.

Based on the results obtained in hematological malignancies, escalating doses of DLI have been utilized in patients with solid tumors not responding to NST and without signs of GVHD for promoting the GVT effect. While some patients experienced tumor regression following this procedure, the role of post-transplantation DLI infusion is not clearly defined in this setting [30, 45]. In particular, the dose of T cells to induce a clinical response and the optimal time interval from allograft to DLI remain to be determined.

The heterogeneous results in different tumor histotypes using NST indicate that GVT effects are likely to vary considerably from one disease to another. At present, the lack of biological information on tumor target antigens and immune mediators for GVT does not allow us to predict which diseases will respond to this approach. For this reason increasing numbers of investigators are performing NST in a wide variety of solid cancers. In this regard, it is mandatory that patients be treated in institutions with proven experience in this setting and in the context of specific clinical trials designed to address major clinical and biological issues. In particular, great attention should be paid to understanding better the mechanisms of immune escape used by tumor cells and how to restore immune competence against cancer. This information is necessary for designing future trials.

Most investigations of NST in solid tumors have required an HLA-matched sibling as stem cell source therefore greatly limit-

Table 1. Published studies of allogeneic stem cell transplantation in solid tumors other than renal cell cancer. Reports in abstract form were excluded. Patients with incomplete data were not considered for evaluation of response. Duration of response was assessed from the time of documented response after transplantation

First author [reference]	Type of tumor	Total number of cases	Evidence of measurable tumor response related to GVT effect	Duration of response (months) ^a	Comment
Eibl et al. [33]	Breast carcinoma	1	CR of metastatic liver lesions	2	Myeloablative conditioning; immune suppression given to control GVHD associated with recurrence. Post-transplantation CTLs capable of lysing partially HLA-matched breast cancer cell lines
Ueno et al. [34]	Breast carcinoma	10	Regression of metastatic liver lesions in two patients	2 and 20+	Myeloablative conditioning associated with chemotherapy-related early responses in five patients
Bregni et al. [30]	Breast carcinoma	6	Two PR	9 and 6+	One additional patient in SD at 1000 days from transplantation; DLJ effective
Carella [35]	Breast carcinoma	14	One CR	21+	Long-lasting responses in two patients with bone metastases only; one patient receiving NST in CR still disease-free at day 890. NST preceded by HDC and autografting
Bay et al. [36]	Ovarian carcinoma	5	One CR	12	One myeloablative, four non-myeloablative conditioning; two patients with short-lasting PR; SD after DLJ in one patient
Moscardo et al. [38]	Non-small-cell lung carcinoma	1	CR	37+	Incidental evidence of lung cancer in the early post-transplantation phase for acute leukemia
Zetterquist et al. [39]	Colorectal carcinoma	1	PR	4	Early death due to transplantation-related complications. Post-mortem histopathological findings of tumor necrosis
Pedrazzoli et al. [32]	Sarcoma	3	One CR (rhabdomyosarcoma)	3	Short-lasting response in the absence of GVHD; two patients having disease stabilization
Peccatori et al. [37]	Ovarian carcinoma	4	One PR	1+	One additional patient achieving non-measurable response. Decrease of serum marker CA-125 in both patients
	Prostate carcinoma	2	One very good PR	6+	One additional patient experiencing reduction of serum marker following transplantation was not evaluable due to early death

^aSymbol '+' denotes continuation of response at the time of publication.

CR, complete response; CTLs, cytotoxic T-lymphocytes; DLI, donor lymphocyte infusion; GVHD, graft-versus-host disease; GVT, graft-versus-tumor; HDC, high-dose chemotherapy; NST, non-myeloablative stem cell transplantation; PR, partial response; SD, stable disease.

ing the number of candidates for this procedure. However, because of the increased risk of GVHD and TRM associated with allogeneic HSCT from HLA-matched unrelated donor (MUD) and the limited experience of NST in solid tumors so far available, it would be very desirable that MUD transplants should not be performed at this stage to avoid any possible confounding element for the evaluation of results.

Allogeneic HSCT for solid tumors in perspective

Clinical studies reported so far provide proof-of-principle that allogeneic T cells can induce clinically relevant GVT effects in RCC and other solid tumors. However, it is already clear that NST without the addition of innovative improvements is unlikely to be a major therapeutic breakthrough for patients with metastatic solid tumors. In the near future the major challenge for investigators in this field will be to understand how to drive the donor immune system in a specific fashion against antigens exclusively or preferentially presented by tumor cells without damaging normal somatic host cells [46]. In other words: can the GVT effect be maximized while GVHD is avoided or minimized? Several lines of research are exploring these issues from different sides.

One possibility to enhance GVT is utilizing donor cells activated against tumor alloantigens. Animal studies have shown that immunization of allogeneic bone marrow transplant recipients with tumor cell vaccines enhances GVT activity without exacerbating GVHD [47, 48]. Furthermore, in a mouse model of mammary carcinoma, Morecki et al. [23] demonstrated that pre-immunization with minor histocompatibility antigen-mismatched tumor or spleen cells was capable of activating effector cells to induce an anti-tumor response. These experimental results provide the scientific background to considering the possibility of using donor lymphocytes primed *ex vivo* against tumor cells as a new clinical tool for eradicating residual disease or in cases in which naive allogeneic cells have not been able to effectively reduce the tumor mass. In this setting immunization of healthy donors with recipient-derived tumor products before collecting stem cells appear less applicable both for safety considerations and because of the risk of enhancing GVHD along with GVT effects [49].

In hematological malignancies it has been already shown that leukemia-specific CTLs generated after allogeneic HSCT are important in maintaining the state of remission [50]. More importantly, Falkenburg et al. [51] demonstrated that in a patient with CML relapsing after allogeneic HSCT and resistant to DLI, treatment with *ex vivo*-generated leukemia-reactive CTLs achieved complete response. Several antigens potentially targeted by allo-reactive lymphocytes have been identified in selected solid tumors. However, within the same tumor and at different stages of the disease, the expression of tumor-associated antigen may vary considerably, thus rendering the production of antigen-specific CTLs a rather difficult task. It has been recently shown that CTLs can be effectively generated using the whole tumor cell, which allows epitopes to be selected that are immunogenic in the context of the individual CTL repertoire [52]. The generation of CTLs with multiple specificities should theoretically diminish the possibility

of selecting for escape variants by the poor immunogenicity of cancer cells *in vivo*. This approach could well be applicable in solid tumors when target antigens are unknown. In this regard, the same authors have recently demonstrated the feasibility of inducing *ex vivo* the generation of donor-derived CTLs directed towards malignant cells from different solid tumor histotypes and displaying low reactivity towards patients' non-malignant cells [53]. From this point of view, preclinical evidence suggests that a lymphopenic host may represent a favorable clinical setting for active immunization or cellular therapy [54]. Although conducted in the autologous setting, a recent study by Dudley et al. [55] has provided for the first time evidence of cancer regression by the adoptive transfer of tumor-reactive T cells directed against melanoma antigens in patients receiving a non-myeloablative, highly immunosuppressive conditioning regimen. This novel approach resulted in the persistent clonal repopulation of cancer patients, with the transferred cells proliferating *in vivo*. The adoptive transfer of specifically immune rather than naive tumor-reactive T-cell populations may reduce the risk of GVHD.

While the adverse manifestations of GVHD are related to the number of T cells in the stem cell inoculum, T-cell depletion strategies designed to avoid excessive GVHD are currently under refinement. They include the delayed T-cell add-back strategy [56], the use of suicide gene systems [57] and selective CD8+ depletion [58]. The latter approach is based on the observation that both CD4+ and CD8+ cells contribute to GVHD but that CD4+ cells in the absence of CD8+ cells can still provide a GVT effect [59]. Overall, T-cell depletion approaches can significantly reduce the risk of GVHD but do not provide definitive evidence of improving the therapeutic index of allogeneic transplantation. In fact, they may increase the risk of rejection and delay T-cell chimerism, thus reducing the GVT effect.

Administration, after HSCT, of various interleukins, including IL-1 [60] and IL-11 [61], or procedures capable of interfering with immunoregulatory mechanisms [62, 63], proved to be effective in animal models in inhibiting GVHD while preserving or enhancing GVT. Studies in humans are ongoing. Finally, investigation of allo-reactive NK cells is desirable as these cells are capable of mediating GVL effects in acute myeloid leukemia without inducing GVHD [64].

In summary, the ability of NST to implant a new immune system into a recipient opens new possibilities for treating hematological malignancies and hopefully also solid tumors. NST can be viewed, in perspective, as a platform for adoptive immunotherapy (Figure 1). The donor immune system provided by NST can in fact permit the repeated infusion of alloimmune lymphocytes, tumor-specific T cells or NK cells from the donor without risking their rejection. These new concepts will have to be developed within carefully planned clinical studies. We must keep in mind that NST for solid tumors is still in its infancy, as many basic clinical questions have yet to be answered.

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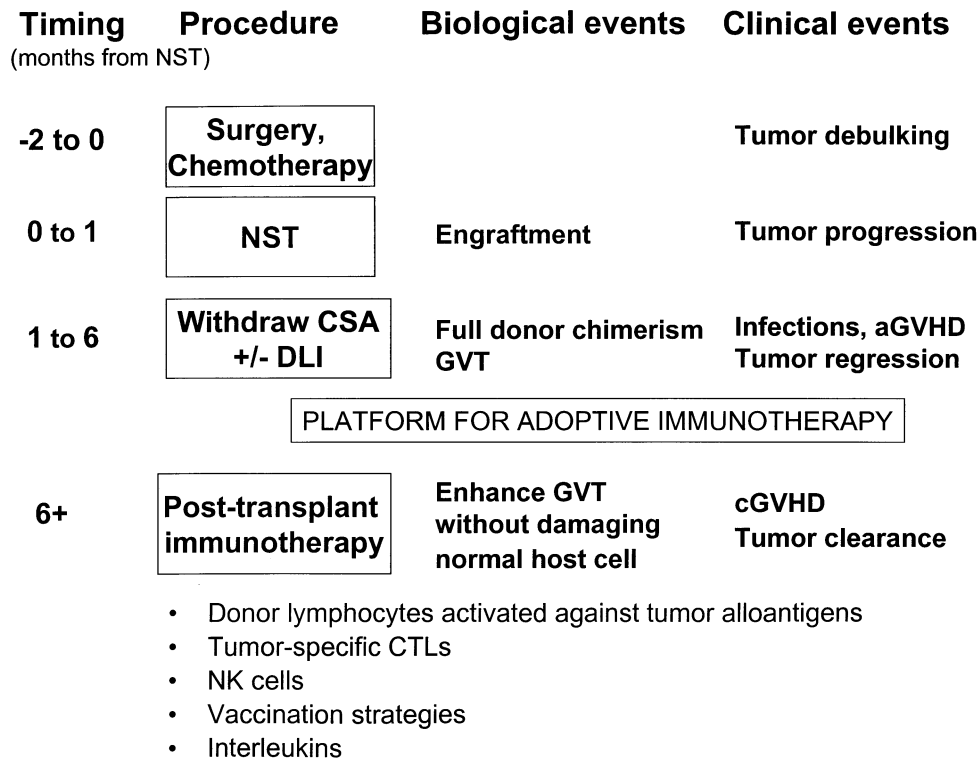


Figure 1. Working hypothesis for future developments in the clinical use of non-myeloablative stem cell transplantation as a platform for adoptive immunotherapy. aGVHD, acute graft-versus-host disease; cGVHD, chronic GVHD; CSA, ciclosporin A; CTLs, cytotoxic T-lymphocytes; GVT, graft-versus-tumor; NK, natural killer; NST, non-myeloablative stem cell transplantation.

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Note added in proof

Additional evidence of a GVT effect in patients with RCC and other solid tumors (including pancreatic carcinoma) has been reported recently.