

MEETING REPORT

The Second International Meeting on Allogeneic Transplantation in Solid Tumors

M Bregni¹, NT Ueno² and R Childs³

¹Oncology-Hematology-Bone Marrow Transplant Unit, Department of Oncology, Istituto Scientifico San Raffaele, San Raffaele Hospital, Milan, Italy; ²Department of Blood and Marrow Transplantation, The University of Texas MD Anderson Cancer Center, Houston, TX, USA and ³NHLBI, National Institutes of Health, Bethesda, MD, USA

In October 2005, the second international meeting on allogeneic transplantation in solid tumors was convened in Stresa (Italy). The aim of this second meeting was to share clinical experiences of allografting in solid tumors, to discuss preclinical data on the mechanisms of graft-versus-tumor (GVT) effect, and to review methods for more efficacious transplant approaches. On the first day, the most recent results in cancer immunotherapy were reviewed; head-to-head comparisons of clinical results achieved by standard therapy and by allografting in renal, breast, and ovarian cancer were presented. On the second day, GVT mechanisms and preclinical models were examined; anecdotal reports of a GVT effect in sarcoma, pancreatic cancer, prostate cancer, colorectal cancer and lung cancer were presented; new strategies for optimizing transplant outcome were discussed, including patient selection, tumor debulking, auto-allo approaches, selective T-cell depletion, targeting with monoclonal antibodies, use of killer cell immunoglobulin-like receptor-ligand mismatched natural killer cells. In conclusion, allografting in solid tumors is feasible with limited toxicities and transplant-related mortality; a GVT effect has been documented in many different solid tumors; targeting of the immune response to the tumor by new strategies and identification of the target antigen(s) of the GVT effect are promising areas of research.

Bone Marrow Transplantation (2006) 38, 527–537. doi:10.1038/sj.bmt.1705479; published online 4 September 2006

Keywords: solid tumors; non-myeloablative allografts; renal cell cancer; breast cancer; ovarian cancer; immunotherapy

In October 2005, the second international meeting on allogeneic transplantation in solid tumors (ATST) was

convened in Stresa (Italy) by Marco Bregni, Naoto Ueno, and Richard Childs under the auspices of the European Group for Blood and Marrow Transplantation (EBMT) Solid Tumor Working Party. The first meeting took place in Milano, Italy, in June 2002, and was a chance for people involved to communicate and discuss initial clinical and biological studies of reduced-intensity allografting in solid tumors.¹ The aim of this second meeting was to share experiences of allografting for solid tumors, update data accumulated in this field over the past 3 years, and to discuss methods to develop more efficacious transplant approaches. In the meeting introduction, *Richard Childs* reported that there were approximately 100 attendees from more than 10 different countries present at the meeting. At the last meeting in 2002, there were only a handful of case reports and a few small case series of allotransplants for solid tumors. Since then, there has been a steady increase in the number of publications describing clinical outcome in patients with select solid tumors undergoing mostly reduced intensity transplantation, including: (i) 12 case series describing graft-versus-tumor (GVT) effects in renal cell carcinoma (RCC); (ii) four small series reporting breast cancer regression as a consequence of a GVT effect, including a report in this year's *Lancet* describing long-term disease-free survival (DFS) in breast cancer patients following a tandem autologous followed by allogeneic transplant approach;² (iii) Numerous case reports and a few case series reporting evidence for GVT effects in other solid tumors including metastatic pancreatic carcinoma, colon carcinoma, ovarian carcinoma and other tumors. Importantly, indirect evidence suggests disease regression occurring after allogeneic hematopoietic cell transplantation (HCT) could potentially prolong survival, as a number of series have reported superior survival in patients having GVT effects compared to nonresponders.

A better understanding of the target antigens and immune mechanisms through which GVT effects occur could make transplant outcome more predictable allowing for enrollment of patients who are most likely to respond.

For the first time, we are now gaining insights into the mechanisms through which GVT effects occur. In this meeting, evidence will be presented supporting GVT effects being mediated by donor T cells targeting minor histocompatibility antigens expressed broadly on recipient

Correspondence: Dr M Bregni, Oncology-Hematology-Bone Marrow Transplant Unit, Department of Oncology, Istituto Scientifico San Raffaele, San Raffaele Hospital, Milan I-20132, Italy.

E-mail: marco.bregni@hsr.it

Received 6 April 2006; accepted 1 July 2006; published online 4 September 2006

tissues and malignant cells as well as antigens that appear to have expression restricted to the tumor.

Despite these advances, there currently exist a number of obstacles that have impeded progress in this field, including restrictions by third party payers to fund these clinical trials, particularly in the US, and difficulties in obtaining referrals of patients who are appropriate candidates for the procedure. Of note, the failure of autologous transplantation to prolong survival in women with breast cancer appears to have had a particularly deleterious impact on referral of patients with solid tumors for investigational allogeneic transplant trials. Remarkably, many practicing oncologist that are in a position to refer appropriate candidates for these types of clinical trials still do not comprehend the immunotherapeutic nature of this strategy. Also, advances in targeted drug therapy for RCC and other solid tumors have recently decreased accrual of patients on transplant clinical trials. The dramatic reduction in transplant procedures for chronic myelogenous leukemia that occurred following the success of imatinib therapy seems likewise to be occurring for RCC corresponding to the success and now recent FDA approval of the tyrosine kinase inhibitors sorafenib and sunitinib. Furthermore, although complete responses do occasionally occur, the vast majority of metastatic tumor patients who have had a GVT effect have only achieved a PR, with most partial responders ultimately developing progressive disease. Despite these limitations, allogeneic transplantation brings to the table something that most drugs cannot yet offer, namely the potential to cure. This meeting will bring us up to date on important observations gleaned from pilot transplant trials conducted over the past 5 years. One of the themes of this meeting will be to develop new transplant strategies that will allow investigators to move from proof of principle to more effective allogeneic immunotherapy regimens for metastatic solid tumors. Better markers of disease prognosis and treatment response are needed in order to prognosticate those patients who are mostly likely to benefit from an allogeneic HCT. It is hoped that the discussions of *in vitro* and preclinical data presented in this meeting by experts in the fields of tumor immunology and allogeneic transplantation will lay the knowledge foundation for the development of more effective transplant strategies utilizing pretransplant tumor debulking approaches and methods to enhance GVT effects using tumor-reactive donor T cells and natural killer (NK) cells.

Session 1 – What is new in immunotherapy for cancer?

Luca Gattinoni (NIH, Bethesda, MD, USA) introduced the session on lymphodepleting therapy and tumor-infiltrating lymphocyte (TIL) by reviewing the current status of cancer vaccines:³ an overall 3.3% objective response rate has been observed in 59 trials that involved 1366 patients, mostly melanoma. By contrast, lymphodepletion followed by adoptive cell transfer reproducibly induced objective responses in half of the patients.⁴ In the pmel-1 ACT murine melanoma model, lymphodepletion enhances the antitumor efficacy of adoptively transferred CD8+ T cells;⁵

in this model, sublethal irradiation acts via indirect mechanisms rather than direct tumor killing. Lymphodepletion does not result in increased number of transferred CD8+ T cells, but it augments their effector function. Endogenous CD4+ T cells (but not CD8+) suppress the antitumor activity of transferred CD8+ T cells, and this activity can be ascribed to CD4+/CD25+ regulatory T cells.⁶ They suppress pmel-1 CD8+ T cells *in vivo*. Sublethal irradiation as well as removal of NK cells enhances the antitumor efficacy of transferred CD8+ T cells even in the genetic absence of Tregs. Levels of interleukin (IL)-15 correlated inversely with NK and activated CD8+ cell populations, and together with IL-7 enhances the antitumor activity of transferred CD8+ T cells. Both IL-7 and IL-15 appear to be necessary for the proliferative response of transferred CD8+ T cells; also lipopolysaccharide, which translocates into bloodstream after damage of mucosal barriers by total body irradiation (TBI), augments the antitumor activity of transferred CD8+ T cells (Paulos CM, MS in preparation). From these data and from clinical experience,⁴ one can assume that a rapid hematological recovery after lymphodepleting therapy may be harmful to antitumor response, because of competition for cytokines, presence of Tregs and other inhibitory elements. This suggests that a myeloablative, rather than a lymphodepleting therapy, may improve adoptive cell transfer-based immunotherapy (Wrzesinski *et al.*, MS in preparation). Moreover, hematopoietic stem cells reinfused after myeloablative therapy produce, or induce the production, of cytokines, growth factors and antiapoptotic factors. This strategy will soon be tested in the clinic.

Maria Grazia Roncarolo (HSR, Milano, Italy) discussed the role of regulatory T cells in inducing tolerance after allogeneic transplantation. She focused her presentation on the characteristics of different Tregs, and on the clinical exploitation of these T-cell subsets for mediating peripheral tolerance to the host and to support immune reconstitution after allografting. Human naturally occurring CD4+ CD25+ T cells are a heterogeneous population of effector and regulatory cells; suppressive clones can be identified based on the high expression of CD25 and FOXP3. For cell therapy purposes, they can be isolated from peripheral blood and *ex vivo* cultured in presence of rapamycin, that selectively expands suppressive subsets that prevent allograft rejection.⁷ Adoptive immune therapy may take advantage also of Tr1 cells that can be *ex vivo* induced by IL-10. IL-10 induces antigen-specific, reproducible anergy in CD4+ T cells while preserving their capacity to respond to nominal and viral antigens. A clinical protocol of add-back reinfusions of IL-10 anergized cells to provide immune reconstitution without graft-versus-host disease (GVHD) in haploidentical transplant is currently ongoing at San Raffaele Institute, having as primary end points incidence of GVHD and of BM aplasia. Six patients have been included so far, without major adverse events. Clinical applications of *ex vivo* IL-10 anergized T cells may expand to human leukocyte antigen (HLA)-matched unrelated transplant: host monocytes can be induced in culture by IL-4 plus granulocyte-macrophage colony-stimulating factor and IL-10 to differentiate into specia-

lized tolerogenic immature dendritic cells (DCs), that can induce anergic CD4+ T cells after *in vitro* stimulation.

Anergic CD4+ Tr1 cells induced by immature/IL-10 DCs suppress proliferation of naïve T cells from the donor, without compromising the normal repertoire of other T cells (e.g., cytomegalovirus (CMV)).

Andrea Velardi (University of Perugia, Italy) discussed the role of NK cells in haploidentical transplantation. Excellent results of haploidentical transplant in high-risk acute myeloid leukemia⁸ were obtained through the engraftment of high doses of T-cell depleted progenitors; problems arising with T depletion are leukemia relapse and delayed immune recovery. NK cells express inhibitory receptors on the cell surface that recognize major histocompatibility complex (MHC) Class I alleles on autologous target cells.

In a mouse model of haploidentical transplantation, human alloreactive NK clones are also able to eliminate human leukemia from SCID mouse. Moreover, infusion of killer cell immunoglobulin-like receptor (KIR)-incompatible NK cells from a H-2d donor into a H-2b recipient was able to condition the host to receive a T-replete transplant from a H-2d donor, thanks to the killing of host antigen-presenting cells by NK alloreactive cells. A further function of alloreactive NK cells is to eliminate host T cells, thus improving engraftment. Besides improving ablation, alloreactive NK cell-based conditioning may contribute to rebuild post-transplant donor immunity, as rapidly reconstituting donor DCs efficiently present pathogens to donor T cells, and increase thymus cellularity (*Ruggeri et al.*, unpublished, 2005). Also, B-cell reconstitution is faster with a NK-based conditioning. In summary, NK-based conditioning in mice enhances engraftment, protects from GVHD, and boosts immune reconstitution (DCs, thymocytes, B-cell precursors). In the future, NK alloreactive cells may be used also for clinical conditioning. The human haploidentical stem cell transplant trial rely on the post-transplant generation of alloreactive NK cell repertoire in patients receiving megadose stem cells and no post-transplant immune suppression. In acute myeloid leukemia (AML) in relapse ($N=50$), alloreactive NK haploidentical transplant does not induce better results than nonalloreactive transplant; in patients with AML in any complete remission (CR) at transplant ($N=60$), alloreactive NK transplant achieves significantly less relapses ($P=0.046$). In the Perugia series of 178 transplants for acute leukemia from 1993 to 2005, GVHD occurred in 8% and in 17% of alloreactive vs nonalloreactive transplant, respectively, and rejection in 5 vs 11%, respectively. Event-free survival (EFS) was significantly better for patients with AML in CR who received a haploidentical transplant from a NK-alloreactive donor ($P=0.03$ vs nonalloreactive). Activating NK receptors, although less studied than inhibitory receptors, may also play a role in leukemia control: in an analysis of 74 haploidentical recipients, multivariate analysis of prognostic factors for relapse found alloreactivity and disease status as the only significant factors, while a multivariate analysis of causes of death in remission found a significant influence of a Haplo-a vs KIR2DS1 + 2 genes ($P=0.046$). Thus, the donor may need to bear activating KIR genes.

Session 2 – Update on standard therapy and allografting for renal cancer, breast cancer and ovarian cancer

In this session, for each disease the first speaker described the state-of-the art treatment, and then one or two speakers reported the results of allografting. Renal cancer was first addressed by *Bernard Escudier* (IGR, Villejuif, France). Cytokine treatment with interferon gives a very small survival benefit in renal cancer; interferon plus IL-2 gives higher response rate (18.6 vs 6.5 and 7.5% of either drug) but no proven survival benefit. The probability of response to cytokine treatment is conditioned by the number of metastatic sites, liver metastasis, mediastinal metastasis, time interval to relapse; in 20% of patients, a 70–80% probability of progression can be predicted at the time of relapse. It is uncertain if treatment with cytokines will have a future in renal cell cancer: while in good prognosis patients the *i.v.* administration seems to ameliorate survival over the *sq* formulation (40 vs 25% overall survival (OS) at 4 years, PERCY DUO study), the survival of intermediate prognosis patients is 15% at 2 years, irrespective of therapy. The biology of renal cell cancer offers new and exciting opportunities of treatment: the central role of vascular endothelial growth factor (VEGF) as mediator of tumor vasculature development and maintenance has been clarified. Growth factors (epidermal growth factor (EGF), platelet-derived growth factor (PDGF), insulin-like growth factor (IGF)-I, basic fibroblast growth factor (bFGF), among others) promote angiogenesis by an mTOR-dependent upregulation of hypoxia-inducible factor (HIF)-1 α , which in turn promotes transcription of VEGF and PDGF- β , and other hypoxia-inducible genes. Bevacizumab is a recombinant humanized monoclonal antibody to VEGF. In a randomized, placebo-controlled trial at NIH, bevacizumab at high dose (10 mg/kg) has reported a 10% partial response (PR) rate, and an increase in progression-free survival (PFS) as compared to placebo and to low-dose (3 mg/kg) bevacizumab. SU11248 (sunitinib) is a small molecule that inhibits VEGF- and PDGF-mediated signaling. In a phase II trial PR in 33% and stable disease >3 months in 37% of the patients have been reported. BAY43-9006 (sorafenib) is a potent, oral inhibitor of RAF kinase and VEGFR-2. In a randomized phase III study vs placebo in patients who have failed one systemic treatment (TARGET study), sorafenib has induced a low rate (2%) of PR, but a high rate of stable disease and minor responses not qualifying as PR (78%). The PFS was doubled in the sorafenib arm compared to placebo (24 vs 12 weeks, P -value < 0.000001). Other new compounds (AG-013736, temsirolimus) have demonstrated antitumor activity in phase II–III trials in advanced renal cancer. Renal cancer treatment is moving quickly from cytokines to targeted therapy; these drugs, though not achieving CRs, probably prolong survival.

On the side of allografting, *Richard Childs* (NIH, Bethesda, USA) updated the rationale and the results of nonmyeloablative allografting in renal cancer. Studies of nonmyeloablative transplantation for renal cancer were initiated at NIH in 1997, and in 1999 the first case report of a GVT effect in renal cancer was published. As of today, 10 series have been published: most of them are small (less

Table 1 Allogeneic transplantation for renal cancer: major series

Authors and reference no.	No. of patients	GVHD (%)	TRM (%)	Evidence for GVT (%)
Childs <i>et al.</i> ⁹	19	74	12	Yes-53
Rini <i>et al.</i> ¹⁰	18	50	14	Yes-22
Bregni <i>et al.</i> ¹¹	7	86	0	Yes-57
Pedrazzoli <i>et al.</i> ¹²	7	0	29	No
Hentschke <i>et al.</i> ¹³	10	50	20	Yes-30
Ueno <i>et al.</i> ¹⁴	15	60	20	Yes-20
Nakagawa <i>et al.</i> ¹⁵	9	56	0	Yes-11
Massenkeil <i>et al.</i> ¹⁶	6	67	0	Yes-33
Blaise <i>et al.</i> ¹⁷	25	—	—	Yes-8
Tykodi <i>et al.</i> ¹⁸	8	63	0	Yes-13
Rini <i>et al.</i> (in press)	22	50	9	No

Abbreviations: GVT = graft-versus-tumor; GVHD = graft-versus-host disease; TRM = transplant-related mortality.

than 10 patients), and eight report a GVT effect, albeit with highly variable response rates (8–57%) (Table 1). Notably, a lack of tumor response has been reported in two transplant series (Pedrazzoli and Rini, Table 1), where death, mostly from tumor progression, occurred early at a median 3.0 and 5.5 months, respectively. As the transplant conditioning regimen is ineffective in cyoreducing the tumor burden, and since GVT effects against renal cancer are delayed relative to hematological malignancies, patients often succumb to their tumor before a GVT effect can occur. The fact that GVT effects against RCC appear delayed relative to hematological malignancies cannot be ignored. New transplant trials that incorporate strategies to control disease until a donor immune-mediated antitumor effect occurs such as through the use of tumor angiogenesis inhibition after the transplant period, could potentially overcome this limitation.

Overall, proof-of-concept that renal cancer is susceptible to a GVT effect has been established; GVT effects in RCC are frequently associated with GVHD, cyclosporine withdrawal, and conversion to full-donor chimerism, with responses being observed in cytokine refractory patients. Although most responses are partial, complete responses can be durable. The pilot trial conducted at the National Heart, Lung, and Blood Institute started in 1997 enrolling patients with progressive metastatic disease with an Eastern Cooperative Oncology Group performance status of <2 without central nervous system metastasis. Patients were required to have failed some form of cytokine-based immunotherapy before transplantation, most commonly IL-2 or interferon-alpha. At present 75 patients with a median of two metastatic sites have been enrolled onto study. The GVHD prophylaxis has consisted of cyclosporine A, given alone in the initial cohort of patients, then later in combination with mycophenolate mofetil (MMF) or mini-dose methotrexate (MTX). The cyclophosphamide/fludarabine-based conditioning regimen has proven to induce rapid T-cell chimerism with a more delayed conversion from mixed to full-donor myeloid chimerism. Tumor responses have been delayed in onset (130–160 days from transplant), frequently preceded by tumor progression, occurring sometimes after the administration of post-transplant interferon in patients who had previously failed

interferon, and in some cases have been durable. Overall, sustained engraftment was achieved in 74/75 patients. Acute and chronic GVHD has been observed in approximately 50% of patients. Death as a consequence of transplant-related mortality has occurred in 8% of patients, half of which died from complications related to GVHD. To date, 38% of patients have had radiographic evidence of tumor regression (27% PR, 9% CR) with responses occurring at median day +160 from transplant (range 30–425). Several prognostic factors have been associated with response including a limited number of metastatic sites, lung only metastasis, clear cell histology and slowly progressive disease. Liver metastasis appeared to be a negative prognostic factor (11% response rate in those transplanted with liver metastasis), while lung metastasis was a positive factor (55% response rate); Responses in nonclear histologies including papillary tumors have not been observed.

Progressive/refractory disease after transplant was treated in a number of ways. Thirty-six patients were treated with donor lymphocyte infusions (median 2); 17% with primary progressive RCC responded including five PRs, and one CR). Eighteen patients received α -interferon with four (22%) PRs being observed; in contrast no patients treated with IL-2 following transplantation had a tumor response. The use of post-transplant angiogenesis inhibition with bevacizumab or VEGF-R thymidine kinase inhibitors in nonresponding patients is currently being evaluated. As observed by others, patients who achieved a disease response had a significant prolongation in survival compared to nonresponding patients. In the last part of his talk on mechanisms of GVT effects, Dr Childs reported exciting new data on a CD8+ cytotoxic T lymphocyte (CTL) clone, isolated from a patient who had a GVT effect that appeared to be targeting a yet-to-be identified RCC restricted antigen. In the second talk on renal cancer, Andrew Artz (University of Chicago) summarized studies reporting on responses to allografting. Out of 14 studies reported in the literature, a total of 163 evaluable patients had 32 PR and seven CR, for an overall response rate of 24%. Donor lymphocyte infusions (DLI) were utilized in 37 patients with two responses (5%). He concluded that a 24% response rate is modest but promising in such a cytokine-refractory population, and that data do not allow conclusions on the best conditioning regimen and on GVHD prophylaxis. He next reported the University of Chicago experience of allografting in renal cancer. Eighteen patients received 19 transplants, with a fludarabine-cyclophosphamide-based conditioning regimen.¹⁹ PRs were 22%, time to response 180 days, and duration of response was 20 months. Transplant-related mortality (TRM) was high at 26, and 47% had a progression of the disease. Hemoglobin <12 g and PS >0 were identified as negative pretransplant prognostic factors.

In reviewing the published literature to identify prognostic factors, he used a novel indirect method to show that patient selection was a critical determinant to transplant outcome. Patients enrolled earlier on given trial had better outcome than those enrolled later on the same trial (33 vs 19%). Independent of GVHD, earlier enrollment was significantly associated with both fewer responses and

inferior OR. He argued also that published studies may not be representative of the real results, as the International Bone Marrow Transplant Research (IBMTR) data report that only 28% of patients receiving a matched sibling donor allograft are alive at 1 year. He concluded that identification of appropriate candidates, availability of novel agents and insurance approval are the major challenges.²⁰ The breast cancer session was introduced by *Marco Colleoni* (Istituto Europeo di Oncologia, Milano). Metastatic breast cancer (MBC) is a heterogeneous disease; survival time has a median duration in excess of 2 years. Treatment end point is to increase the duration of time without disease-related symptoms, with the lowest costs in terms of side effects. The choice of treatment depends on: extent of the disease, related symptoms, estimation of survival, time to expected response to treatment, subjective attitude of the patient and the physician, personal preference and considerations about quality of life, comorbid medical conditions. Factors predictive of response include ER expression, Her2/neu overexpression, shorter disease-free survival, multiple organ involvement, poor performance status.

Chemotherapy is used for all patients whose aggressive disease requires a fast objective tumor regression. Response rate range between 40 and 90%, the median time to response is 7–14 weeks, and the median duration of response is 6–12 months. It is uncertain if it prolongs survival. There are no data supporting the superiority of any particular regimen, nor a sequential vs a concurrent approach; there is no standard for patients requiring second or further line treatment.

Few chemotherapy clinical trials in MBC have claimed an OS benefit for a particular regimen compared with another. Two studies showed an advantage in OS (docetaxel plus capecitabine vs docetaxel, and gemcitabine plus paclitaxel vs paclitaxel), but neither included a mandatory crossover for patients randomly assigned to the single-agent arm. Innovative chemotherapy schedules and dosing (dose intensity; dose density; dose escalation; metronomic dosing) marginally improves survival; notably, the updated Philadelphia trial did not show a survival advantage for the high-dose arm. Other less toxic therapeutic options (hormonal therapy; trastuzumab for the Her2/neu+ tumors; bevacizumab) should be considered and discussed with the individual patient, taking into consideration the subjective attitude and her personal preferences. In the near future, a patient-tailored approach will be possible thanks to identification of breast cancer molecular subtypes.

Naoto T Ueno (MD Anderson Cancer Center, Houston) reviewed the recent advances in allogeneic transplantation for breast cancer. He described the first experiences of myeloablative transplant at MD Anderson in the 1990s with the preparative regimen CBT (cyclophosphamide, BCNU, thiotepa) followed by donor PBSC for patients with breast cancer metastatic to liver or bone marrow. He observed a GVT effect (one CR, two PR) at tapering of immunosuppression, as other investigators (Bregni, Carella, Bishop, Rizzieri, Blaise, Cheng) have done after a nonmyeloablative regimen. He then described the joint CIBMTR/EBMT analysis of allogeneic transplantation in metastatic breast cancer. This analysis was done to

determine feasibility and efficacy of allogeneic hematopoietic stem cell transplantation (HSCT) for metastatic breast cancer, and to describe outcomes of allogeneic HSCT for metastatic breast cancer (TRM, relapse rate, disease-free survival, and OS). Seventy-five patients who have received an allografting between 1992 and 2000 from a HLA-identical or unrelated donor from 16 centers were included. A GVT effect was suggested by the occurrence of disease response, according to a preliminary data analysis. For future studies, reduced conditioning regimens, selection of optimal candidates, and combination of other approaches with transplant are the major issues that need to be addressed. In the MD Anderson DM97-268 study which uses reduced conditioning regimen, only patients with responsive disease (in CR or PR) were considered eligible. So far 16 patients were included, median 41-years old, most ($n=12$) in PR, already treated with ≥ 2 regimens. At a median follow-up of 592 days, the median PFS is 337 days; several long-term responses are observed. In the future, allogeneic transplant should be preceded by debulking of the disease, and combined with antigen-specific T cells or vaccines for inducing GVT.

Michael Bishop (NIH, Bethesda, MD, USA) reviewed the utilization of allogeneic T cells as adoptive immunotherapy for metastatic breast cancer. He first presented the results of the ABP1 study, in which the cytotoxic antitumor effects of the reduced-intensity regimen were separated from the potential GVT effect, thanks to a T-cell depleted graft and to delayed donor lymphocyte infusions. In this study, all responses ($n=6$, four PR and two minor response (MR)) occurred following lymphocyte infusion and after the disease had progressed, suggesting an immune-associated GVT effect.²¹ Optimization of the GVT effect against breast cancer is being pursued in the phase I–II trial of administration of *in vitro* generated Th2/Tc2 cells to augment a TCD allograft ('T cell exchange'). Th2/Tc2 cells have been shown to abrogate graft rejection,²² to decrease GVHD, and to exert activity against breast cancer.²³ Other potential combinations with allogeneic cell therapy include TILs and vaccine (PANVAC-F) expressing MUC-1 and/or CEA, which are expressed on >90% of breast cancer cells. Future plans include identification and expansion of T-cell populations with tumor specificity; building tumor-specific donor lymphocytes (TDL) therapy into allografting protocols; assessment of the effect of TDL in combination with standard and novel cancer therapies, and of tumor vaccine administration on TDL potency.

The third and final part of the session was dedicated to ovarian cancer. *Jonathan Ledermann* (University College, London, UK) presented the state-of-the-art management of ovarian carcinoma, and the trends for the future. Ovarian cancer accounts for 6% of all cancer deaths; in 80% of the cases it presents as advanced disease. Surgical cytoreduction (bilateral salpingo-oophorectomy and omentectomy) followed by a chemotherapy doublet with platinum compounds is regarded as standard of care. Despite a 75% response rate to chemotherapy (40–50% CR) 75% of the patients relapse and die of their disease. Several therapeutic strategies have been tried to improve on these results, that is, triple drug combinations, sequence of non-cross resistant drugs, maintenance/consolidation therapy,

monoclonal antibodies and high-dose chemotherapy with autograft support. There have been two European randomized phase III studies of high-dose therapy (the GINECO/FNCLCC/SFGM-Italy trial and the HODOC-EIS trial). The first showed a superior PFS in the high-dose arm, but neither study showed an improvement in OS. Molecular targeted therapy and immunotherapy are strategies that hold promise for the future.

Jacques-Olivier Bay (Clermont-Ferrand, France) presented the results of allografting in ovarian cancer on behalf of the EBMT. He analyzed 24 patients who had undergone 26 transplants, two with a myeloablative regimen (Cy + busulfan) and 22 with one of three different nonmyeloablative, fludarabine-based regimens. Patients were in median 51-years old, and had received allograft a median of 41 months from diagnosis; disease status at transplant was PD in nine, stable disease (SD) in 10, PR in six, and CR in one patient. The graft was bone marrow in five (19%) and PBPC in 21 (81%). Engraftment and chimerism were rapid and complete, irrespective of the conditioning. aGVHD was observed in 15 patients (≥ 2 in 11 cases, 44%), and correlated with tumor response in eight cases. cGVHD occurred in eight patients, in three after DLI. Seven patients received DLI for PD, and three obtained a PR associated with cGVHD. Responses were as follows: 0 CR, 13 PR, seven SD, four PD. TRM was relatively high (six patients, 28%), and 16 patients died for disease progression. Median survival was 10 months. He concluded that a graft-vs-ovarian cancer effect was suggested by the data, and that TRM should be lowered to present allografting as a viable therapeutic option.

Session 3 – Graft-versus-solid tumor: from mouse to man

Shimon Slavin (Hadassah University, Jerusalem, Israel) examined how to maximize the GVT effect and minimize antihost reactivity. He discussed four topics: (1) nonspecific activation of donor lymphocytes by cytokines; (2) alloactivation of donor lymphocytes by host alloantigens; (3) using specifically immune donor lymphocytes as anticancer effector cells; (4) target-activated lymphocytes to cancer cells to maximize GVT effects and minimize GVHD. Immunization against leukemia-specific minor histocompatibility antigens cure leukemia in the mouse model (in the BALB/c mouse inoculated with BCL1 murine leukemia cells and receiving BCL1-immune B10.D2 cells, survival is over 90%). A GVT effect is visible against 4T1 murine breast cancer by alloactivated or tumor-activated donor lymphocytes. He then addressed the role of lymphoablation, and/or high-dose chemotherapy and autologous transplant, in combination with allogeneic cell therapy. High-dose chemotherapy may be useful as debulking to accomplish minimal residual disease, to establish a niche for donor lymphocytes, and to eliminate suppressor T cells; allogeneic cell therapy may follow with cytokine-activated or specifically immune DLI. He mentioned the results of his pilot trial of haploidentical, IL-2-activated donor lymphocytes after autologous transplant in patients with acute promyelocytic leukemia (APL), in remission since 1992. Immunotherapy by allogeneic lymphocytes may be per-

formed by naïve cells, in this case their antitumor effect may be slow and tolerance may be required, or by activated lymphocytes, that are faster and tolerance is not mandatory. Also disease burden may play a role in this decision (minimal residual disease may require a short-term immunotherapy and no tolerance, while bulky disease needs more prolonged immunotherapy and tolerance induction is necessary).²⁴ He then described the use of bispecific, trifunctional antibodies for serotherapy and allogeneic cell-mediated immunotherapy. These antibodies are chimeric mouse-rat constructs with specificity for CD3 and for an epithelial tumor-associated determinant (EpCAM or Her-2/neu), and they can mediate better targeting of allogeneic cells in the murine C57Bl/6 model infused with melanoma cells transduced with human EpCAM. He concluded that (a) preclinical models and pilot clinical trials suggest that targeted cancer immunotherapy is feasible; (b) both allogeneic T lymphocytes and NK cells induce GVL and GVT effects: immunotherapy mediated by NK cells may be devoid of GVHD, and GVT effects mediated by T cells can be optimized by targeting T cells to the tumor site; (c) specifically immune T cells can induce effective and relatively selective cancer immunotherapy; (d) immunotherapy of both T cell and NK cells can be amplified by IL-2, cox-2 inhibition, and by prior chemoradiotherapy for both tumor debulking and lymphodepletion. *Michael Nishimura* (The University of Chicago, Chicago, IL, USA) reported exciting results on the generation of large numbers of tumor antigen-reactive T cells for transfer into cancer patients. Genes encoding tumor and viral antigen-specific T-cell receptors can be introduced into primary human T cells by retroviral-mediated gene transfer as a potential method of providing any patient with a source of autologous tumor-reactive T cells. A T-cell receptor-specific for a class I MHC (HLA-A2)-restricted epitope of the Hepatitis C virus (HCV) gene NS3 was isolated from a CD8+ T cells clone isolated from a liver transplant patient. T-cell receptor-transduced T cells secreted various cytokines when cocultured with HCV NS3:1406–1415 peptide-loaded antigen-presenting cells as well as tumor cells expressing HCV sequences in an HLA-A2-restricted manner. Furthermore, purified CD8+ and CD4+ T-cells isolated from these cultures showed the HCV TCR transduced T cells could recognize HLA-A2+ melanoma cells, giving us the possibility of engineering class I MHC-restricted effector and T helper cells against HCV+ hepatocellular carcinoma cells. The ability to confer class I MHC-restricted tumor cell recognition to CD4+ T cells makes this HCV reactive TCR an attractive candidate for T-cell receptor gene transfer-based immunotherapy. *Yoshi Takahashi* (NIH, Bethesda, MD, USA) discussed efforts at the NHLBI to characterize the mechanism through which GVT effects occur against metastatic RCC following allogeneic transplantation. Clinical observations have provided indirect evidence that allogeneic T cells play a central role in mediating graft-vs-RCC effects including: (1) tumor regression being delayed in onset and typically not occurring until cyclosporine or tacrolimus has been tapered or withdrawn; (2) the association of GVHD with GVT effects (3) Disease regression following donor lymphocyte infusions (4) Disease regression following

interferon-alpha therapy in patients who failed to respond to interferon prior to transplantation. *In vitro* studies to investigate the mechanisms through which GVT effects occur have provided more direct evidence associating donor T cells with GVT effects including (1) lineage-specific T-cell chimerism being predominantly or 100% donor in origin during tumor regression; (2) the observation that interferon-gamma producing T cells expand in patients at the time of tumor regression; (3) the isolation of donor CD8+ T-cell clones specific for patient minor histocompatibility antigens (mHa) that lyse RCC cells *in vitro*.

At the NHLBI, efforts have focused on establishing tumor cells lines in patients undergoing transplantation so that the exact target antigen of GVT effects can be characterized in responding patients. Using patient tumor cells to stimulate peripheral blood mononuclear cells (PBMCs) collected from patients at multiple time points post-transplant the following observations have been made (1) interferon-gamma-based ELISPOT analysis has shown donor CD8+ T cells reactive against the patient's RCC cells are absent at baseline but increase in frequency in patients having disease regression. In contrast, no sustained increase in RCC reactive CD8+ T cells was observed in nonresponders; (2) in some responders RCC-specific T cells could not be expanded *in vitro* although mHa-specific CD8+ T-cell clones capable of lysing both the patient's RCC cells and B cells were identified; (3) in one responder, a donor CD8+ CTL line was established that appeared to have tumor-specific cytotoxicity.

Work is currently focusing on identifying the exact target antigen/s of donor T cells with cytotoxicity profiles consistent with recognition of an RCC-specific antigen. An HLA-A11 restricted CD8+ T-cell clone derived from the above-mentioned CTL line by limiting dilution was found to kill patient RCC cells *in vitro* but not to have cytotoxicity against either patient Epstein-Barr virus transformed B cells or fibroblasts. Importantly, approximately 50% of RCC cells expressing HLA-A11 were also lysed *in vitro* by this T-cell clone, suggesting this antigen may be commonly expressed on RCC tumor cells. Using cDNA expression cloning, work is currently underway to identify the gene and its encoded target peptide recognized by this donor T-cell clone. The identification of an antigen restricted to solid tumor cells using PBMC isolated from responding patient has provided further evidence that disease regression

occurring after transplantation is likely mediated by donor T cells. More importantly, if this antigen is confirmed to have a tumor restricted pattern and to be expressed on a high percentage of RCCs, it potentially could be used in future tumor vaccine trials.

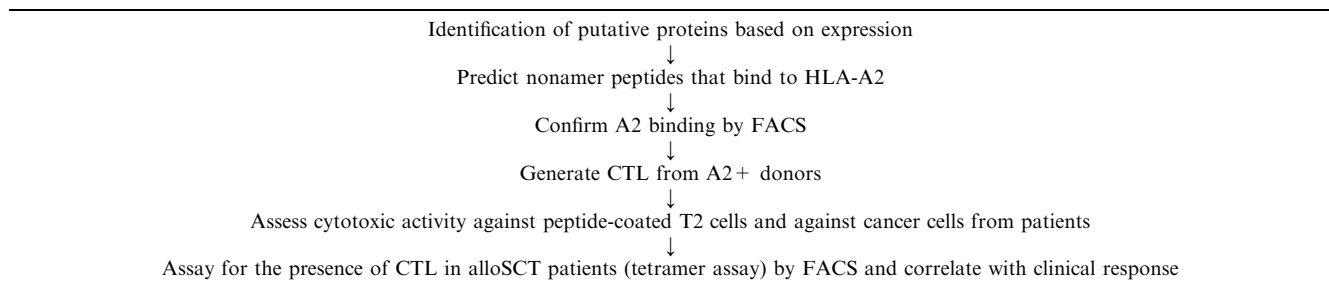
Eric Wieder (MD Anderson Cancer Center, Houston, TX, USA) discussed the rationale and the strategies for identifying RCC-associated antigens. Their identification may enhance therapy by vaccination strategies or by infusing specific CTLs, with the overall goal of increasing GVT while reducing GVHD in the allograft setting. In their prior experience of leukemia-associated antigens (Proteinase 3 and neutrophil elastase) they had evidence that PR1-specific CTL participate in the control of leukemia.²⁵ A deductive strategy for cancer antigen discovery is depicted in Table 2. A number of proteins (G250/CA9; EGLN3), overexpressed in RCC, have been identified as putative tumor-associated antigens; five nonameric peptides from CA9 and EGLN3 with high predicted binding to HLA-A2 were synthesized. Two EGLN3 and one CA9 peptides elicited CTLs with specific cytolytic activity; in the MD Anderson trial of allograft in RCC (see Ueno, second Session) 7/15 patients were A2+, and five were evaluable. CA9- and EGLN3-specific peptides are absent in normal donors, but detectable in RC patients, and preliminary results suggest a correlation with clinical responses. Additional patients need to be studied.

Session 4 – Graft-versus-solid tumors: anecdotal reports

In this session, preliminary clinical evidences of GVT effect in various solid tumors were described. Paolo Pedrazzoli (Niguarda-Ca' Granda Hospital, Milano, Italy) reported the data of a retrospective analysis of EBMT of allografting in advanced soft-tissue sarcoma. The incidence of TRM was higher in the reduced-intensity group compared to myeloablative regimens, emphasizing the importance of a careful patient selection; the long-term survival observed in some patients suggests that a graft-versus-sarcoma effect may exist, but should be substantiated in prospective studies.

Yoshinobu Kanda (The University of Tokyo Hospital, Japan) described a series of seven patients with advanced pancreatic cancer, who received a conditioning regimen based on gemcitabine, busulfan, and fludarabine and

Table 2 Deductive strategy for cancer antigen discovery



Abbreviations: CTL = cytotoxic T lymphocytes; FACS = fluorescence-activated cell sorting; HLA = human lymphocyte antigen.

allograft from a HLA-compatible sibling donor; he observed two minor responses of short duration, and a median survival of 229 days. He concluded that the antitumor effect appeared at discontinuation of immunosuppression, concurrently with GVHD, but was transient. In the next study, they propose to associate a CEA652 peptide vaccine to allografting in patients at an earlier stage of disease. *Marco Bregni* (San Raffaele Institute, Milano, Italy) reported six patients with hormono-refractory prostate cancer, median 61-year old, with progressive disease in 5/6 cases, in good PS at transplant. After a fludarabine/cyclophosphamide/thiotepa conditioning regimen, they developed aGVHD in 5/6 cases that was the cause of TRM in one patient. cGVHD was observed in 4/6 cases (two limited/two extended). Three prostate-specific antigen (PSA) declines at cyclosporine (CSA) tapering and one CR of 4-month duration, documented with PSA decline, CT and TB-PET were reported. He concluded that toxicity was substantial in such an old patient population, and that it is uncertain that PSA responses are clinically meaningful. *Lisbeth Barkholt* (Karolinska Institute, Stockholm, Sweden) reported two patients with prostate cancer, one with prostate and nodal disease, the other free of disease after surgery, RT, and hormonal therapy. They had received an allograft from a HLA-identical sibling donor and from a matched unrelated donor, respectively. The first patient is alive at 29 months, the second died of infection associated to GVHD at 14 months. *Massimo Aglietta* (IRCC Candiolo, Torino, Italy) reviewed recent advances in the treatment of metastatic colon cancer, and described their experience in 15 patients with unfavorable characteristics (high tumor burden, PD at transplant). They utilized the Seattle regimen (fludarabine, 2 Gy TBI, CSA and MMF), and observed a >50% donor chimerism in 13 patients at 90 days after transplant. Transplant-related morbidity was low, TRM occurred in one patient. They observed a PR of 3-month durations in one patient, median OS was 9 months, not different from patients not transplanted. However, in three patients the *ex vivo* detection of CEA-specific T cells was associated with the development of GVHD, and in one case with a PR, that is consistent with a graft-versus-colon cancer effect. They plan to utilize anti-CEA vaccine to boost a tumor-specific response. *Giuseppe Banna* (Istituto Clinico Humanitas, Rozzano, Italy) described the clinical case of a patient with a squamous tracheo-bronchial carcinoma in which a presumable graft-versus lung cancer effect has occurred. This young patient presented with a HPV16-positive tumor unresponsive to four lines of chemotherapy, but had a disease response 4 months after allografting, concurrently with aGVHD and a noninfectious lung failure. Banna speculated that HPV16 infection could have enhanced T-cell recognition in this case, and that a GVT effect against lung cancer should be assessed on future disease-oriented phase II studies. Last case report was presented by *Jacopo Peccatori* (Istituto San Raffaele, Milano, Italy), who illustrated a reduced-intensity haploidentical transplant from a familial donor in a patient with metastatic, cytokine-refractory renal cell cancer. Conditioning regimen utilized was based on thiotepa 10 mg/kg, fludarabine 150 mg/msq, and TBI 200 cGy; T-cell and B-cell depletion

were accomplished with ATG, 25 mg/kg over 5 days, and Mabthera, 750 mg/msq in 2 days. The graft was T-cell depleted, and no other GVHD prophylaxis was utilized. The patient received an unmodified DLI (10^4 CD3+ cells/kg) 3 months after transplant, and 3 months later a PR of the abdominal mass was observed. The duration of response was 6 months, then a slow progression was apparent; he received two further DLIs at 3-month intervals, without GVHD. He is alive at 16 months from transplant, with a PS 90, and a slowly progressing disease. Peccatori concluded that reduced-intensity conditioning for haploidentical transplant is feasible, that a durable donor engraftment can be achieved, and that this GVT effect after haploidentical transplant in RCC confirms that already observed by Satoh and Childs.²⁶ Also, further investigation in this area, including NK alloreactivity, seems warranted.

Session 5 – Optimizing transplant outcome

Didier Blaise (Institut Paoli-Calmettes, Marseille, France) reported a large series of 116 patients recruited in six different ongoing French trials.²⁷ He emphasized the importance of disease status for response in patients with different solid tumors (RCC, breast, ovarian cancer); they have observed 10/25 responses in patients with stable or responding disease (40%) vs 1/52 responses in patients with progressive disease (1.9%). By analyzing responses in single diseases, he confirmed that a GVT effect was apparent in breast and in RCC in 40% of the cases, provided that the tumor is stable or responding. He concluded that procedure-related toxicity is low that responses can be seen in nonprogressive disease, and that responses can lead to a survival benefit.

In his second presentation, *Richard Childs* underlined the importance of tumor debulking before allografting. Both theoretical reasons and preclinical data sustain this assumption: tumors can be immunosuppressive, and allogeneic GVT effects are more effective in murine models with minimal residual disease. In the Balb/C H-2d hosting RENCA RCC tumor and transplanted with B10.d2 H-2d donor cells, survival is inversely proportional to tumor burden. Practical limitations may however apply to this concept: tumor kinetics may exceed the pace of the GVT effect, such as in metastatic melanoma, or tumor growth after transplant may necessitate immediate immunosuppressive treatment, as in case of steroids for CNS metastasis. Clinical experience to date has been anecdotal in patients with RCC, surgically debulked before transplant; another promising strategy may be the autologous transplant followed by allografting in breast cancer, as in Genoa experience (see Carella, below). To optimize transplant outcome, it is conceivable to provide allografting to patients with disease in CR or near CR achieved by autologous transplant or by surgery.

Angelo M Carella (San Martino Hospital, Genoa, Italy) described his strategy of tandem transplant in advanced breast cancer, where autologous transplant has the scope of inducing short-term tumor cytoreduction, and reduced-intensity allograft that of achieving a durable remission through the immune-mediated GVT effect. This strategy

could reduce toxicities associated with either procedure. Seventeen patients have been already included in a pilot study;² they have received a high-dose therapy with mitoxantrone and thiotepa with autologous cell support, followed by an allograft with fludarabine and cyclophosphamide. TRM has been 23% (4/17), due to extensive cGvHD and to infections. One-third of patients had a tumor response, and five patients (29%) are alive at a median of 1957 days after allograft. He concluded that reducing tumor burden can enhance the GVT effect, and that high-risk patients who achieved CR after conventional chemotherapy might have the greatest benefit chance from this approach. In a lively discussion thereafter, it was claimed that patients in CR after therapy most probably would not be eligible in the US for such a trial, given the reported TRM.

John Barrett (NIH, Bethesda, MD, USA) discussed selective depletion of alloresponding T cells as a means to reduce GVHD without compromising GVT effect. The strategy he outlined was to selectively remove host-reacting cells and to eliminate immunosuppression post-transplant, allowing remaining T cells to exert their GVT effect and to create a platform for further boosting of GVT response. As Michalek *et al.*²⁸ have shown, CD25+ cell depletion after donor–recipient mixed lymphocytic reaction (MLR) spares leukemia-reactive cells. In a scale-up of the procedure, recipient cells are collected by apheresis, and CD3+ cells are selected, *ex vivo* expanded in the presence of OKT3 and IL-2, and irradiated. This product is incubated at 1:1 ratio with donor CD34-negative cell fraction, and after 24 h an anti-CD25 immunotoxin is added to the MLR. The cell suspension is then washed and cryopreserved, and utilized for transplant. In a pilot study of selective depletion in older (>50-year old) SCT recipients with leukemia, patients are reinfused with CD34+ cells and allodepleted cells from the donor, after a conditioning regimen based on fludarabine plus alkylating agents. Twenty patients with standard/intermediate ($N=9$) or high-risk ($N=11$) hematological malignancies have been included.²⁹ All patients had a primary engraftment, without adverse events or autoimmune phenomena; T-cell engraftment was rapid (100% donor at day 30), and myeloid engraftment was even faster than in historical controls (100 and 38% at day 30, respectively). Immune recovery of CD4+ and CD8+ cells was rapid, and no CMV disease or death occurred. Median survival was 482 days; 43% had a disease relapse, and day-200 mortality was $28 \pm 11\%$. Acute GVHD grade 0–II was manageable in 90% as outpatients, however, two patients (11%) died of grade III–IV aGvHD. These individuals appeared to have received products that were not selectively depleted. Historical controls had a clearly higher incidence of grade III–IV aGvHD ($41 \pm 11\%$). In summary, selective allodepletion with anti-CD25 immunotoxin is feasible and safe; engraftment is not affected, and GVHD incidence and severity are lessened. Technical improvement of the method will imply an automated procedure and/or photodepletion with a photosensitizer (TH9402) which delivers free radicals to activated T cells. Future developments of graft manipulation will involve *ex vivo* shaping of donor repertoire by eliminating GVH-causing T

cells and boosting CMV-specific CTLs. *Shimon Slavin* (Hadassah University, Jerusalem, Israel) stressed that targeting activated alloreactive donor lymphocytes with monoclonal or bispecific antibodies may not only enhance the cytotoxic effects of killer T cells and NK cells against targeted cancer cells, but also minimize untowards GVHD, due to preferential targeting of alloreactive T cells to cancer cells. In this regards, only the T cells need to be diverted, because activated NK cells, even if fully haploidentically mismatched, are unlikely to result in GVHD, as was documented in mice across MHC (C57BL/6 into BALB/c or (BALB/cxC57BL/6-F1) and in pilot clinical trials, in patients receiving donor-derived rIL-2-activated blood lymphocytes positively selected for NK cells or negatively selected for CD3+ cells.^{30,31}

Francesca Re (San Raffaele Institute, Milano, Italy) presented her findings on the lysis of primary tumor cells by KIR-ligand mismatched, alloreactive NK cells. There is evidence that NK cells play a role in tumor rejection through the mechanism of ‘missing self’, that is, the lack on target cell of the HLA molecule that binds to the inhibitory KIR receptor; this mechanism has been implicated in leukemia control after haploidentical stem cell transplant.³² It is also known that RCC and melanoma cell lines are susceptible to lysis mediated by KIR-incompatible NK clones;³³ it is not clear, however, if fresh tumor cells from primary tumors are lysed by NK cells as well. She designed a technique for purification of fresh cells from different primary tumors (colo-rectal ovarian, renal), and she obtained highly purified tumor cells in nine cases from 69 samples; cells were HLA-typed, and assayed with KIR-mismatched NK cells. A single-cell cytotoxicity assay (SCCA) was utilized, that allowed faster and better cell viability than standard ⁵¹Cr release assay. Specific lysis from alloreactive NK cells was observed in different tumor types (Re *et al.*, in press, 2006). These results open the possibility of clinical utilization of NK cells in the setting of transplantation and/or allogeneic cell therapy.

In the last presentation of the afternoon, *Olle Ringden* (Karolinska Institute, Stockholm) described the clinical model of patients with large primary liver tumors, in whom they wanted to induce mixed chimerism, tolerance and antitumor effects by combining liver and bone marrow transplantation. The indications were hepatocellular carcinoma >10 cm in its largest diameter, or >3 nodules, and cholangiocarcinoma any size. Hematopoietic stem cell procurement from cadaveric donors gave a good yield. Their first patient was a 46-years old woman with a hepatocellular carcinoma. Donor and recipient were HLA-A and -B incompatible, and had a partial match at locus DR. She received TLI 800 cGy, cyclophosphamide 120 mg/kg, TBI 7.5 Gy, antilymphocyte globulin, and syngeneic plus allogeneic T-cell depleted marrow. Immune suppression after liver transplant consisted of tacrolimus and steroids; after marrow transplant, also MTX at days 1, 3, 6, 11 was used. Four patients had received so far a combined liver and marrow transplant; one is alive at a median follow-up of 48 months, three died with disease recurrence.^{34,35}

At the conclusion of the meeting, *Marco Bregni* acknowledged that allografting for solid tumors has

increased its accrual, and that some points of evidence can be made:

1. Allografting in solid tumors is feasible with limited toxicities and TRM. However, further improvement of toxicities and TRM is needed for acceptance by the oncologic community, particularly in diseases which have many therapeutic options (e.g., breast cancer, RCC);
2. A GVT effect can be documented in many different solid tumors. Further research is needed to translate the GVT effect into meaningful clinical benefit;
3. Advanced renal cancer is the solid tumor which seems the best target for allografting; other tumors with promising response rates are breast cancer and ovarian cancer;
4. There is some evidence that small volume disease by debulking of the tumor before transplant, or cytoreduction with chemotherapy with or without autologous transplant, may be of benefit;
5. Targeting of the immune response to the tumor by specific immune lymphocytes, or by NK cells, or by vaccines, is a promising area of research;
6. Unrelated or haploidentical donor transplant for solid tumors is still in a developmental phase;
7. The search for target antigens of the immune response should be the end point of translational research that should be associated to every clinical trial.

All these issues will be the topic of the next 2007 ATST meeting.

Acknowledgements

We thank the sponsors that have made possible this meeting, and all the Speakers, the Chairmen and the Organizing Secretariat. A special thanks to EBMT Solid Tumor Working Party and to its chairman, Dr Taner Demirer, for the patronage of this event.

Note added in proof

The report by Rini *et al.* referenced as 'in press' in Table 1 has been published (Rini BI *et al.*, Adoptive immunotherapy by allogeneic stem cell transplantation for metastatic renal cell carcinoma: A CALGB Intergroup Phase II Study. *Biol Blood Marrow Transplant* 2006 Jul; **12**: 778–785).

References

- 1 Allogeneic hematopoietic cell transplantation for solid tumors. *Haematologica* 2002; **87** (Suppl. 1 to n.6): 1–33.
- 2 Carella AM, Beltrami G, Corsetti MT, Nati S, Musto P, Scalzulli P *et al.* Reduced intensity conditioning for allograft after cytoreductive autograft in metastatic breast cancer. *Lancet* 2005; **366**: 318–320.
- 3 Rosenberg SA, Yang JC, Restifo NP. Cancer immunotherapy: moving beyond current vaccines. *Nat Med* 2004; **10**: 909–915.
- 4 Dudley ME, Wunderlich JR, Yang JC, Sherry RM, Topalian SL, Restifo NP *et al.* Adoptive cell transfer therapy following non-myeloablative but lymphodepleting chemotherapy for the treatment of patients with refractory metastatic melanoma. *J Clin Oncol* 2005; **23**: 2346–2357.
- 5 Gattinoni L, Finkelstein SE, Klebanoff CA, Antony PA, Palmer DC, Spiess PJ *et al.* Removal of homeostatic cytokine sinks by lymphodepletion enhances the efficacy of adoptively transferred tumor-specific CD8+ T cells. *J Exp Med* 2005; **202**: 907–912.
- 6 Antony PA, Piccirillo CA, Akpınarli A, Finkelstein SE, Speiss PJ, Surman DR *et al.* CD8+ T cell immunity against a tumor/self-antigen is augmented by CD4+ T helper cells and hindered by naturally occurring T regulatory cells. *J Immunol* 2005; **174**: 2591–2601.
- 7 Battaglia M, Stabilini A, Roncarolo MG. Rapamycin selectively expands CD4+CD25+FoxP3+ regulatory T cells. *Blood* 2005; **105**: 4743–4748.
- 8 Aversa F, Terenzi A, Tabilio A, Falzetti F, Carotti A, Ballanti S *et al.* Full haplotype-mismatched hematopoietic stem-cell transplantation: a phase II study in patients with acute leukemia at high risk of relapse. *J Clin Oncol* 2005; **23**: 3447–3454.
- 9 Childs R, Chernoff A, Contentin N, Bahceci E, Schrupp D, Leitman S *et al.* Regression of metastatic renal-cell carcinoma after nonmyeloablative allogeneic peripheral-blood stem-cell transplantation. *N Engl J Med* 2000; **343**: 750–758.
- 10 Rini BL, Zimmerman T, Stadler WM, Gajeski TF, Vogelzang NJ. Allogeneic stem-cell transplantation of renal cell cancer after nonmyeloablative chemotherapy: feasibility, engraftment, and clinical results. *J Clin Oncol* 2002; **20**: 2017–2024.
- 11 Bregni M, Doderio A, Peccatori J, Pescarollo A, Bernardi M, Sassi I *et al.* Reduced-intensity hematopoietic cell allografting and escalating-dose donor lymphocyte infusions in advanced renal and breast cancer. *Blood* 2002; **99**: 4234–4236.
- 12 Pedrazzoli P, Da Prada GA, Georgiani G, Schiavo R, Zimbelli A, Giralì E *et al.* Allogeneic blood stem cell transplantation after a reduced-intensity, preparative regimen: a pilot study in patients with refractory malignancies. *Cancer* 2002; **94**: 2409–2415.
- 13 Hentschke P, Barkholt L, Uzunel M, Mattsson J, Wersall P, Pisa P *et al.* Low-intensity conditioning and hematopoietic stem cell transplantation in patients with renal and colon carcinoma. *Bone Marrow Transplant* 2003; **31**: 253–261.
- 14 Ueno NT, Cheng YC, Rondon G, Tannir NM, Gajewski JL, Couriel DR *et al.* Rapid induction of complete donor chimerism by the use of a reduced-intensity conditioning regimen composed of fludarabine and melphalan in allogeneic stem cell transplantation for metastatic solid tumors. *Blood* 2003; **102**: 3829–3836.
- 15 Nakagawa T, Kami M, Hori A, Kim SW, Murashige N, Hamaki T *et al.* Allogeneic hematopoietic stem cell transplantation with a reduced-intensity conditioning regimen for treatment of metastatic renal cell carcinoma: single institution experience with a minimum 1-year follow-up. *Exp Hematol* 2004; **32**: 599–606.
- 16 Massenkeil G, Roigas J, Nagy M, Wille A, Stroszczyński C, Mapara MY *et al.* Nonmyeloablative stem cell transplantation in metastatic renal cell carcinoma: delayed graft-versus-tumor effect is associated with chimerism conversion but transplantation has high toxicity. *Bone Marrow Transplant* 2004; **34**: 309–316.
- 17 Blaise D, Bay JO, Faucher C, Michallet M, Boiron JM, Choufi B *et al.* Reduced-intensity preparative regimen and allogeneic stem cell transplantation for advanced solid tumors. *Blood* 2004; **103**: 435–441.
- 18 Tykodi SS, Warren EH, Thompson JA, Riddell SR, Childs RW, Otterud BE *et al.* Allogeneic hematopoietic cell transplantation for metastatic renal cell carcinoma after non-myeloablative conditioning: toxicity, clinical response, and immunological response to minor histocompatibility antigens. *Clin Cancer Res* 2004; **10**: 7799–7811.

- 19 Artz AS, Van Biesen K, Zimmerman T, Gajewski TF, Rini BI, Hu HS *et al*. Long-term follow-up of nonmyeloablative allogeneic stem cell transplantation for renal cell carcinoma: the University of Chicago Experience. *Bone Marrow Transplant* 2005; **35**: 253–260.
- 20 Artz AS, Kocherginsky M, Van Besien K. Order of patient entry influences outcome for metastatic renal cell cancer after non-myeloablative allogeneic stem cell transplantation. *Br J Haematol* 2006; **132**: 747–754.
- 21 Bishop MR, Fowler DH, Marchigiani D, Castro K, Kasten-Sportes C, Steinberg SM *et al*. Allogeneic lymphocytes induce tumor regression of advanced metastatic breast cancer. *J Clin Oncol* 2004; **22**: 3886–3892.
- 22 Erdmann AA, Jung U, Foley JE, Toda Y, Fowler DH. Costimulated/Tc2 cells abrogate murine marrow graft rejection. *Biol Blood Marrow Transplant* 2004; **10**: 604–613.
- 23 Jung U, Foley JE, Erdmann AA, Eckhaus MA, Fowler DH. CD3/CD28-costimulated T1 and T2 subsets: differential *in vivo* allosensitization generates distinct GVT and GVHD effects. *Blood* 2003; **102**: 3439–3446.
- 24 Slavin S. Allogeneic cell-mediated immunotherapy at the stage of minimal residual disease following high-dose chemotherapy supported by autologous stem cell transplantation. *Acta Haematologica* 2005; **114**: 214–220.
- 25 Molldrem JJ, Lee PP, Wang C, Felio K, Kantarjian HM, Champlin RE *et al*. Evidence that specific T lymphocytes may participate in the elimination of chronic myelogenous leukemia. *Nat Med* 2000; **6**: 1018–1023.
- 26 Satoh M, Miyamura K, Yamada M, Ishidoya S, Childs RW, Arai Y. Haploidentical, non-myeloablative stem-cell transplantation for advanced renal-cell carcinoma. *Lancet Oncol* 2004; **5**: 125–126.
- 27 Blaise D, Bay JO, Faucher C, Michallet M, Boiron JM, Choufi B *et al*. Reduced-intensity preparative regimen and allogeneic stem cell transplantation for advanced solid tumors. *Blood* 2004; **103**: 435–441.
- 28 Michalek J, Collins RH, Durrani HP, Vaclavkova P, Ruff LE, Douek DC *et al*. Definitive separation of graft-versus-leukemia- and graft-versus-host-specific CD4+ T cells by virtue of their receptor beta loci sequences. *Proc Natl Acad Sci USA* 2003; **100**: 1180–1184.
- 29 Solomon SR, Mielke S, Savani BN, Montero A, Wisch L, Childs R *et al*. Selective depletion of alloreactive donor lymphocytes: a novel method to reduce the severity of graft-versus-host disease in older patients undergoing matched sibling donor stem cell transplantation. *Blood* 2005; **106**: 1123–1129.
- 30 Slavin S, Shapira MY, Morecki S, Samuel S, Ackerstein A, Gelfand Y *et al*. Immunotherapy for resistant hematologic malignancies using matched or mismatched rIL-2 activated donor lymphocytes positively selected for CD56+ after allogeneic stem cell transplantation for allogeneic cell therapy without GVHD. *Blood* 2003; **102**: 400b (Abs#5329).
- 31 Slavin S, Morecki S, Shapira M, Bitan S, Samuel A, Ackerstein A *et al*. Use of matched or mismatched rIL-2 activated donor lymphocytes positively selected for CD56+ for immunotherapy of resistant leukemia after allogeneic stem cell transplantation. *Am Soc Clin Oncol* 2004; **23**: 560 (Abs#6516).
- 32 Ruggeri L, Capanni M, Urbani E, Perruccio K, Shlomchik WD, Tosti A *et al*. Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. *Science* 2002; **295**: 2097–2100.
- 33 Igarashi T, Wynberg J, Srinivasan R, Becknell B, McCoy Jr JP, Takahashi Y *et al*. Enhanced cytotoxicity of allogeneic NK cells with killer immunoglobulin-like receptor ligand incompatibility against melanoma and renal cell carcinoma cells. *Blood* 2004; **104**: 170–177.
- 34 Ringdén O, Söderdahl G, Mattsson J, Uzunel M, Remberger M, Hentschke P *et al*. Transplantation of autologous and allogeneic bone marrow with liver from a cadaveric donor for primary liver cancer. *Transplantation* 2000; **69**: 2043–2048.
- 35 Söderdahl G, Barkholt L, Hentschke P, Mattsson J, Uzunel M, Ericzon BG *et al*. Liver transplantation followed by adjuvant nonmyeloablative hematopoietic stem cell transplantation for advanced primary liver cancer in humans. *Transplantation* 2003; **75**: 1061–1066.