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**ALLOGENEIC HEMATOPOIETIC CELL  
TRANSPLANTATION FOR SOLID TUMORS**

**June 28, 2002**

**Milan, Italy**



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4. Red cell aplasia (Editorial). *Lancet* 1982; 1:546-7.
5. Karlsson S, Humphries RK, Gluzman Y, Nienhuis AW. Transfer of genes into hemopoietic cells using recombinant DNA viruses [abstract]. *Blood* 1984; 64(Suppl 1):58a.

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6. Ferrata A, Storti E. *Le malattie del sangue*. 2nd ed. Milano: Vallardi, 1958.
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9. DuPont B. Bone marrow transplantation in severe combined immunodeficiency with an unrelated MLC compatible donor. In: White HJ, Smith R, eds. *Proceedings of the third annual meeting of the International Society for Experimental Hematology*. Houston: International Society for Experimental Hematology, 1974:44-6.
10. Bieber MM, Kaplan HS. T-cell inhibitor in the sera of untreated patients with Hodgkin's disease (Abstract). Paper presented at the International Conference on Malignant Lymphoma Current Status and Prospects, Lugano, 1981:15.
11. Worwood M. Serum ferritin. In: Cook JD, ed. *Iron*. New York: Churchill Livingstone, 1980:59-89. (Chanarin I, Beutler E, Brown EB, Jacobs A, eds. *Methods in hematology*; vol 1).
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# Allogeneic Hematopoietic Cell Transplantation for Solid Tumors

Milan, June 28, 2002  
San Raffaele Congress Center

Under the auspices of



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*“La medicina dispone da tempo sia di un principio sia di un metodo in base ai quali [...] sono state fatte le sue scoperte, che sono molte e utili; e tutte le altre potranno essere fatte se qualcuno che ne abbia le capacità e conosca le precedenti scoperte continuerà la ricerca ponendole come punto di partenza”*

*Ippocrate, Opere, trad. ital. 1961*

The observation that the benefits of allogeneic bone marrow transplantation in hematology depend, to a large extent, on an immunologic effect, has opened the way to exploitation of the same effect in oncology. The transfer of allografting to the solid tumor area has opened a new field of clinical research, focused on the alloreactive T-cell, and more generally, on adoptive immunotherapy as a treatment modality for selected malignancies.

This is the meaning of the sentence reported above, that Hippocrates wrote approximately 400 BC, which means *many discoveries will be made if someone, who knows the methodology of medicine and is familiar with the previous discoveries, uses these as a starting point.*

We know that several solid tumors are susceptible to the graft-versus-tumor (GVT) effect.

We also know that T-cells can eradicate tumor cells of host origin, but are also responsible of graft-versus-host disease, which still represents a major problem in allogeneic transplant. Many efforts are being devoted to the understanding of the GVT effect, and more specific strategies are being developed to increase selectivity of the allogeneic transplant.

The major issues that will be addressed during this workshop are: the identification of new solid tumors amenable to the GVT effect, the dissection of the mechanisms of the graft-versus-tumor effect, the relative merits of conditioning regimens and of GvHD prophylaxis, and the role of donor lymphocyte infusions in inducing responses. The question we are faced with is whether the clinical and immunological responses we observe after allografting will translate in a durable clinical benefit for our patients, with an acceptable toxicity. Moreover, the information gathered with solid tumor allografting may be useful to develop a more specific, antigen-targeted cellular therapy. We hope this workshop will contribute to refine this new and promising approach further.

*Marco Bregni*

## Exploring the role of allogeneic immunotherapy for non-hematologic malignancies: proof of concept and potential immune mechanisms of graft-vs-tumor effects in solid tumors

TAKEHITO IGARASHI,\* OTHON MENA,<sup>o</sup> FRANCESCA RE,\*  
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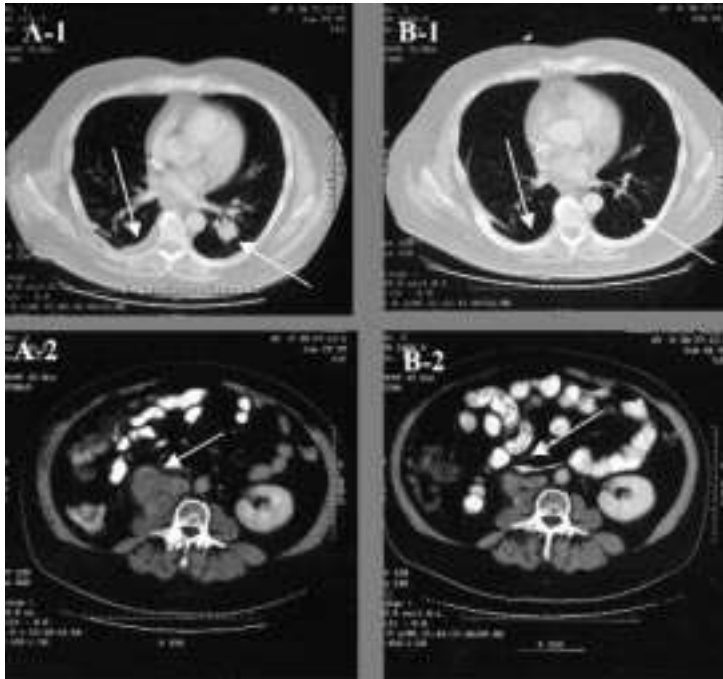
The graft-vs-leukemia effect, mediated by engrafting donor immune cells following allogeneic hematopoietic stem cell transplantation, is capable of inducing disease regression in a variety of different hematologic malignancies.<sup>1-3</sup> Indeed, over the past 15 years the list of hematologic cancers that have been shown to be susceptible to this effect has grown substantially, from chronic leukemias such as chronic myeloid leukemia (CML) to now include acute leukemias, post-transplant EBV-associated lymphoproliferative disorder, chronic lymphocytic leukemia, Hodgkin's and non-Hodgkin's lymphoma, and multiple myeloma. The realization of the powerful nature of the graft-vs-tumor (GVT) effect recently led investigators to test whether malignant hematopoietic cells might be eradicated completely through this donor immune-mediated anti-tumor effect, without the need for toxic dose intensive conditioning. The early clinical results of such *non-myeloablative* or *low intensity* allogeneic stem cell transplants have been encouraging, demonstrating that such procedures are typically well tolerated, with a lower risk of regimen-related toxicity and mortality than is typically seen in conventional *myeloablative* regimens.<sup>4-9</sup> More importantly, the demonstration that molecular remissions can be achieved against a variety of different hematologic cancers following this approach has provided definitive proof that GVT alone is sufficient to cure some cancers.

The powerful nature of the graft-versus-leukemia (GVL) effect in hematologic cancers has recently lured investigators to explore the use of allogeneic stem cell transplantation as a therapeutic modality in treatment-refractory solid tumors.<sup>10-19</sup> Although these studies are very much

in their infancy, case reports and small case series of solid tumors such as kidney cancer, breast cancer and ovarian cancer regressing after non-myeloablative stem cell transplantation have already provided proof of concept and laid the foundation for the development of tumor targeted allogeneic approaches.

### Results of clinical trials

We began exploring the use of non-myeloablative stem cell transplantation in solid tumors at the National Institute of Health in 1997. Initial studies focused on tumors that we knew were a target for autologous-based immune attack, namely metastatic renal cell carcinoma (RCC) and melanoma. All patients were conditioned with cyclophosphamide (60 mg/kg x 2) and fludarabine (25 mg/m<sup>2</sup> x 5), and were then transplanted with a granulocyte colony-stimulating factor (G-CSF)-mobilized blood stem cell allograft from their HLA identical or single antigen mismatched sibling donor. Cyclosporin (CSA) alone or in combination with mycophenolic acid (MMF) was used as graft-versus-host disease (GVHD) prophylaxis and was withdrawn as early as day 30 in patients with mixed T-cell chimerism or disease progression. While no clinically meaningful GVT effects were seen in our patients with metastatic melanoma, renal cell carcinoma was quickly identified as being a target for GVT effects.<sup>12,13</sup> Ten of the first 19 and subsequently 23 of the first 55 patients had regression of metastatic disease compatible with a GVT effect (Figure 1). Four patients who had previously failed to benefit from interferon- $\alpha$  treatment had a disease response when they were re-treated with interferon- $\alpha$  post-transplant. Additionally, in the non-responding cohort, a mixed response was observed in 6 (10%) patients.



**Figure 1.** Regression of metastatic RCC in multiple metastatic sites. Day 100 post-transplant CT images (A1, A2) of a patient with RCC with diffuse metastatic disease involving the lungs, pleura, and retroperitoneum. Four weeks later (B1+B2), following the withdrawal of CSA, repeat CT scans revealed complete resolution of all pulmonary metastatic disease (B1) and partial regression of metastasis in the retroperitoneum (B2).

Five patients died from transplant-related causes. Acute GVHD has been the major toxicity associated with the procedure, occurring in approximately 50% of patients, and has been fatal in 3 cases. The first patient transplanted with metastatic RCC remains in complete remission 4.5 years since undergoing treatment. The observation of GVT effects in RCC led us to initiate similar trials of non-myeloablative allogeneic transplantation for patients with a variety of different treatment-refractory solid tumors. Recently we observed evidence of a GVT effect in a patient with colon carcinoma as well as in a patient with metastatic pancreatic carcinoma.

#### *Mechanisms of graft-vs-tumor in renal cell carcinoma*

Responses in RCC patients following non-myeloablative transplantation provide proof of concept and are a testament to the power of the GVT effect. Unfortunately, however, the vast majority of responding patients with solid tumors who have shown evidence of a GVT effect have failed to achieve a complete remission. While these partial responses may translate into clinically meaningful disease regression, most of the patients will ultimately still succumb to disease progression. Therefore, characterizing the immune mechanisms in patients achieving a major disease response is

important as it may provide the level of understanding of GVT required for the development of possibly more efficacious tumor-targeted allogeneic transplant approaches.

Indirect evidence supporting regression of metastatic RCC as being mediated through the transplanted donor's immune system includes the findings that, typically, metastatic tumor regression: 1) was delayed following transplant (median 5-6 months); 2) followed CSA/MMF withdrawal; 3) occurred after a donor lymphocyte infusion (DLI) (5 patients); 4) was favorably associated with a history of GVHD; and 5) did not occur until T-cell chimerism had changed from mixed to predominantly donor. Figure 2 illustrates the time course of common post-transplant events observed following NST using our conditioning regimen. As has been reported previously for hematologic malignancies, acute GVHD was favorably associated with, but not required to achieve, a clinical response. One patient had a complete response without acute GVHD, and several others whose responses were temporally distant from periods of GVHD illustrate the separation of GVHD from GVT. The engraftment profile and clinical history of a patient with metastatic RCC who had a GVT effect temporally distant from acute GVHD is shown in Figure 3.

A detailed functional analysis of lymphocyte sub-

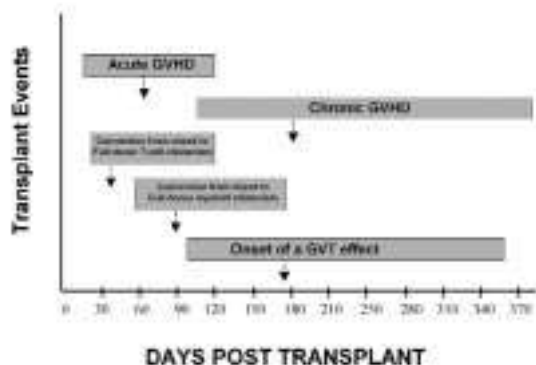


Figure 2. Time course of common post-transplant events following NST using cytoxan/fludarabine conditioning. Boxes show the time range and arrows represent the median time of onset of individual events.

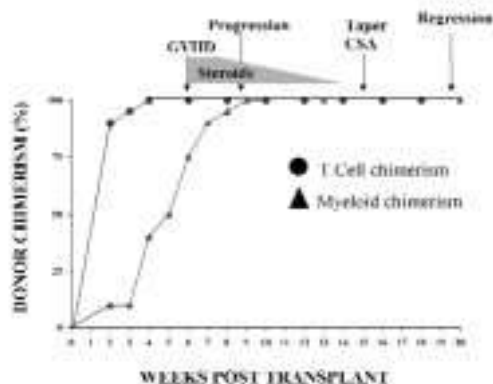


Figure 3. GVT effect temporally distant from acute GVHD. T-cell engraftment (circles), and myeloid engraftment (triangles), shown as percentage donor, was initially mixed, although complete donor T-cell engraftment occurred before complete donor myeloid engraftment. Acute GVHD resolved quickly following a brief treatment with oral corticosteroids. Shortly following the withdrawal of CSA and in the absence of GVHD, regression of metastatic disease occurred in multiple metastatic sites.

sets conducted *in vitro* in responding patients has provided data indicating that these anti-tumor immune responses may be mediated through both *allo-reactive* and tumor-restricted donor T-cell responses. FACS analysis of T-cell subsets obtained from peripheral blood lymphocytes collected from responding patients at the time of disease regression revealed an increase (3-11 fold) in activated

cytotoxic T-cells (CD3<sup>+</sup>, CD8<sup>+</sup>, DR<sup>+</sup>, CD38<sup>+</sup>) from pre-transplant baselines, in contrast to NK cell populations (CD16/56<sup>+</sup>, CD3<sup>-</sup>), which typically declined from baseline during the same period. An analysis of the V $\beta$  repertoire by FACS and TCR spectra typing revealed one or more V $\beta$  subfamilies to be skewed from baseline in these activated CD8<sup>+</sup> populations in all responders analyzed. Using B7.1 transduced autologous RCC cell lines as stimulators *in vitro*, donor-derived CD8<sup>+</sup> T-cell clones have been isolated from several responding RCC patients: these cells have direct MHC class I restricted cytotoxicity against the patients' tumor cells.<sup>19,20</sup> Although we have occasionally expanded cytotoxic T-lymphocytes *in vitro* which appear to have a tumor-restricted pattern of cytotoxicity (isolated from patients with disease regression in the absence of GVHD), the majority of these CD8<sup>+</sup> T-cell clones recognize both the patient's tumor cells as well as autologous EBV-transformed B-cells, strongly implying that minor histocompatibility antigens that are expressed on, but not restricted to, tumor cells are potential target antigens for a GVT effect. Furthermore, when viewed in the context of the patients' clinical histories, our findings suggest that distinct tumor antigens might be targeted in patients who experience tumor regression in the context of GVHD as opposed to those without GVHD. The target antigens of these tumor reactive T-cell clones are currently being pursued. Once defined, these antigens might be used to develop tumor-targeted strategies for future allogeneic immunotherapy approaches.

#### Future development

The clinical response observed in RCC patients refractory to cytokine therapy highlights the power of the alloimmune response that may occur following allogeneic stem cell transplantation. Future investigations will focus on limiting the toxicity related to the procedure, and directing the immune response specifically to the tumor. The major challenge with these approaches will be to obtain blockade of GVHD without compromising GVT effects.

Even though dramatic tumor regression has been noted in some patients with RCC, a very small subset of donor lymphocytes are probably responsible for the anti-tumor effect. Tumor-specific immune responses might be enhanced by vaccination strategies employing a variety of platforms. *Ex vivo* generation of tumor-reactive T-cells followed by their adoptive transfer, or *targeted DLI* could also enhance anti-tumor responses without increasing

the risk of GVHD. Laboratory and clinical data suggest that GVT responses may also be enhanced by the use of cytokines such as GM-CSF and interferons in conjunction with donor lymphocyte infusions. The framework has been laid for future innovative immunotherapy approaches which will attempt to harness the powerful potential of the GVT effect in many different types of metastatic solid tumors.

## References

1. Thomas ED, Blume KG. Historical markers in the development of allogeneic hematopoietic cell transplantation. *Biol Blood Marrow Transplant* 1999; 5:341-6.
2. Weiden PL, Flournoy N, Thomas ED, Prentice R, Fefer A, Buckner CD, et al. Antileukemic effect of graft-versus-host disease in human recipients of allogeneic-marrow grafts. *N Engl J Med* 1979; 300:1068-73.
3. Kolb HJ, Schattenberg A, Goldman JM, Hertenstein B, Jacobsen N, Arcese W, et al. Graft-versus-leukemia effect of donor lymphocyte transfusions in marrow grafted patients. *European Group for Blood and Marrow Transplantation Working Party Chronic Leukemia. Blood* 1995; 86:2041-50.
4. Khouri IF, Keating M, Korbling M, Przepiorka D, Anderlini P, O'Brien S, et al. Transplant-lite: induction of graft-versus-malignancy using fludarabine-based nonablative chemotherapy and allogeneic blood progenitor-cell transplantation as treatment for lymphoid malignancies. *J Clin Oncol* 1998; 16:2817-24.
5. Giralot S, Estey E, Albitar M, van Besien K, Rondon G, Anderlini P, et al. Engraftment of allogeneic hematopoietic progenitor cells with purine analog-containing chemotherapy: harnessing graft-versus-leukemia without myeloablative therapy. *Blood* 1997; 89:4531-6.
6. Slavin S, Nagler A, Naparstek E, Kapelushnik Y, Aker M, Cividalli G, et al. Nonmyeloablative stem cell transplantation and cell therapy as an alternative to conventional bone marrow transplantation with lethal cytoreduction for the treatment of malignant and nonmalignant hematologic diseases. *Blood* 1998; 91:756-63.
7. Sykes M, Preffer F, McAfee S, Saidman SL, Weymouth D, Andrews DM, et al. Mixed lymphohaemopoietic chimerism and graft-versus-lymphoma effects after non-myeloablative therapy and HLA-mismatched bone-marrow transplantation. *Lancet* 1999; 353:1755-9.
8. Childs R, Clave E, Contentin N, Jayasekera D, Hensel N, Leitman S, et al. Engraftment kinetics after nonmyeloablative allogeneic peripheral blood stem cell transplantation: full donor T-cell chimerism precedes alloimmune responses. *Blood* 1999; 94:3234-41.
9. McSweeney PA, Niederwieser D, Shizuru JA, Sandmaier BM, Molina AJ, Maloney DG, et al. Hematopoietic cell transplantation in older patients with hematologic malignancies: replacing high-dose cytotoxic therapy with graft-versus-tumor effects. *Blood* 2001; 97:3390-400.
10. Eibl B, Schwaighofer H, Nachbaur D, Marth C, Gachter A, Knapp R, et al. Evidence for a graft-versus-tumor effect in a patient treated with marrow ablative chemotherapy and allogeneic bone marrow transplantation for breast cancer. *Blood* 1996; 88:1501-8.
11. Ueno NT, Rondon G, Mirza NQ, Geisler DK, Anderlini P, Giralot SA, et al. Allogeneic peripheral-blood progenitor-cell transplantation for poor-risk patients with metastatic breast cancer. *J Clin Oncol* 1998; 16:986-93.
12. Childs RW, Clave E, Tisdale J, Plante M, Hensel N, Barrett J. Successful treatment of metastatic renal cell carcinoma with a nonmyeloablative allogeneic peripheral-blood progenitor-cell transplant: evidence for a graft-versus-tumor effect. *J Clin Oncol* 1999; 17:2044-9.
13. 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; 343:750-8.
14. Bay JO, Choufi B, Pomel C, Dauplat J, Durando X, Tournilhac O, et al. Potential allogeneic graft-versus-tumor effect in a patient with ovarian cancer. *Bone Marrow Transplant* 2000; 25:681-2.
15. Rini BI, Zimmerman TM, Gajewski TF, Stadler WM, Vogelzang NJ. Allogeneic peripheral blood stem cell transplantation for metastatic renal cell carcinoma. *J Urol* 2001; 165:1208-9.
16. Bregni M, Doderio A, Peccatori J, Bernardi M, Pescarollo A, Bordignon C, et al. Graft-vs-tumor effect in advanced solid tumors following reduced-intensity stem cell allografting. *Proc Am Soc Clin Oncol* 2001; 20:45.
17. Makimoto A, Mineishi S, Tanosaki R, Kanda Y, Saito T, Nakai K, et al. Nonmyeloablative stem cell transplantation (NST) for refractory solid tumors. *Proc Am Soc Clin Oncol* 2001; 20:44.
18. Pedrazzoli P, Da Prada GA, Giorgiani G, Schiavo R, Zambelli A, Giralot E, et al. Allogeneic blood stem cell transplantation after a reduced-intensity, preparative regimen. *Cancer* 2002; 94:2409-15.
19. Childs R, Srinivasan R. Advances in allogeneic stem cell transplantation: directing graft-versus-leukemia at solid tumors. *Cancer J* 2002; 8:2-11.
20. Mena O, Igarashi T, Srinivasan R. Immunologic mechanisms involved in the graft-vs-tumor effect in renal cell carcinoma following non-myeloablative allogeneic peripheral blood stem cell transplantation. *Blood* 2001; 98:856a [abstract].

**Non-myeloablative allogeneic  
peripheral blood progenitor cell  
transplantation for metastatic breast  
cancer and metastatic renal cell  
carcinoma: the M.D. Anderson Cancer  
Center experience**

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**A**t the M.D. Anderson Cancer Center, we have been exploring the use of allogeneic stem cell transplantation for metastatic breast cancer (BC) and metastatic renal cell carcinoma (RCC). These two diseases have unique characteristics in the sense that kidney cancer is more immunogenic<sup>1</sup> than breast cancer in the setting of autologous immunotherapy. According to the American Cancer Society,<sup>2</sup> the estimated number of new cases of RCC in 2002 is 31,800, with 11,600 expected deaths. Early stage RCC can be cured by nephrectomy, but most cases present as advanced disease.<sup>3</sup> The standard treatment for advanced disease is immunotherapy with interleukin-2 or  $\alpha$ -interferon, used either singly or in combination. However, the best response rates do not exceed 20%.<sup>4-9</sup> Among those patients who show a complete response to immunotherapy, a significant fraction could remain disease-free. Nevertheless, the 5-year survival rate is less than 10%, with little hope of cure for patients in whom standard immunotherapy failed.

Breast cancer is the leading cause of cancer among women in the United States and worldwide. In the United States, 205,000 new cases are expected in 2002, with an estimated 40,000 deaths.<sup>2</sup> The recent decrease in mortality rate combined with an increase in incidence is due to advances in adjuvant treatment and screening. However, metastatic disease remains a significant challenge to treat. Among the treatment options for metastatic BC are combination chemotherapy, hormonal therapy, monoclonal antibody treatment, and stem cell transplantation.<sup>10</sup> High-dose chemotherapy with autologous stem cell transplant for metastatic breast cancer produces a high tumor response rate; the median duration of

response is about 9-12 months, with 15-20% of carefully selected patients achieving durable complete remission.<sup>11,12</sup> However, the recurrence rate from residual disease is still high.<sup>13-15</sup> After recurrence, the median survival duration is 8-9 months, with 70-80% of patients dying within 2 years. Previous clinical trials of autologous immunotherapy with agents such as interferon, BCG, or interleukin-2 suggest that BC generally does not respond to such an approach.<sup>16</sup> However, since breast tumors have been shown to express major histocompatibility complex (MHC) class I and/or II antigens,<sup>17-19</sup> infusing allogeneic lymphocytes that target variable expression of MHC antigen in the donor could target minor histocompatibility antigens presented by the malignant breast cells.<sup>20,21</sup> Moreover, the use of allogeneic peripheral blood progenitor cells (PBPCs) ensures that the infused cells are free of tumor. At the M.D. Anderson Cancer Center, we have explored this hypothesis by infusing allogeneic stem cells into patients with metastatic BC since 1995. In a pilot study of allogeneic PBPC transplantation after a conditioning regimen of high-dose chemotherapy (cyclophosphamide, carmustine, and triethylenethiophosphoramide) in 10 patients with metastatic BC, we demonstrated regression of metastatic liver lesions in association with skin graft-versus-host disease as the immunosuppressive therapy was being tapered off in 2 patients.<sup>22</sup> This phenomenon, also observed by Eibl *et al.*,<sup>23</sup> suggests the existence of a graft-versus-breast cancer effect. We and Dr. Niederwieser are currently analyzing 61 patients with metastatic BC who underwent allogeneic stem cell transplantation and are registered in the databases of the *European Group for Blood and Marrow Transplantation* and the *International*

*Bone Marrow Transplant Registry.* We hope that this analysis will reveal the rate of graft-versus-tumor effect in metastatic BC.

Given the high morbidity and mortality associated with full myeloablative stem cell transplantation, we developed a reduced-dose conditioning regimen for allogeneic PBPC transplantation with the goal of minimizing major toxic effects among patients who are undergoing a second transplant, are elderly, or have comorbid conditions while still allowing the engraftment of allogeneic PBPCs. The regimen consists of 5 days of fludarabine and 2 days of melphalan. High-dose melphalan followed by transplantation has led to durable remissions in patients with hematologic malignancies, with little extramedullary toxicity.<sup>24,25</sup> Melphalan also produced a high tumor response rate among patients who underwent autologous transplant for metastatic BC<sup>26</sup> or Wilms' tumor.<sup>27,28</sup> Fludarabine, a potent immunosuppressant, produces lymphopenia. We have found that, in combination with high-dose melphalan, fludarabine allows complete chimerism with relatively mild toxicity.<sup>29</sup> We hypothesized that the use of fludarabine with melphalan for metastatic BC or RCC would produce complete donor chimerism, thereby obviating the need for donor lymphocyte infusion (DLI) for full donor engraftment and allow the use of unrelated donors. With this goal, we chose a more intensive regimen than the combination of fludarabine and cyclophosphamide that was used by Childs *et al.*<sup>30</sup>

In our study, patients with metastatic RCC were given a conditioning regimen of fludarabine 25 mg/m<sup>2</sup> IV daily from day -6 to -2 and melphalan 70 mg/m<sup>2</sup> IV daily from day -3 to -2. Patients with metastatic BC received the same conditioning regimen except that the dose of fludarabine was higher (30 mg/m<sup>2</sup>). On day 0, PBPCs that had been mobilized by granulocyte colony-stimulating factor from either an HLA-compatible related donor or an unrelated donor marrow/cord blood sample were infused. The graft-versus-host disease prophylaxis regimen was tacrolimus and micro-methotrexate. If disease recurrence or progression was evident at day 100 without graft-versus-host disease, the tacrolimus was then tapered off rapidly. If mixed chimerism was present at any time, or if the disease recurred or progressed after cessation of the tacrolimus and no evidence of graft-versus-host disease was present, the patient was given DLI. A maximum of 3 DLIs, infused every 6 weeks, was allowed.

From January 1999 to March 2001, we completed transplants for 7 patients with metastatic BC

and 14 with metastatic RCC. For the BC group, the median age was 47 years (range, 36-53) and the median follow-up period was 268 days (range, 118-721); for the RCC group, the median age was 53 (range, 45-64) and the median follow-up period was 105.5 days (range, 19-715). All 7 patients with BC received PBPCs from a 6/6 match-related donor. Of the 14 patients with RCC, 10 received 6/6 match-related PBPCs, 1 received 5/6 match-related PBPCs, 1 received 5/6 match-related bone marrow, 1 received 6/6 match-unrelated bone marrow, and 1 received a 4/6 match-unrelated cord blood sample.

All 7 patients with metastatic BC had successful 100% donor chimerism at day 30 and 100. When this report was written, 2 patients had died from progressive disease, 1 had a complete remission (at a follow up of 721 days), and no DLIs have been needed for donor engraftment or disease control.

Of the RCC group, 7 patients have died, 5 of whom in the first 100 days after the transplant. Among these 5 patients, 2 died of acute graft-versus-host disease, 1 died of complications from primary graft failure, 1 died on day 76 because of a complication of acute myocardial infarction and the other one died of respiratory failure on day 85. Two patients died more than 100 days after the transplant, 1 on day 245 with progressive disease despite an attempt at disease control with a DLI and the other on day 175 of sepsis. All of the patients who survived until day 30 or day 100 after the transplant, except for the 1 patient who had primary graft failure, had 100% donor chimerism. No DLIs were needed for donor engraftment, and DLI was given to 2 patients for disease control.

We conclude that allogeneic PBPC transplantation, with reduced-dose fludarabine and melphalan used as a conditioning regimen, is feasible for metastatic BC or RCC. The treatment-related mortality rate within the first 100 days after the transplant was 5 cases in 21 patients, and 1 of those patients had primary graft failure. The most significant result of our study was our ability to achieve full donor engraftment without further DLI. All patients in whom engraftment was successful retained full donor chimerism. This is important because DLI may not be readily available in some situations, i.e., when samples used are from cord blood or an unrelated donor. Moreover, full donor engraftment is necessary for a significant graft-versus-tumor effect. We had good results with donor engraftment, and thus DLI could be reserved for later use if needed to control disease. Of the 5 cases of treatment-related mortality within 100

days of the transplant, all were in the RCC group. Since the RCC patients had all undergone a previous nephrectomy, they may have been particularly vulnerable to treatment-related renal toxicity. Therefore we recommend testing kidney function with a 24-hour urine creatinine clearance test before beginning the conditioning regimen for such patients. Doses can then be adjusted as necessary depending on the results. Further study is needed to determine the relationship between donor chimera status and tumor response.

## References

- Oliver RT, Nethersell AB, Bottomley JM. Unexplained spontaneous regression and  $\alpha$ -interferon as treatment for metastatic renal carcinoma. *Br J Urol* 1989; 63:128-31.
- Jemal A, Thomas A, Murray T, Thun M. Cancer statistics, 2002. *CA Cancer J Clin* 2002; 52:23-47.
- Stenzl A, deKernion JB. The natural history of renal cell carcinoma. *Semin Urol* 1989; 7:144-8.
- Buzio C, Andrulli S, Santi R, Pavone L, Passalacqua R, Potenzoni D, et al. Long-term immunotherapy with low-dose interleukin-2 and interferon- $\alpha$  in the treatment of patients with advanced renal cell carcinoma. *Cancer* 2001; 92:2286-96.
- Schmidinger M, Steger GG, Wenzel C, Locker GJ, Brodowicz T, Budinsky AC, et al. Sequential administration of interferon  $\gamma$  and interleukin-2 in metastatic renal cell carcinoma: results of a phase II trial. *Austrian Renal Cell Carcinoma Study Group. Cancer Immunol Immunother* 2000; 49:395-400.
- Locatelli MC, Facendola G, Pizzocaro G, Piva L, Pegoraro C, Pallavicini EB, et al. Subcutaneous administration of interleukin-2 and interferon- $\alpha$  2b in advanced renal cell carcinoma: long-term results. *Cancer Detect Prev* 1999; 23:172-6.
- Dutcher JP, Fisher RI, Weiss G, Aronson F, Margolin K, Louie A, et al. Outpatient subcutaneous interleukin-2 and interferon- $\alpha$  for metastatic renal cell cancer: five-year follow-up of the Cytokine Working Group Study. *Cancer J Sci Am* 1997; 3:157-62.
- Piga A, Giordani P, Quattrone A, Giulioni M, De Signoribus G, Antognoli S, et al. A phase II study of interferon alpha and low-dose subcutaneous interleukin-2 in advanced renal cell carcinoma. *Cancer Immunol Immunother* 1997; 44:348-51.
- Whitehead RP, Wolf MK, Solanki DL, Hemstreet GP 3rd, Benedetto P, Richman SP, et al. A phase II trial of continuous-infusion recombinant interleukin-2 in patients with advanced renal cell carcinoma: a Southwest Oncology Group study. *J Immunother Emphasis Tumor Immunol* 1995; 18:104-14.
- Danova M, Porta C, Ferrari S, Riccardi A. Strategies of medical treatment for metastatic breast cancer. *Int J Oncol* 2001; 19:733-9.
- Nieto Y, Cagnoni PJ, Shpall EJ, Matthes S, Baron A, Jones RB, et al. Phase II trial of high-dose chemotherapy with autologous stem cell transplant for stage IV breast cancer with minimal metastatic disease. *Clin Cancer Res* 1999; 5:1731-7.
- Antman KH, Rowlings PA, Vaughan WP, Pelz CJ, Fay JW, Fields KK, et al. High-dose chemotherapy with autologous hematopoietic stem-cell support for breast cancer in North America. *J Clin Oncol* 1997; 15:1870-9.
- Laport GF, Grad G, Grinblatt DL, Bitran JD, Williams SF. High-dose chemotherapy consolidation with autologous stem cell rescue in metastatic breast cancer: a 10-year experience. *Bone Marrow Transplant* 1998; 21:127-32.
- Fields KK, Elfenbein GJ, Trudeau WL, Perkins JB, Janssen WE, Moscinski LC. Clinical significance of bone marrow metastases as detected using the polymerase chain reaction in patients with breast cancer undergoing high-dose chemotherapy and autologous bone marrow transplantation. *J Clin Oncol* 1996; 14:1868-76.
- Rill DR, Santana VM, Roberts WM, Nilson T, Bowman LC, Krance RA, et al. Direct demonstration that autologous bone marrow transplantation for solid tumors can return a multiplicity of tumorigenic cells. *Blood* 1994; 84:380-3.
- Hadden JW. The immunology and immunotherapy of breast cancer: an update. *Int J Immunopharmacol* 1999; 21:79-101.
- Sgagias MK, Nieroda C, Yannelli JR, Cowan KH, Danforth DN Jr. Upregulation of DF3, in association with ICAM-1 and MHC class II by IFN- $\gamma$  in short-term human mammary carcinoma cell cultures. *Cancer Biother Radiopharm* 1996; 11:177-85.
- Jerome KR, Barnd DL, Bendt KM, Boyer CM, Taylor-Papadimitriou J, McKenzie IF, et al. Cytotoxic T-lymphocytes derived from patients with breast adenocarcinoma recognize an epitope present on the protein core of a mucin molecule preferentially expressed by malignant cells. *Cancer Res* 1991; 51:2908-16.
- Whitwell HL, Hughes HP, Moore M, Ahmed A. Expression of major histocompatibility antigens and leucocyte infiltration in benign and malignant human breast disease. *Br J Cancer* 1984; 49:161-72.
- Linehan DC, Goedegebuure PS, Peoples GE, Rogers SO, Eberlein TJ. Tumor-specific and HLA-A2-restricted cytotoxicity by tumor-associated lymphocytes in human metastatic breast cancer. *J Immunol* 1995; 155:4486-91.
- Baxevas CN, Dedoussis GV, Papadopoulos NG, Missitzis I, Stathopoulos GP, Papamichail M. Tumor specific cytotoxicity by tumor infiltrating lymphocytes in breast cancer. *Cancer* 1994; 74:1275-82.
- Ueno NT, Rondon G, Mirza NQ, Geisler DK, Anderlini P, Giralt SA, et al. Allogeneic peripheral-blood progenitor-cell transplantation for poor-risk patients with metastatic breast cancer. *J Clin Oncol* 1998; 16:986-93.
- Eibl B, Schwaighofer H, Nachbaur D, Marth C, Gachter A, Knapp R, et al. Evidence for a graft-versus-tumor effect in a patient treated with marrow ablative chemotherapy and allogeneic bone marrow transplantation for breast cancer. *Blood* 1996; 88:1501-8.
- Olivieri A, Capelli D, Montanari M, Brunori M, Massidda D, Poloni A, et al. Very low toxicity and good quality of life in 48 elderly patients autotransplanted for hematological malignancies: a single center experience. *Bone Marrow Transplant* 2001; 27:1189-95.
- Attal M, Harousseau JL, Stoppa AM, Sotto JJ, Fuzibet JG, Rossi JF, et al. A prospective, randomized trial of autologous bone marrow transplantation and chemotherapy in multiple myeloma. *Intergroupe Francais du Myelome.*

- N Engl J Med 1996; 335:91-7.
26. Vincent MD, Powles TJ, Coombes RC, McElwain TJ. Late intensification with high-dose melphalan and autologous bone marrow support in breast cancer patients responding to conventional chemotherapy. *Cancer Chemother Pharmacol* 1988; 21:255-60.
  27. Pein F, Michon J, Valteau-Couanet D, Quintana E, Frappaz D, Vannier JP, et al. High-dose melphalan, etoposide, and carboplatin followed by autologous stem-cell rescue in pediatric high-risk recurrent Wilms' tumor: a French Society of Pediatric Oncology study. *J Clin Oncol* 1998; 16:3295-301.
  28. Saarinen-Pihkala UM, Wikstrom S, Vettenranta K. Maximal preservation of renal function in patients with bilateral Wilms' tumor: therapeutic strategy of late kidney-sparing surgery and replacement of radiotherapy by high-dose melphalan and stem cell rescue. *Bone Marrow Transplant* 1998; 22:53-9.
  29. Giralt S, Estey E, Albitar M, van Besien K, Rondon G, Anderlini P, et al. Engraftment of allogeneic hematopoietic progenitor cells with purine analog-containing chemotherapy: harnessing graft-versus-leukemia without myeloablative therapy. *Blood* 1997; 89:4531-6.
  30. Childs R, Chernoff A, Contentin N, Bahceci E, Schrump D, Leitman S, et al. Regression of metastatic renal-cell carcinoma after nonmyeloablative allogeneic peripheral-blood stem-cell transplantation. *N Engl J Med* 2000; 343:750-8.

## Autografting and non-myeloablative allogeneic stem cell transplantation in metastatic breast cancer

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**N**on-myeloablative hematopoietic stem cell transplantation has been used to treat hematologic neoplasias and certain genetic diseases, such as enzyme-deficiency disorders, Fanconi's anemia and thalassemia major.<sup>1</sup> With growing clinical experience, it became evident that this approach could also be used for immunotherapy of metastatic solid tumors.<sup>2</sup> This idea was reinforced by the demonstration of graft-versus-tumor effects of allogeneic lymphocytes in animals pre-treated with non-myeloablative conditioning.<sup>3</sup> For this reason, the success of the non-myeloablative protocol used by the Boston team to treat metastatic renal-cell cancer is a source of hope. It has been clearly demonstrated that the greater potential benefit of allografting could be exploited if the tumor burden is minimized prior to the allograft and conditioning mortality decreased. One method

to do this would be to combine high-dose therapy (HDT) and autografting (ASCT) followed by an immunosuppressive non-myeloablative allogeneic transplant mainly in patients with advanced disease. This strategy, successfully employed by us in lymphomas,<sup>4</sup> attempts to achieve both maximal tumor reduction with HDT and immune-mediated control of disease with allografting. Moreover, HDT induces significant host immunosuppression, which should contribute to the enhancement of donor engraftment during the allogeneic transplant procedure. Fourteen consecutive patients with advanced metastatic breast cancer who had suitable donors received an immunosuppressive non-myeloablative conditioning regimen of fludarabine and cyclophosphamide (13 patients) or melphalan (1 patient), followed by an infusion of a peripheral blood stem cell allograft from an HLA-identical

**Table 1. Characteristics of patients with metastatic breast cancer undergoing ASCT followed by NST.**

UPN	Age	Dx-Tx (Days)	No. of previous treatments	Disease sites	Previous ASCT	Status at NST	Conditioning	GvHD		Relapse	Outcome	F/U (Days)
								acute	chronic			
8	46	1030	3/HT	B	yes	stable	Flu/Cy	III	-	no	CR/rb*	1742
9	38	856	3/RT	Lu,B,Li	yes	progressive	Flu/Cy	-	-	progressive	PD/died	75
10	36	1661	4/HT/RT	B,Li	yes	progressive	Flu/Cy	-	-	progressive	PD/died	86
11	46	4681	2/HT/RT	Li,B,P	yes	stable	Flu/Cy	III	-	no	PR/Died	517
36	42	1760	5/HT	Med,Ln	yes	CR	Flu/Cy	II	extensive	no	CR	890
41	57	2833	4/HT/RT	Ln	yes	progressive	Flu/Cy	II	extensive	no	PD/Died	33
38	50	498	4/HT	B,Li	yes	progressive	Flu/Cy	-	-	progressive	CR	828
47	38	1160	4/HT	B,Li	yes	progressive	Flu/Cy	-	-	progressive	PD/Died	134
52	56	2363	4/HT/RT	B	yes	stable	Flu/Cy	II	limited	no	CR/rb*	630
62	49	1585	3/HT	Lu	yes	stable	Flu/Cy	-	-	no	SD	448
67	38	950	3/HT	B,Li	yes	stable	Flu/Cy	I	-	no	SD	360
80	40	1426	4/HT	B	yes	stable	Flu/Cy	-	-	progressive	PD	243
93	42	2153	3/HT	Li,B,P	no	progressive	Flu/Mel	IV	-	progressive	PD/Died	50
100	45	2690	4/HT	Med,B	yes	stable	Flu/Cy	-	-	no	SD	79

CR/rb\* : clinical remission and reduction of bone metastases; B: bone, Li: liver, Lu: lung, Med: mediastinum, P: pleural, HT: hormone-therapy; RT: radiotherapy; ASCT: Auto-grafting; NST: Non myeloablative stem cell transplantation.

sibling. Cyclosporin A, used to prevent GVHD, was withdrawn early in patients with full chimerism or disease progression. Patients with no response received infusions of donor lymphocytes. The characteristics of the patients and the outcome of their transplantations are shown in Table 1. At the time of the last follow-up (March 2002), 8 of the 14 patients were alive 79 to 1,742 days after transplantation (median, 539 days). None had died of transplant-related causes. Six patients died of progressive disease 33 to 517 days (median 80 days), after the allograft. All alive patients achieved clinical remission or stabilization of their disease. In three patients metastatic bone regression was also observed (UPN 08, 36, 52). Regression of metastases was delayed, occurring at a median of 160 days after transplantation and was always linked to full chimerism achievement and acute/chronic GVHD. These results are, therefore, consistent with a graft-versus-tumor effect. In conclusion, our double procedure can induce sustained regression of metastatic breast cancer in patients with advanced disease. Because no transplant-related deaths were observed, the above data confirm the feasibility of the procedure. Furthermore, the sustained *clinical benefit* and the disappearance of pain in most patients are consistent with the widening of the experience.

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## References

1. Carella AM, Champlin R, Slavin S, McSweeney P, Storb R. Mini-allografts: ongoing trials in humans. *Bone Marrow Transplant* 2000; 25:345-50.
2. Ueno NT, Rondon G, Mirza NQ, Geisler DK, Anderlini P, Giralt SA, et al. Allogeneic peripheral-blood progenitor-cell transplantation for poor-risk patients with metastatic breast cancer. *J Clin Oncol* 1998; 16:986-93.
3. Childs R, Chernoff A, Contentin N, Bahceci E, Schrump D, Leitman S, et al. Regression of metastatic renal-cell carcinoma after nonmyeloablative allogeneic peripheral-blood stem-cell transplantation. *N Engl J Med* 2000; 343:750-8.
4. Carella AM, Cavaliere M, Lerma E, Ferrara R, Tedeschi L, Romanelli A, et al. Autografting followed by nonmyeloablative immunosuppressive chemotherapy and allogeneic peripheral-blood hematopoietic stem-cell transplantation as treatment of resistant Hodgkin's disease and non-Hodgkin's lymphoma. *J Clin Oncol* 2000; 18:3918-24.

## Evidence of allogeneic graft-versus-tumor effect in prostate and ovarian cancer

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Allogeneic stem cell transplantation has emerged as a potentially curative form of immunotherapy for patients with hematologic malignancies that are resistant to conventional chemo-radiotherapy.<sup>1</sup> Donor T-cell populations targeting allogeneic minor histocompatibility antigens expressed on the patient's malignant cells are felt to be the driving force of the graft-versus-leukemia reaction, although to date only a handful of these antigens have been fully characterized.<sup>2</sup> Moreover, the use of non-myeloablative immunosuppressive conditioning regimens offers the opportunity to achieve a full-donor engraftment with reduced transplant-related complications and mortality.<sup>3</sup> Recent data from experimental animal models<sup>4</sup> and limited clinical data in humans suggest that graft-versus-tumor effects, analogous to the graft-versus-leukemia reaction, may be generated against solid tumors such as renal cell cancer, breast cancer and other malignancies.<sup>5-10</sup>

In 2000, Bay reported the case of a 33-year old woman with progressive chemo-resistant ovarian cancer who achieved a complete remission after allogeneic stem cell transplant following a myeloablative regimen. The conditioning regimen may have contributed to disease regression, although no regression was observed until the onset of acute graft-vs-host disease (GVHD) four weeks after the allograft.<sup>11</sup>

In 2001 we started a clinical phase II trial of allogeneic hematopoietic cell transplantation after a reduced intensity/immunosuppressive regimen in patients with metastatic hormone-refractory prostate cancer and advanced ovarian cancer. Approval was obtained from the *Institutional Review Board* for these studies. Here we present

our preliminary results.

Four patients with ovarian cancer and two patients with prostate cancer were treated with a reduced-intensity regimen including thiotepa (5 mg/kg for prostate cancer, 10 mg/kg for ovarian cancer), fludarabine (60 mg/m<sup>2</sup>) and cyclophosphamide (60 mg/kg) followed by allogeneic peripheral blood cell transplantation from an HLA-identical sibling. GVHD prophylaxis consisted of cyclosporin A and short-course methotrexate (Figure 1).

All the four patients with ovarian cancer are alive at a median follow-up of nearly 6 months. The first one, now over 300 days from transplant, had a transient remission after the conditioning regimen, but then progressed and needed further chemotherapy; she did not develop GVHD after cyclosporin withdrawal or after donor lymphocyte infusion (DLI). The other three patients developed GVHD after cyclosporin A withdrawal: one had a grade I acute GVHD and, concurrently, a reduction of serum marker CA125, but then progressed despite the occurrence of limited chronic GVHD; the other two patients developed a grade II-III acute GVHD, and they both achieved a partial remission. We add further details on the last patient, a 46-year old woman with a serous adenocarcinoma FIGO stage IIIC. She had a transient decrease of serum marker CA125 after the conditioning regimen, but then the disease progressed with the occurrence of multiple liver metastases. Four months after the allograft she developed grade III acute GVHD (skin, liver and gut): CA125 began to decrease (from 4067 to 327 mU/mL) (Figure 2), and liver metastases regressed.

Two patients with prostate cancer were treated with the above described program. The first one

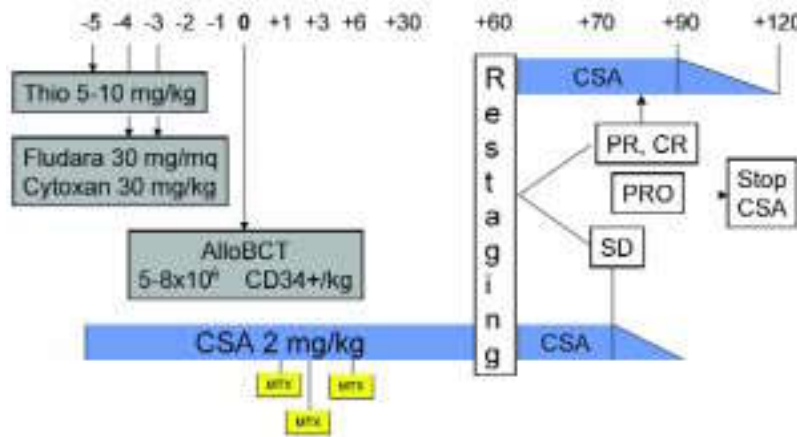


Figure 1. Protocol outline.

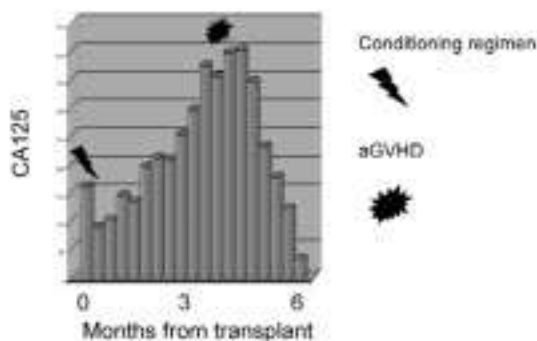
Patient No	Age/ Sex	Diagnosis/ stage/busto	Site(s) of disease	Status Pre Tx	PD after Tx (day)	AGVHD Grad(day)	cGVHD	Response	Outcome
1	44/F	OC/IIIC	Nodes	SD	+165	-	-	MR (+60)	307- PD
2	36/F	OC/IIIB	Peritoneal surfaces, surrenal gland	CR	-	I (+130)	limited	-	198- PD
3	59/F	OC/IIIC	Peritoneal surfaces, ascites	PR	-	II (+135)	-	PR (-150)	163- PR (Ca 125 438 → 110)
4	46/F	OC/IIIC	Peritoneal surfaces, ascites	SD	-120 (liver)	III (+120)	-	PR (-130)	149+ PR (Ca 125 4000 → 327)
1	59/M	PC	bone	SD	-	IV (+60)	-	NE	93 death, SD (bur PSA)
2	66/M	PC	Nodes	PD	-	II (+148)	Limited	PR (-150)	346+ VGPR

Table 1. Patient's characteristics and outcome of treatment. **Abbreviations.** OC: ovarian cancer; CR: complete remission; PR: partial remission; SD: stable disease; PD: progressive disease; PC: prostate cancer.

developed grade IV GVHD after cyclosporin A withdrawal, and died of cytomegalovirus enteritis three months after the allograft. Concurrently with GVHD we observed a decrease in the serum marker, PSA. The second patient, a 66-year old man with advanced prostate cancer refractory to hormonal therapy, developed a grade III skin acute GVHD during cyclosporin A dose reduction and, following that, we observed a continuous reduction of the

value of PSA to normal, and a progressive resolution of lymph node metastases confirmed both by PET and CT imaging. The patient is now alive and well 12 months after transplantation (*Peccatori et al., personal communication*).

These results clearly point to the existence of a graft-versus-tumor effect in ovarian and prostate cancer, and therefore suggest that allografting with non-myeloablative conditioning as a new treat-



**Figure 2.** Effect of acute GVHD on CA<sup>125</sup> serum marker in ovarian cancer.

ment option for these diseases deserves further investigation. Our findings clearly outline the close association between GVHD and GVT, suggesting that the anti-tumor response is probably mediated via the same *allo-reactive* donor T-cells responsible for GVHD. Steroid treatment can control GVHD, but switches GVT off. The delicate balance between GVHD and GVT is very difficult, and future strategies to induce tumor-specific T-cell responses are urgently required.

## References

1. Weiden PL, Flournoy N, Thomas ED, Prentice R, Fefer A, Buckner CD, et al. Antileukemic effect of graft-versus-host disease in human recipients of allogeneic-marrow grafts. *N Engl J Med* 1979; 300:1068-73.
2. Kolb HJ, Mittermuller J, Clemm C, Holler E, Ledderose G, Brehm G, et al. Donor leukocyte transfusions for treatment of recurrent chronic myelogenous leukemia in marrow transplant patients. *Blood* 1990; 76:2462-5.
3. Barrett J, Childs R. Non-myeloablative stem cell transplants. *Br J Haematol* 2000; 111:6-17.
4. Morecki S, Yacovlev E, Diab A, Slavin S. Allogeneic cell therapy for a murine mammary carcinoma. *Cancer Res* 1998; 58:3891-5.
5. 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; 343:750-8.
6. Eibl B, Schwaighofer H, Nachbaur D, Marth C, Gachter A, Knapp R, et al. Evidence for a graft-versus-tumor effect in a patient treated with marrow ablative chemotherapy and allogeneic bone marrow transplantation for breast cancer. *Blood* 1996; 88:1501-8.
7. Ueno NT, Rondon G, Mirza NQ, Geisler DK, Anderlini P, Giralt SA, et al. Allogeneic peripheral-blood progenitor-cell transplantation for poor-risk patients with metastatic breast cancer. *J Clin Oncol* 1998; 16:986-93.
8. Zetterquist H, Hentschke P, Thorne A, Wernerson A, Mattsson J, Uzunel M, et al. A graft-versus-colonic cancer effect of allogeneic stem cell transplantation. *Bone Marrow Transplant* 2001; 28:1161-6.
9. Moscardo F, Martinez JA, Sanz GF, Jimenez C, Cervera J, Sanchis J, et al. Graft-versus-tumour effect in non-small-cell lung cancer after allogeneic peripheral blood stem cell transplantation. *Br J Haematol* 2000; 111:708-10.
10. Bregni M, Doderio A, Peccatori J, Pescarollo A, Bernardi M, Sassi I, et al. Nonmyeloablative conditioning followed by hematopoietic cell allografting and donor lymphocyte infusions for patients with metastatic renal and breast cancer. *Blood* 2002; 99:4234-6.
11. Bay JO, Choufi B, Pomel C, Dauplat J, Durando X, Tournilhac O, et al. Potential allogeneic graft-versus-tumor effect in a patient with ovarian cancer. *Bone Marrow Transplant* 2000; 25:681-2.

## Allogeneic immunotherapy in patients suffering from advanced solid tumors

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Despite continuous progress in the treatment of patients suffering from solid tumor (ST), a large number of them die from cancer evolution. Meanwhile, allogeneic stem cell transplantation (ASCT) has shown its potential to eradicate malignant blood diseases. It is now known that this major effect is due to the immune activity of the allogeneic graft on leukemic cells: this means that allogeneic transplant represents a unique form of cellular immunotherapy.<sup>1</sup> However little is known about the impact of ASCT on solid tumors and experience is limited (Table 1). On this basis, in 1996 we started a program assessing ASCT in ST. There have been recent advances in this field: use of peripheral blood cells rather than bone marrow as graft source,<sup>2</sup> use of donor lymphocyte infusion to increase allogeneic control<sup>3</sup> and a shift from cytotoxic conditioning regimens to *non-myeloablative regimens*.<sup>4</sup> Such advances are leading to a substantial reduction in transplant-related mortality (TRM). For these reasons, after using a myeloablative regimen (busulfan-cytosin) for the first 6 patients, we changed to a non-myeloablative regimen in order to decrease TRM. This non-myeloablative regimen consisted of fludarabine (30 mg/m<sup>2</sup> x 6), busulfan (1 mg/kg x 8) and anti-thymocyte globulin (ATG) (thymoglobulin: 2.5 mg/kg/d): ATG was progressively decreased to lower infections and increase alloreactivity (4 days: 8; 3-2 days: 8; 1 day: 24). All patients [age: 44 years (28-60) and male/female: 20/46] were transplanted from an HLA identical sibling (bone marrow: 20; peripheral blood stem cells: 26). These patients have been treated in 8 centers. The diseases treated were: renal cell carcinoma (RCC) (16); breast cancer (10); melanoma (7); ovarian cancer (6); colorectal carcinoma (2); others (5). All patients had been extensively pre-treated and the majority had

very active disease. The primary goals of this program were to demonstrate an acceptable toxicity in this field and to optimize the procedure. Of the 6 patients who received the myeloablative regimen, 2 experienced grade 2 acute graft-vs-host disease (GVHD) and 2 died from TRM. One patient with ovarian cancer obtained a partial remission. All progressed while 1 patient with melanoma survived 2 years. Among the 40 patients who were given a non-myeloablative regimen, 31% developed grade  $\geq$  2 GVHD. TRM occurred in one patient (GVHD after cyclosporin A withdrawal). Response evaluation is still under study but at least 4 had an objective response (2 patients with RCC, 1 each with ovarian and breast carcinoma) and at least 3 patients (RCC, breast) have stable disease with GVHD (Table 2).

Although complete evaluation is still pending, some first conclusions can be drawn:

1. TRM is minimal with the non-myeloablative regimen in the context of patients treated for ST (no TRM among the last 24 patients).
2. All responses but one have been documented with the lowest pre-transplant immunosuppressive treatment (see Table 2) (ATG: 1 day) and conditioning immunosuppression is presently being further lowered.
3. Very active disease is unlikely to be controlled: pre-graft tumor kinetics must be slowed down.
4. Response is associated with clinical expression of GVHD.
5. Early post-graft immunomodulation is crucial (notably for cyclosporin A decrease) to reach rapid antitumor alloreactivity.
6. Not all diseases have the same level of sensitivity (RCC<sup>5</sup> and ovarian carcinoma<sup>6</sup> have a high sensitivity, melanoma a low sensitivity).

**ALLOGENEIC TRANSPLANTATION AND SOLID TUMORS :  
A limited experience**

	Auto	Auto for ST	Allo	Allo for ST
EBMT 97	12199	4139	4751	15
EBMT 98	12494	3788	5182	24
EBMT 99	12481	3066	5879	43
EBMT 00	12775	2258	6428	106

**ALLO FOR SOLID TUMORS : RESPONSE EVALUATION**

	BUCY N=6	FBS ATG 3-4 N=15	FBS ATG 1-2 N=24
Response			
- CR			1 (ovarian)
- PR	1 (ovarian)		3 (2 RCC, Breast)
- mixte			1 (RCC)
- minimal		2 (Ovarian; Melanoma)	3* (2 Breast, RCC)
			OR = 17 %

\* Alive in progressing response at 5, 5 and 6 months

Based on these preliminary data, we are presently investigating the real impact of ASCT in various situations: a national trial, in conjunction with the group of immunotherapy of RCC, will assess the impact of ASCT on the survival of patients suffering from RCC in comparison with that of a control group. In addition, two phase II trials in patients suffering from advanced ovarian and breast cancer will be started to determine disease control. Finally a prospective evaluation of two preparation modalities will try to determine the optimal regimen for solid tumors.

## References

1. Appelbaum FR. Haematopoietic cell transplantation as immunotherapy. *Nature* 2001; 411:385-9.
2. Blaise D, Kuentz M, Fortanier C, Bourhis JH, Milpied N, Sutton L, et al. Randomized trial of bone marrow versus lenograstim-primed blood cell allogeneic transplantation in patients with early-stage leukemia: a report from the Societe Française de Greffe de Moelle. *J Clin Oncol* 2000; 18:537-46.
3. Kolb HJ, Schattenberg A, Goldman JM, Hertenstein B, Jacobsen N, Arcese W, et al. Graft-versus-leukemia effect of donor lymphocyte transfusions in marrow grafted patients. European Group for Blood and Marrow Transplantation Working Party Chronic Leukemia. *Blood* 1995; 86:2041-50.
4. McSweeney PA, Niederwieser D, Shizuru JA, Sandmaier BM, Molina AJ, Maloney DG, et al. Hematopoietic cell transplantation in older patients with hematologic malignancies: replacing high-dose cytotoxic therapy with graft-versus-tumor effects. *Blood* 2001; 97:3390-400.
5. 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; 343:750-8.
6. Bay JO, Choufi B, Pomel C, Dauplat J, Durando X, Tournilhac O, et al. Potential allogeneic graft-versus-tumor effect in a patient with ovarian cancer. *Bone Marrow Transplant* 2000; 25:681-2.

## Allogeneic hematopoietic stem cell transplantation for solid tumors other than renal cell cancer

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Despite well established laboratory evidence of the therapeutic role of allogeneic hematopoietic stem cell transplantation (HSCT) for solid tumors, this approach has been investigated in the clinical setting only recently, following the evidence that sustained engraftment of donor hematopoietic stem cells can be accomplished with the use of preparative regimens that cause immunosuppression, without ablating host hematopoiesis. The latter observation is clinically relevant because, due to the morbidity and mortality associated with myeloablative high-dose chemotherapy, allogeneic transplantation preceded by the latter aggressive preparative regimen was restricted to young, medically fit patients, thus excluding the vast majority of potential candidate cancer patients from this therapy. In addition, high-doses of cytotoxic drugs were not required as patients with chemo-refractory diseases are treated.

Early clinical trials aimed to explore a graft-versus tumor (GVT) effect following allogeneic stem cell transplantation were focused on metastatic renal cell carcinoma (RCC) as this tumor is known to be immunogenic and respond to autologous immune-based approaches. Several studies, which are widely discussed by other authors in this supplement of *Haematologica*, have now proven that donor lymphocytes transferred with the graft may produce a clinically meaningful GVT effect in RCC.<sup>1</sup>

### *Allogeneic hematopoietic stem cell transplantation for solid tumors*

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 (Table 1).

In a pivotal article, Eibl *et al.*<sup>2</sup> described a GVT

effect in a woman with refractory metastatic breast cancer treated with allogeneic HSCT. The patient experienced regression of liver metastases in parallel with the onset of acute GVHD. In addition, the authors demonstrated, in the post-transplant phase, an expansion of cytotoxic T-lymphocytes (CTLs) reactive against the patient's hematopoietic minor HA which were capable of lysing partially HLA-matched breast cancer cell lines. More recently, further evidence of a clinically meaningful GVT effect in breast cancer has been reported by Ueno *et al.*<sup>3</sup> and Bregni *et al.*<sup>4</sup>

There are single case reports and small series of patients with cancer of various histologies treated with allogeneic HSCT. The cancers, carcinoma of the ovary,<sup>5,6</sup> lung,<sup>7</sup> colorectum,<sup>8</sup> and prostate,<sup>6</sup> as well as sarcoma,<sup>9</sup> have all been demonstrated to be susceptible to allogeneic immune attack following engraftment of donor immune cells. In most cases responses were accompanied by the occurrence of acute GVHD, thus suggesting that the anti-tumor effect might be mediated by the same allo-reactive T-cells as those responsible for GVHD. Preliminary results in metastatic melanoma are discouraging as death from disease progression occurred before day 100 in 5 out of 10 patients treated at the National Cancer Institute in USA.<sup>10</sup> Based on the latter experience, subsequent trials have considered selected patients with less advanced or indolent melanoma.

While tumor regression has been reported in patients with solid tumors following allogeneic HSCT, it is important to consider these responses in the context of the risk of transplant. Although in non-myeloablative stem cell transplantation (NST) regimen-related toxicities appear to be less than in conventional myeloablative transplants, the risk of

**Table 1. Published studies of allogeneic stem cell transplantation in solid tumors other than renal cell cancer. Reports in abstract form are not included.**

<i>First author/Ref</i>	<i>Type of Tumor</i>	<i>Number of cases</i>	<i>Evidence of GVT</i>	<i>Comment</i>
Eibl/2	Breast Carcinoma	1	regression of metastatic liver lesions with acute GvHD	Myeloablative conditioning; immune suppression given to control GvHD associated with recurrence. Post-transplant CTLs capable of lysing partially HLA-matched BC cell lines
Ueno/3	Breast Carcinoma	10	regression of metastatic liver lesions in two patients with GvHD	myeloablative conditioning associated with early responses in 6 patients
Bregni/4	Breast Carcinoma	6	two PR, one SD (>1,000 days from transplant)	Responses associated with GvHD, DLI effective
Bay/5	Ovarian Carcinoma	1	CR	Myeloablative conditioning; GvHD; durable response.
Moscardo/7	Lung Carcinoma	1	CR	Patient transplanted for leukemia. Regression of lung tumor associated with GvHD; long-lasting remission
Zetterquist/8	Colorectal Carcinoma	1	Clinical PR; post-mortem histopathologic findings (tumor necrosis)	GvHD; early death due to transplant-related complications
Pedrazzoli/9	Sarcoma	3	one CR	Short-lasting response in the absence of GvHD; two patients having disease stabilization along with GvHD
Peccatori/6	Ovarian Carcinoma	4	two PR	Regression of liver metastases in one patient; decrease of serum marker CA125 in both patients; GvHD
	Prostate Carcinoma	2	one CR	Long-lasting CR (serum marker and CT scan), GvHD; one patient not evaluable due to early death

*Abbreviations: GVT, graft versus tumor; GvHD, graft-versus-host disease; DLI, donor lymphocyte infusion; CR, complete response; PR, partial response; SD, stable disease; CTLs, cytotoxic T-lymphocytes; BC, breast cancer.*

transplant-related mortality (TRM) is still in the range of 10-20% before day 100. Studies of NST in RCC<sup>1,4</sup> used substantial doses of alkylating agents in their preparative regimens placing all patients at risk of bleeding and infection. The Seattle Group has also seen responses of RCC after non-ablative transplantations using only low-dose fludarabine and 2 Gy of total body irradiation.<sup>11</sup> This lower-dose regimen avoids pancytopenia and can be managed on an outpatient basis. Whether response rates will be different with the less toxic Seattle regimen is unknown.

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 poor performance status (PS), who had failed to benefit from prior treatments. Such patients are more *fragile* and the risk of transplant-related complications is extremely high.<sup>9</sup> In addition, the severe post-transplant immunodeficiency may potentially inhibit any immune antitumor mechanisms, thus promoting cancer progression. For these reasons 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 allogeneic HSCT. Because of the limited number of patients with solid tumors so far treated with NST, the incidence of long-term complications, including GVHD, infections, cannot be clearly defined.

#### *The Milano Niguarda Ca' Granda and Pavia experience*

Our group began exploring the use of NST in solid tumors in 1999. Initial studies focused on patients who had failed to benefit from conventional treatments and had progressive disease. Preliminary data, while confirming the need for accurate patient selection and adequate conditioning to reduce TRM and achieve full donor engraftment, showed that a GVT effect can be generated *in vivo* against sarcoma cells.<sup>9</sup>

As of April 2002, 7 patients with advanced-stage sarcoma have been treated with NST at Niguarda Ca' Granda Hospital in Milan and Policlinico San Matteo in Pavia. All patients had metastatic disease: two patients had Ewing's sarcoma (ES) and the remaining patients had rhabdomyosarcoma, gastric sarcoma, liposarcoma (LS), renal sarcoma, and malignant schwannoma. Six patients had progressive disease not amenable to further treatment, one patient with very high risk (bone and lung

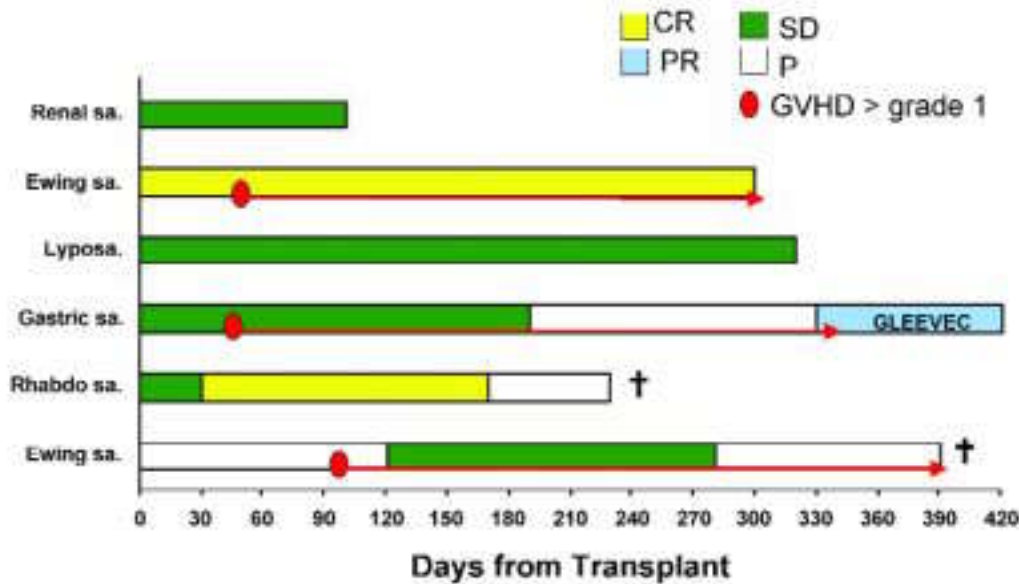


Figure 1. Tumor course and outcome of patients with follow-up longer than 100 days. Patients are listed according to their disease. Abbreviations: CR, complete response; PR, partial response; SD, stable disease; P, progression; GVHD, graft-versus-host disease.

metastases at onset) ES was transplanted after obtaining response to standard dose chemotherapy. Patients received either fludarabine (FLU)/cyclophosphamide (CY)<sup>1,9</sup> or thiotepa/FLU/CY<sup>4</sup> as conditioning regimens and were transplanted with blood stem cells from HLA-identical or one antigen mismatch siblings. GVHD prophylaxis consisted of cyclosporin A (CSA) and short-course methotrexate. Treatment-related complications were limited to short-lasting cytopenia and no organ toxicity. Acute GvHD  $\geq$  grade 2 occurred in 4 cases. The outcome of patients with a follow-up longer than 100 day is reported in Figure 1. Tumor regression or disease stabilization occurred in five patients following CSA withdrawal. In three cases disease progressed within 6 months. One child with very high-risk ES and one patient with LS remain with no evidence of progressive disease at almost 1 year after their transplants.

### Conclusions

Clinical studies so far reported allow recognition that donor lymphocytes can survive in the host after non-myeloablative conditioning and can induce clinically relevant responses in patients with solid tumors. However, because of the limited number of individuals treated so far, data on the clinical outcome should be considered with great cau-

tion. It is mandatory that patients continue to be treated in Institutions with proven experience in this setting and in the context of specific clinical trials designed to address still unsolved major clinical and biological issues. A clinical improvement in this setting is likely to depend, in the near future, on the possibility of driving the donor immune system in a specific fashion against antigens exclusively or preferentially presented by tumor cells without damaging normal somatic host cells.<sup>12</sup>

### References

- 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: 750-8.
- Eibl B, Schwaighofer H, Nachbaur D, Marth C, Gachter A, Knapp R, et al. Evidence for a graft-versus-tumor effect in a patient treated with marrow ablative chemotherapy and allogeneic bone marrow transplantation for breast cancer. *Blood* 1996; 88:1501-8.
- Ueno NT, Rondon G, Mirza NQ, Geisler DK, Anderlini P, Giralt SA et al. Allogeneic peripheral-blood progenitor-cell transplantation for poor-risk patients with metastatic breast cancer. *J Clin Oncol* 1998; 16:986-93.
- Bregni M, Doderio A, Peccatori J, Pescarollo A, Bernardi M, Sassi I et al. Nonmyeloablative conditioning followed by

- hematopoietic cell allografting and donor lymphocyte infusions for patients with metastatic renal and breast cancer. *Blood* 2002; 99:4234-6.
5. Bay JO, Choufi B, Pomel C, Dauplat J, Durando X, Tournilhac O, et al. Potential allogeneic graft-versus-tumor effect in a patient with ovarian cancer. *Bone Marrow Transplant* 2000; 25:681-2.
  6. Peccatori J, Ciceri F, Bernardi M, Corti C, Rigatti P, Mangili G, et al. Evidence of allogeneic graft-versus-tumor effect in prostate and ovarian cancer. *Haematologica* 2002; 87 (suppl. to n.6):.
  7. Moscardo F, Martinez JA, Sanz GF, Jimenez C, Cervera J, Sanchis J, et al. Graft-versus-tumour effect in non-small-cell lung cancer after allogeneic peripheral blood stem cell transplantation. *Br J Haematol* 2000;111:708-10.
  8. Zetterquist H, Hentschke P, Thorne A, Wernerson A, Mattsson J, Uzunel M, et al. A graft-versus-colonic cancer effect of allogeneic stem cell transplantation *Bone Marrow Transplant* 2001; 28:1161-6.
  9. Pedrazzoli P, Da Prada GA, Giorgiani G, Schiavo R, Zambelli A, Giraldi E, et al. Allogeneic blood stem cell transplantation after a reduced-intensity, preparative regimen. *Cancer* 2002; 94:2409-15.
  10. Childs R, Bradstock KF, Gottlieb D, et al. Nonmyeloablative stem cell transplantation as immunotherapy for metastatic melanoma: results of a pilot study. *Blood* 2000; 96(suppl. 1):abstract 3536.
  11. Appelbaum FR, Sandmaier B. Sensitivity of renal cell cancer to non-myeloablative allogeneic hematopoietic cell transplantations: unusual or unusually important? *J Clin Oncol* 2002; 20:1965-7.
  12. Schiavo R, Comoli P, Pedrazzoli P, Carminati O, Oreste P, Gelosa G, et al. Epstein-Barr virus (EBV)-specific cytotoxic T-Lymphocytes (CTLs) for therapy of nasopharyngeal carcinoma (NPC). *J Clin Oncol* 2002; 21(suppl. 1):27a (abstract 103).

## Mechanisms of graft-versus-malignancy in humans

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One of the most important messages derived from clinical science over the last decade is that some hematologic malignancies, especially chronic myeloid leukemia (CML) can be cured by the administration of allogeneic T-lymphocytes. This evidence has prompted investigators to develop novel strategies of adoptive immunotherapy for the clinical treatment of malignancies and identify the safest and most effective approach to exploit such a powerful tool. Allogeneic stem cell transplantation (SCT) is a very effective therapeutic option for a variety of hematologic malignancies. The actuarial probability of relapse is very low for patients allografted with unmanipulated marrow cells using cyclosporine alone as prophylaxis for graft-versus-host disease (GvHD). However, the incidence of relapse becomes much higher when the GvHD prophylaxis is more intensive.<sup>1</sup> In particular, the observation that the use of T-cell-depleted stem cells significantly increases the incidence of relapses provided *prima facie* evidence that allogeneic T-cells play a pivotal role in eradicating leukemic cells and/or maintaining remission.<sup>1,2</sup>

### Adoptive allogeneic immunotherapy: clinical results

This graft-versus-leukemia (GvL) effect provides the rationale for the use of donor lymphocyte infusions (DLI) to treat patients who relapse after conventional allografting or as an adjuvant therapy after reduced intensity conditioning allografts. The extensive employment of adoptive immunotherapy for tumor relapses after allografting has clearly demonstrated that CML is exquisitely sensitive to immune recognition and that DLI should be considered as the first choice therapeutic option.<sup>3</sup> The results with other hematologic malignancies, such as acute leukemia, multiple myeloma and lym-

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phomas, are less convincing or require more extensive investigations. Although the evidence in favor of a graft-versus-tumor effect in solid cancer remains circumstantial, the findings reported with the use of allogeneic SCT for renal cell carcinoma are convincing.<sup>4</sup> The experience acquired in CML has shed light on the mechanisms underlying the effect of DLI and has provided important information for designing effective and safe procedures to administer donor cells. A large proportion of CML patients (70-90%) respond to DLI treatment. Numerous studies have consistently shown that the most important factor predictive of response to DLI is the disease stage at the time of DLI. Patients whose disease is detected only at the molecular or cytogenetic level fare better than those with hematologic evidence of disease and among hematologic relapses, patients in chronic phase respond better than those with advanced disease.<sup>5,6</sup> The intervals between transplant to DLI<sup>6</sup> or between transplant to relapse<sup>7</sup> have also been demonstrated to be predictive of response. It is likely that these intervals reflect the activity of the disease and/or the tumor burden. However, it remains to be elucidated whether advanced disease is refractory because of an unfavorable ratio between donor T-cells and recipient leukemic cells or because leukemic cells become intrinsically resistant to effector T-cells. In most of the current studies patients have been treated with variable numbers of donor cells, thus making it impossible to address this question. We have tried to identify the dose of DLI required to achieve remission (effective cell dose, ECD) in 45 patients with CML at different stages of disease. DLI was administered according to an escalating dose regimen ( $10^6/10^7/5 \times 10^7/10^8$  CD3<sup>+</sup> cells/kg) at 20-week intervals between the infusions. Forty-two patients (93%) achieved molecular remission (M-Rem). We observed a statisti-

cally significant correlation between the ECD and disease stage at DLI. ECD also depended on the donor type. In fact, although the response rates for sibling (Sib) and volunteer unrelated (VUD) transplants were similar, the ECD for VUD was lower than for Sib transplants. These data show that the effect of DLI is subject to a dose-response effect, and that the ECD correlates with disease stage and donor type.

Acute graft-versus-host disease complicates donor lymphocyte infusion treatment in 40–60% of cases.<sup>5,6</sup> Although factors predictive of GvHD after allogeneic stem cell transplantation<sup>8</sup> could also be valid after DLI, the details of the transplant procedure differ substantially from those of DLI. The myeloablative therapy, the infusion of stem cells, and the immunosuppressive regimen which strongly influence the occurrence of GvHD are not part of DLI treatment. Moreover, at the time of DLI a substantial proportion of recipient dendritic cells has been replaced by dendritic cells of donor origin and this appears to ameliorate GvHD.<sup>9</sup> We investigated the role of possible factors in the development of acute GvHD post-DLI in 63 CML patients receiving DLI for leukemia relapse. The majority of patients who developed acute GvHD following DLI treatment did not have a history of acute GvHD after their original transplant. Recipient-donor sex mismatch, patient-donor cytomegalovirus seropositivity, and increasing patient age (>35 yrs) were found to be significantly associated with acute GvHD in the DLI setting. In accord with previous observations, patients who received T-cell-replete allografts suffered less acute GvHD post-DLI as compared to those who received a T-cell-depleted stem cell preparation. The method of DLI administration (bulk dose or escalating dose regimen) was highly significant. In a multivariate logistic analysis, any positive patient-donor cytomegalovirus serostatus, use of the bulk dose regimen and T-cell-depletion at transplant were found to be independent predictors of GvHD post-DLI. These findings demonstrate that most of the factors associated with acute GvHD after allogeneic SCT are not predictive of acute GvHD following DLI. Furthermore, DLI can be safe if the effective cell dose is administered late after transplant,<sup>10</sup> suggesting that GvHD may be particularly severe in an inflammatory context, i.e. when the *danger signals*<sup>11</sup> produced by the cytokine storm following the conditioning regimen<sup>12</sup> are maximal. This carries important implications for the use of DLI in the context of reduced intensity conditioning SCT.

### The molecular basis of the GvL effect

Although elimination of leukemic cells (GvL) is often associated with the attack on normal cells (GvH), the notion that the clinical effects of DLI may be mediated by distinct T-cell clones is supported by the clinical observations that the GvL effect is not always associated with GvHD.<sup>13</sup> The linked occurrence of GvL and GvHD may reflect the simultaneous activity of two discrete donor T-cell populations with distinct specificities: T-cell clones specific for leukemia antigens and T-cell clones specific for antigens of normal cells. Alternatively, the same population of donor T-cell clones specific for shared antigens expressed in leukemic and normal cells may mediate both GvL as well as GvHD.

### Minor histocompatibility antigens

In the majority of cases the stem cell allograft is matched for the MHC of the recipient. Therefore, the GvH effect is caused by disparities at the level of transplantation antigens that are not encoded by the MHC, namely the minor histocompatibility (H) antigens. Minor H antigens are polymorphic (generally di-allelic) cell-derived self-peptides that are inherited independently from the MHC. They are presented on the cell surface by MHC molecules and recognized by alloreactive T cells.<sup>14</sup> That these molecules play a major role in allogeneic SCT has been demonstrated by the correlation between mismatches for minor H antigens between donor and recipient and GvHD<sup>15</sup> and, more clearly by the observation that severe GvHD is associated with the expansion of minor H antigen-specific CD8<sup>+</sup> donor T-cells.<sup>16</sup> The reactivity of donor T-cells against minor H antigens on recipient tissues also supports the notion that minor H antigens may be the major target of the GvL effect. Although this would argue against the possibility of dissecting GvH from GvL, some minor H antigens appear to exhibit a restricted tissue distribution. The minor H antigens HA-1 and HA-2 appear to be expressed exclusively on hematopoietic cells. HA-1 and HA-2-specific cytotoxic T-lymphocytes have recently been generated using synthetic peptides, and these are reported to lyse leukemic cells but not skin fibroblasts.<sup>17</sup> They might be used to treat leukemia relapses after allogeneic BMT with low risk of GvHD. However, it is unclear why these *leukemia-specific* minor H antigens have been found to induce high level of specific T-cells in patients developing acute GvHD.<sup>16</sup> An attractive explanation for the selective anti-leukemia activity of donor T-cells may derive from recent data obtained in an animal model, whereby the infusion of T-cells

directed against a single immunodominant minor H antigen exerts a profound anti-tumor effect but fails to cause GvHD. In contrast, the infusion of donor T-cells containing precursors against additional minor H antigens expressed by the recipient produce severe GvHD possibly by a mechanism of *epitope spreading*.<sup>18</sup> Such an explanation would be consistent with the hypothesis that GvHD is greatly favored by the cytokine storm occurring soon after transplantation.<sup>12</sup>

#### *Tumor-specific antigens*

The major challenge for improving tumor immunotherapy is to identify leukemia-associated antigens suitable to direct T-cell responses selectively against leukemia cells without causing damage to normal cells. A recent study has identified the presence in CML patients of BCR-ABL peptides associated with the relevant HLA class I molecule. These patients can mount a cytotoxic T-lymphocytes (CTL) response against the BCR-ABL junctional peptide expressed on their CML cells.<sup>19</sup> However, CTL responses are limited by restrictions of natural antigen processing pathways and by HLA binding preferences. Differentiation antigens preferentially expressed by tumor cells are much less exposed to these limitations and may thus represent a better target. This is the case for a peptide derived from the primary granule enzyme proteinase 3. Proteinase 3 specific CTL have been detected in patients responding to interferon- $\alpha$  or allogeneic SCT but not in those who failed to do so.<sup>20</sup>

The Wilms' tumour antigen 1 (WT1) is a transcription factor that is expressed in several embryonic tissues and is involved in normal development and differentiation. Interestingly, WT1 is also expressed in CD34<sup>+</sup> hematopoietic stem cells and it is lost when they differentiate. Studies in leukemia patients have demonstrated elevated levels of WT1 expression. Using an allo-restricted CTL approach, it was possible to identify CTL epitopes in the WT1 protein. CTL specific for these epitopes killed primary leukemic cells and leukemic cell lines expressing WT1, but not control cells which did not express this protein.<sup>21</sup> Therefore, adoptive therapy with WT1-specific CTL remains an attractive treatment option, particularly if autologous CTL responses are ineffective.

#### Conclusions

The graft-versus-tumor effect exerted by allogeneic donor T-cells is a very effective approach to eradicate some hematologic malignancies and appears to be valid also for selected solid tumors.

The major targets of this effect are the differences between donor and recipient at the level of minor H antigens. However, tumor-associated antigens have been recently identified which may serve the purpose of designing more selective therapeutic approaches.

#### References

1. Goldman JM, Gale RP, Horowitz MM, Biggs JC, Champlin RE, Gluckman E, et al. Bone marrow transplantation for chronic myelogenous leukemia in chronic phase. Increased risk for relapse associated with T-cell depletion. *Ann Intern Med* 1988; 108:806-14.
2. Fefer A, Sullivan KM, Weiden P, Buckner CD, Schoch G, Storb R, et al. Graft versus leukemia effect in man: the relapse rate of acute leukemia is lower after allogeneic than after syngeneic marrow transplantation. *Prog Clin Biol Res* 1987; 244:401-8.
3. Dazzi F, Szydlo RM, Goldman JM. Donor lymphocyte infusions for relapse of chronic myeloid leukemia after allogeneic stem cell transplant: where we now stand. *Exp Hematol* 1999; 27:1477-86.
4. 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; 343:750-8.
5. Kolb HJ, Schattenberg A, Goldman JM, Hertenstein B, Jacobsen N, Arcese W, et al. Graft-versus-leukemia effect of donor lymphocyte transfusions in marrow grafted patients. European Group for Blood and Marrow Transplantation Working Party Chronic Leukemia. *Blood* 1995; 86:2041-50.
6. Collins RH Jr, Shpilberg O, Drobyski WR, Porter DL, Giral S, Champlin R, et al. Donor leukocyte infusions in 140 patients with relapsed malignancy after allogeneic bone marrow transplantation. *J Clin Oncol* 1997; 15:433-44.
7. Dazzi F, Szydlo RM, Cross NC, Craddock C, Kaeda J, Kanfer E, et al. Durability of responses following donor lymphocyte infusions for patients who relapse after allogeneic stem cell transplantation for chronic myeloid leukemia. *Blood* 2000; 96:2712-6.
8. Przepiorka D, Smith TL, Folloder J, Khouri I, Ueno NT, Mehra R, et al. Risk factors for acute graft-versus-host disease after allogeneic blood stem cell transplantation. *Blood* 1999; 94:1465-70.
9. Shlomchik WD, Couzens MS, Tang CB, McNiff J, Robert ME, Liu J, et al. Prevention of graft versus host disease by inactivation of host antigen-presenting cells. *Science* 1999; 285:412-5.
10. Johnson BD, Truitt RL. Delayed infusion of immunocompetent donor cells after bone marrow transplantation breaks graft-host tolerance allows for persistent anti-leukemic reactivity without severe graft-versus-host disease. *Blood* 1995; 85:3302-12.
11. Gallucci S, Matzinger P. Danger signals: SOS to the immune system. *Curr Opin Immunol* 2001; 13:114-9.
12. Krenger W, Hill GR, Ferrara JL. Cytokine cascades in acute graft-versus-host disease. *Transplantation* 1997; 67:553-8.
13. Dazzi F, Szydlo RM, Craddock C, Cross NC, Kaeda J, Chase

- A, et al. Comparison of single-dose and escalating-dose regimens of donor lymphocyte infusion for relapse after allografting for chronic myeloid leukemia. *Blood* 2000; 95:67-71.
14. Simpson E, Scott D, James E, Lombardi G, Cwynarski K, Dazzi F, et al. Minor H antigens: genes and peptides. *Eur J Immunogenet* 2001; 28:505-13.
  15. Goulmy E, Schipper R, Pool J, Blokland E, Falkenburg JH, Vossen J, et al. Mismatches of minor histocompatibility antigens between HLA-identical donors and recipients and the development of graft-versus-host disease after bone marrow transplantation. *N Engl J Med* 1996; 334: 281-5.
  16. Mutis T, Gillespie G, Schrama E, Falkenburg JH, Moss P, Goulmy E. Tetrameric HLA class I-minor histocompatibility antigen peptide complexes demonstrate minor histocompatibility antigen-specific cytotoxic T lymphocytes in patients with graft-versus-host disease. *Nat Med* 1999; 5:839-42.
  17. Dickinson AM, Wang XN, Sviland L, Vyth-Dreese FA, Jackson GH, Schumacher TN, et al. In situ dissection of the graft-versus-host activities of cytotoxic T cells specific for minor histocompatibility antigens. *Nat Med* 2002; 8: 410-4.
  18. Fontaine P, Roy-Proulx G, Knafo L, Baron C, Roy DC, Perreault C. Adoptive transfer of minor histocompatibility antigen-specific T lymphocytes eradicates leukemia cells without causing graft-versus-host disease. *Nat Med* 2001; 7:789-94.
  19. Clark RE, Dodi IA, Hill SC, Lill JR, Aubert G, Macintyre AR, et al. Direct evidence that leukemic cells present HLA-associated immunogenic peptides derived from the BCR-ABL b3a2 fusion protein. *Blood* 2001; 98:2887-93.
  20. 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; 6:1018-23.
  21. Gao L, Bellantuono I, Elsasser A, Marley SB, Gordon MY, Goldman JM, et al. Selective elimination of leukemic CD34(+) progenitor cells by cytotoxic T lymphocytes specific for WT1. *Blood* 2000; 95:2198-203.

## Non-myeloablative hematopoietic stem cell transplantation for metastatic solid tumors

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The immune system is known to induce tumor regression.<sup>1</sup> Following allogeneic hematopoietic stem cell transplantation (HSCT), graft-versus-host disease (GVHD) has been found to contribute to an anti-leukemic effect.<sup>2</sup> With increasing grade of acute GVHD, and especially with chronic GVHD, there is a lower risk of leukemic relapse.<sup>3,4</sup>

An alloresponse of donor T-lymphocytes is most probably the cause of GVHD and the graft-versus-leukemia (GVL) effect.<sup>3</sup> An allogeneic graft-versus-tumor (GVT) effect has also been reported in breast cancer.<sup>5-7</sup> Recently, HSCT was found to induce tumor regression in half of the patients with renal metastatic carcinoma.<sup>8,9</sup>

When performing HSCT, lethal myeloablative conditioning to eradicate leukemia and induce marked immunosuppression to pave the way for the donor immuno-hematopoietic system has been the routine for several decades.<sup>10-13</sup> However, this approach has been challenged by the use of lower doses and less toxic conditioning to induce immunosuppression and take advantage of the GVT effect later.<sup>14-19</sup>

Inspired by the non-myeloablative conditioning and demonstrated allogeneic antitumor effect in renal carcinoma patients, we used a non-myeloablative protocol and included patients not only with renal but also other solid tumors, such as adenocarcinoma of colon and breast as well as cholangiocarcinoma in the liver. The aim was to evaluate engraftment and complications following HSCT, and whether HSCT could induce an antitumor effect in patients with various solid tumors.

### Design and Methods

Between August 1999 and November 2001 eight patients with metastatic adenocarcinoma of colon (C1-8), eleven with renal (R1-11) and one with

breast adenocarcinoma (B1) as well as one with cholangiocarcinoma in the liver hilum, Klatskin's tumor (L1), were treated with non-myeloablative allogeneic peripheral HSCT at Huddinge University Hospital. All 21 patients had been considered to have tumors not curable with any conventional therapy. The median age of the patients was 58 (range 38-77) years. The study was approved by the Research Ethics Committee at Huddinge University Hospital.

The non-myeloablative conditioning consisted of fludarabine 30 mg/m<sup>2</sup>/day for three or five consecutive days in patients with an HLA-identical sibling donor and in those with a matched unrelated donor (MUD), respectively, followed by 2 Gy of total body irradiation (TBI).<sup>18</sup> Antithymocyte globulin (Thymoglobuline<sup>®</sup>, IMTIX-Sangstat, Lyon, France) was administered at a dose of 2 mg/kg/day for two days to patients with an unrelated donor (n=7).<sup>20</sup> From May 2001 TBI was replaced by cyclophosphamide 60 mg/day for two days in patients with a sibling donor (n=3).

The selection of donors was based on HLA-typing with high-resolution polymerase chain reaction-single-stranded polymorphism (PCR-SSP) for classes I and II.<sup>21</sup> All donors were at least HLA-A, -B and DRβ1 compatible with the recipient. An HLA-matched sibling donor (n=14) was given priority but, if not available, an unrelated donor (n=7) was accepted (Table 1).

Peripheral blood stem cells (PBSC) were collected after granulocyte colony-stimulating factor (G-CSF) (Neupogen<sup>®</sup>, Amgen, Stockholm, Sweden) stimulation of all donors.<sup>22</sup> The median cell yield in grafts was 6.6×10<sup>6</sup> CD34<sup>+</sup> cells/kg recipient body weight (range 1.7-21.8).

Post-transplant immunosuppression consisted of mycophenolate mofetil (MMF, Cellcept<sup>®</sup>, Hoffman LaRoche, Basel, Switzerland), 0.5-1 g twice a day for

one to two months.<sup>18,19,23</sup> From May 2001, MMF was replaced by methotrexate. In addition, cyclosporin A [Sandimmun Neoral® (CsA), Novartis Pharma AG, Stein, Switzerland] was given to all patients for at least three months. The CsA doses ranged between 3-12 mg/kg to achieve a trough level of 100 ng/mL and 200 to 300 ng/mL in patients with a sibling or unrelated donor, respectively.<sup>24,25</sup>

Initial treatment of acute GVHD with prednisolone 2 mg/kg/day was given as early as grade I to avoid more severe GVHD.<sup>26</sup> The supportive therapy was according to our HSCT protocols.<sup>24,27</sup>

Chimerism was analyzed using PCR amplification of variable number of tandem repeats from CD3-, CD19- and CD45-positive peripheral blood cells, separated with immunomagnetic beads (DynaI, Oslo, Norway) as described elsewhere in detail.<sup>28-30</sup>

Donor lymphocyte infusions (DLI) were given in escalating doses, 1×, 5×, 10×, 100×10<sup>6</sup> CD3<sup>+</sup> cells/kg, usually starting three to four months after HSCT and after the immunosuppressive therapy had been discontinued.<sup>31</sup> The indication for DLI was tumor progression and/or mixed chimerism in the absence of GVHD.

Acute GVHD was graded from 0 to IV using standard criteria.<sup>10</sup> Chronic GVHD was evaluated in patients surviving after 90 days, and classified as limited or extensive,<sup>32</sup> or mild, moderate or severe by the physician.<sup>33</sup> Bacteremia, fungemia and cytomegalovirus (CMV) infection were diagnosed as previously defined.<sup>34, 35</sup>

The antitumor effect was monitored by computed tomography (CT) of the thorax and abdomen 3, 6 and 12 months after HSCT. These examinations were compared with those obtained within one month prior to the HSCT.

### Statistical analysis

The Mann-Whitney U method was used to compare the number of days to complete donor chimerism (DC). The probability of survival and acute GVHD were estimated using the method of Kaplan-Meier.

## Results

Four patients were not evaluable for tumor reponse because of graft rejection or death within three months, before the first scheduled CT. The first patient (C1) had regression of all metastases and has been reported in detail.<sup>37</sup> Three patients (C4, R2 and R7), had progression in the liver but regression of lung metastases. Patient R2 developed metastases in the liver, bone and lungs dur-

ing the first six months, but the size and number of metastases in the lungs regressed during the next six months. In contrast, metastases in the liver and bone continued to progress during the same time. Patient R4 had progression of abdominal metastases but unchanged status of the majority of lung metastases on CT at three months. She died of severe GVHD involving the skin, gut and liver at five months. Autopsy showed necrosis of metastases in the lungs. Patient R8 without a tumor to be demonstrated prior to HSCT developed a subcutaneous metastasis of the original renal adenocarcinoma near the nephrectomy scar within three months. This was successfully extirpated.

All patients had initial engraftment. Development of complete DC varied between different cell lineages, with T-cell DC lagging behind myeloid and B-cell DC. In 12 of 18 evaluable patients who developed DC in all cell-lineages, the median time for T-cell DC to occur was 65 (25-223) days, compared to 28 (12-71) days for B-cells and 49 (21-187) days for myeloid cells ( $p=0.003$  and  $p=0.04$ , respectively). Recipients of unrelated HSCT tended to have a faster engraftment of donor CD3<sup>+</sup> cells than recipients of HSCT from HLA-identical siblings ( $p=0.09$ ). In the remaining six patients, two (C3 and R10) rejected the graft at five and two months, respectively. Another three patients (C1, R1, L1) had mixed chimerism up to four months. Patient R6 had mixed chimerism up to 2.5 months when he suddenly developed aplasia involving both the donor and recipient cells. EBV-DNA was found in the bone marrow.

The post-transplant cytopenia was brief and mild. Only three patients, all treated with cyclophosphamide, showed total leukocyte counts  $< 0.5 \times 10^9/L$  within the first month after HSCT. No platelet transfusions were needed in any patient during the first month, whereas ten patients were given from two to six erythrocyte transfusions. Two patients (R6, R7) with blood groups B and A, respectively, and who were given an O blood group graft, developed hemolysis. They required erythrocyte transfusions up to 3 and 9 months after HSCT, respectively.

Most patients received treatment on an outpatient basis. Conditioning was well tolerated. Only two patients (R5 and R6) suffered from septicemia (*Staphylococcus epidermidis*, *Pseudomonas aeruginosa*) within three months after HSCT. No clinically significant fungal infection was detected. CMV reactivation was diagnosed by PCR in 14 patients during the first three months after HSCT. Four of them also developed symptoms of CMV disease

(fever and joint pain) but no tissue invasive disease developed. They were successfully treated with a gancyclovir regimen.

The cumulative incidence of grades II-IV acute GVHD was 57%. Overall grade II-IV acute GVHD occurred in 9/21 (43%) patients. Two of these had grade III and IV, respectively. Chronic GVHD of mild degree was seen in five patients among 14 evaluable patients observed more than three months after HSCT.

Donor lymphocyte infusions (DLI) did not enhance GVHD in any patient. Eleven patients were given from two to five escalating doses of DLI. One of these patients (C4) showed a partial tumor response even before the first DLI was given. Tumor regression after DLI has not been documented so far in any of the 12 patients observed for a median of 2 (range 1.5-9.5) months after the first DLI.

As of 30 April, 2002, four patients were alive 6 to 17 months after transplantation. The causes of death in 17 patients were progressive disease in 12, transplantation-related complications in four and head trauma at home in one patient.

## Discussion

The encouraging results using reduced conditioning and peripheral blood stem cell transplantation for renal cell carcinoma, described by Childs *et al.*, have aroused interest in this approach for patients with metastatic cancer with poor prognosis.<sup>8,9</sup> Since few data are available, we report here our first 21 consecutive patients with solid tumors who underwent HSCT. Seven of them were transplanted with cells from an unrelated donor.

The response of the tumor was dramatic in our first patient,<sup>36</sup> inspiring us to continue the program. Childs *et al.* reported complete tumor response in three patients and partial response in seven of 19 with renal cell carcinoma.<sup>8,9</sup> In total, we noted response in two of eight patients with colon carcinoma and three of ten with renal carcinoma. Three of our patients had response only in lung and lymph node metastases, but progression of liver metastases occurred in parallel, and in one of them, it was also seen in bone. The tumor response in the lymph nodes may be expected if recipient cells of hematopoietic cells are replaced by donor cells which may also induce antitumor activity. The reason for the discrepancy between the higher response seen in the lungs than in the liver is unclear, because both organs are loaded by bone marrow-derived macrophages which could act as antigen-presenting cells for tumor and/or minor

histocompatibility antigens to induce an allogeneic and antitumor effect. It is possible that the i.v. infusion of the graft may entrap many of the lymphoid cells in the lungs rather than the liver.

An overall high rate of tumor progression may be expected because, when a new experimental procedure is used, it is mainly tried in those with advanced disease and a poor prognosis. Since a high tumor load may increase the risk of progression, debulking of the tumor before transplantation was done in all but two of our patients. However, despite debulking, the tumor burden was high and these patients can be considered to resemble those undergoing HSCT in an advanced stage of leukemia. Patients undergoing HSCT even with complete myeloablative therapy and advanced leukemia have a 75% or higher probability of relapse.<sup>10-12,37,38</sup>

This regimen could be given on an out-patient basis in accord with the experience of McSweeney *et al.*<sup>19</sup> Complete donor chimerism of all lymphoid cell lineages examined was seen in most patients. However, unlike Childs *et al.*, we found that B- and myeloid cell donor chimerism occurred faster than donor T-cell chimerism in patients with sibling donors.<sup>39</sup> A faster T-cell chimerism did occur in patients with unrelated donor. This discrepancy may be because Childs *et al.* gave higher doses for conditioning. Furthermore, we used mycophenolate mofetil in addition to cyclosporin, while they used cyclosporin alone as GVHD prophylaxis.<sup>9,19,23,39</sup> However, the incidence of moderate-to-severe acute GVHD was similar with both regimens, being about 50%. This is higher than we have seen in our institution in patients with leukemia, using HLA-identical siblings or unrelated donors and myeloablative regimens, in whom the incidence of acute GVHD II-IV was 11% and 15%, respectively.<sup>24</sup> Higher age may be one reason for the observed increase in acute GVHD since the current tumor patients were older than those with hematologic malignancies. Furthermore, we tried to discontinue immunosuppression after two to three months in order to induce an allogeneic antitumor effect. Interestingly, we recorded acute GVHD in the gut without prior skin involvement which might mirror another phenomenon of the reduced conditioning.

So far, eleven of our patients have been given DLI in two to five escalating doses. DLI in this setting was well tolerated with no GVHD and no aplasia. No beneficial effects of DLI have been seen so far, but the observation time has been relatively short. Childs *et al.* reported similar findings, but the effect of DLI seemed to be confined to one patient.<sup>9</sup>

One reason for the low response hitherto in our patients may be that enhanced GVHD occurred only in three patients. In patients with leukemia who responded to DLI, most developed acute and/or chronic GVHD.<sup>31,38</sup> For instance, in those with chronic myeloid leukemia who relapsed after HSCT, infusion induced long-lasting cytogenetic and molecular remission in 70% to 80% of them.<sup>39</sup>

It is expected that an increasing number of patients with otherwise untreatable metastatic cancer will be treated with HSCT at various institutions in the world. We are just at the beginning of what may develop into a new era for hematopoietic stem cell transplantation. The use of non-myeloablative or reduced conditioning permits this treatment with an acceptable degree of toxicity to older patients with solid tumors, as found in this study. This agrees with previous findings, mainly in hematologic malignancies.<sup>9,14-19</sup> Nevertheless, there is an obvious risk of an advent of tumor metastases during the early immunosuppressive phase as demonstrated in one of our patients (R8) with no tumor or metastases to be demonstrated prior to HSCT. To improve the results, patients need to be selected at an earlier stage with a lower tumor burden and less widespread metastases. Patients with a large tumor mass and/or several metastases might, in addition to debulking, undergo extensive tumor reducing treatment such as precision or radiofrequency irradiation. Apart from adenocarcinoma of kidney, colon, breast and liver, HSCT may also be tried in prostate cancer, ovarian cancer, soft tissue sarcoma, melanoma and lung carcinoma. It is important that this is done in clinical trials, - for instance, in collaboration with the *International Bone Marrow Transplant Registry* and the *European Group for Blood and Marrow Transplantation*. With more worldwide experience, the role of HSCT in solid tumors will be established.

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## References

1. Dermime S, Barrett J, Gambacorti-Passerini C. The role of the immune system in anti-tumour responses. Potential for drug therapy. *Drugs Aging* 1995; 7:266-77.
2. Weiden PL, Flournoy N, Thomas ED, Prentice R, Fefer A, Buckner CD, et al. Antileukemic effect of graft-versus-host disease in human recipients of allogeneic-marrow grafts. *N Engl J Med* 1979; 300:1068-73.
3. Horowitz MM, Gale RP, Sondel PM, Goldman JM, Kersey J, Kolb HJ, et al. Graft-versus-leukemia reactions after bone marrow transplantation. *Blood* 1990; 75:555-62.
4. Ringdén O, Labopin M, Gluckman E, Reiffers J, Vernant JP, Jouet JP, et al. Graft-versus-leukemia effect in allogeneic marrow transplant recipients with acute leukemia is maintained using cyclosporin A combined with methotrexate as prophylaxis. *Acute Leukemia Working Party of the European Group for Blood and Marrow Transplantation. Bone Marrow Transplant* 1996; 18:921-9.
5. Eibl B, Schwaighofer H, Nachbaur D, Marth C, Gachter A, Knapp R, et al. Evidence for a graft-versus-tumor effect in a patient treated with marrow ablative chemotherapy and allogeneic bone marrow transplantation for breast cancer. *Blood* 1996; 88:1501-8.
6. Ben-Yosef R, Or R, Nagler A, Slavin S. Graft-versus-tumor and graft-versus-leukaemia effect in patients with concurrent breast cancer and acute myelocytic leukaemia. *Lancet* 1996; 348:1242-3.
7. Ueno NT, Rondon G, Mirza NO, Geisler DK, Anderlini P, Giralt SA, et al. Allogeneic peripheral-blood progenitor-cell transplantation for poor-risk patients with metastatic breast cancer. *J Clin Oncol* 1998; 16:986-93.
8. Childs RW, Clave E, Tisdale J, Plante M, Hensel N, Barrett J. Successful treatment of metastatic renal cell carcinoma with a nonmyeloablative allogeneic peripheral-blood progenitor-cell transplant: evidence for a graft-versus-tumor effect. *J Clin Oncol* 1999; 17:2044-9.
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; 343:750-8.
10. Thomas E, Storb R, Clift RA, Fefer A, Johnson FL, Neiman PE, et al. Bone-marrow transplantation (first of two parts). *N Engl J Med* 1975; 292:832-43.
11. Appelbaum F, Clift R, Radich J, Anasetti C, Buckner C. Bone marrow transplantation for chronic myelogenous leukemia. *Semin Oncol* 1995; 22:405-11.
12. Appelbaum F. Allogeneic hematopoietic stem cell transplantation for acute leukemia. *Semin Oncol* 1997; 24:114-23.
13. Ringdén O. Allogeneic bone marrow transplantation for hematological malignancies - controversies and recent advances. *Acta Oncol* 1997; 36:549-64.
14. Giralt S, Estey E, Albitar M, van Besien K, Rondon G, Anderlini P, et al. Engraftment of allogeneic hematopoietic progenitor cells with purine analog-containing chemotherapy: harnessing graft-versus-leukemia without myeloablative therapy. *Blood* 1997; 89:4531-6.
15. Slavin S, Nagler A, Naparstek E, Kapelushnik Y, Aker M, Cividalli G, et al. Nonmyeloablative stem cell transplantation and cell therapy as an alternative to conventional bone marrow transplantation with lethal cytoreduction

- for the treatment of malignant and nonmalignant hematologic diseases. *Blood* 1998; 91:756-63.
16. Khouri IF, Keating M, Korbling M, Przepiorka D, Anderlini P, O'Brien S, et al. Transplant-lite: induction of graft-versus-malignancy using fludarabine-based nonablative chemotherapy and allogeneic blood progenitor-cell transplantation as treatment for lymphoid malignancies. *J Clin Oncol* 1998; 16:2817-24.
  17. Sykes M, Preffer F, McAfee S, Saidman SL, Weymouth D, Andrews DM, et al. Mixed lymphohaemopoietic chimerism and graft-versus-lymphoma effects after non-myceloablative therapy and HLA-mismatched bone-marrow transplantation. *Lancet* 1999; 353:1755-9.
  18. McSweeney PA, Storb R. Mixed chimerism: preclinical studies and clinical applications. *Biol Blood Marrow Transplant* 1999; 5:192-203.
  19. McSweeney PA, Niederwieser D, Shizuru JA, Sandmaier BM, Molina AJ, Maloney DG, et al. Hematopoietic cell transplantation in older patients with hematologic malignancies: replacing high-dose cytotoxic therapy with graft-versus-tumor effects. *Blood* 2001; 97:3390-400.
  20. Remberger M, Svahn BM, Hentschke P, Löfgren C, Ringdén O. Effect on cytokine release and graft-versus-host disease of different anti-T cell antibodies during conditioning for unrelated haematopoietic stem cell transplantation. *Bone Marrow Transplant* 1999; 24:823-30.
  21. Olerup O, Zetterquist H. HLA-DR typing by PCR amplification with sequence-specific primers (PCR-SSP) in 2 hours: an alternative to serological DR typing in clinical practice including donor-recipient matching in cadaveric transplantation. *Tissue Antigens* 1992; 39:225-35.
  22. Ringdén O, Remberger M, Runde V, Bornhauser M, Blau IW, Basara N, et al. Peripheral blood stem cell transplantation from unrelated donors: a comparison with marrow transplantation. *Blood* 1999; 94:455-64.
  23. Storb R, Yu C, Wagner JL, Deeg HJ, Nash RA, Kiem HP, et al. Stable mixed hematopoietic chimerism in DLA-identical littermate dogs given sublethal total body irradiation before and pharmacological immunosuppression after marrow transplantation. *Blood* 1997; 89:3048-54.
  24. Ringdén O, Remberger M, Persson U, Ljungman P, Aldener A, Andstrom E, et al. Similar incidence of graft-versus-host disease using HLA-A, -B and -DR identical unrelated bone marrow donors as with HLA-identical siblings. *Bone Marrow Transplant* 1995; 15:619-25.
  25. Carlens S, Aschan J, Remberger M, Dilber M, Ringdén O. Low-dose cyclosporin of short duration increases the risk of mild and moderate GVHD and reduces the risk of relapse in HLA-identical sibling marrow transplant recipients with leukemia. *Bone Marrow Transplant* 1999; 24:629-35.
  26. Ringdén O, Persson U, Johansson SG. Early diagnosis and treatment of acute human graft-versus-host disease. *Transplant Proc* 1983; 15:1490-4.
  27. Ringdén O, Pihlstedt P, Markling L, Aschan J, Baryd I, Ljungman P, et al. Prevention of graft-versus-host disease with T cell depletion or cyclosporin and methotrexate. A randomized trial in adult leukemic marrow recipients. *Bone Marrow Transplant* 1991; 7:221-6.
  28. Mattsson J, Uzunel M, Remberger M, Ljungman P, Kimby E, Ringden O, et al. Minimal residual disease is common after allogeneic stem cell transplantation in patients with B cell chronic lymphocytic leukemia and may be controlled by graft-versus-host disease. *Leukemia* 2000; 14:247-54.
  29. Mattsson J, Uzunel M, Remberger M, Ringdén O. T-cell mixed chimerism is significantly correlated to a decreased risk of acute graft-versus-host disease after allogeneic stem cell transplantation. *Transplantation* 2001; 71:433-9.
  30. Zetterquist H, Mattsson J, Uzunel M, Nasman-Bjork I, Svenberg P, Tammik L, et al. Mixed chimerism in the B cell lineage is a rapid and sensitive indicator of minimal residual disease in bone marrow transplant recipients with pre-B cell acute lymphoblastic leukemia. *Bone Marrow Transplant* 2000; 25:843-51.
  31. Carlens S, Remberger M, Aschan J, Ringdén O. The role of disease stage in the response to donor lymphocyte infusions as treatment for leukemic relapse. *Biol Blood Marrow Transplant* 2001; 7:31-8.
  32. Shulman HM, Sullivan KM, Weiden PL, McDonald GB, Striker GE, Sale GE, et al. Chronic graft-versus-host syndrome in man. A long-term clinicopathologic study of 20 Seattle patients. *Am J Med* 1980; 69:204-17.
  33. Carlens S, Ringdén O, Remberger M, Lonnqvist B, Hagglund H, Klaesson S, et al. Risk factors for chronic graft-versus-host disease after bone marrow transplantation: a retrospective single centre analysis. *Bone Marrow Transplant* 1998; 22:755-61.
  34. Sparrelid E, Hagglund H, Remberger M, Ringden O, Lonnqvist B, Ljungman P, et al. Bacteraemia during the aplastic phase after allogeneic bone marrow transplantation is associated with early death from invasive fungal infection. *Bone Marrow Transplant* 1998; 22:795-800.
  35. Ljungman P, Loré K, Aschan J, Klaesson S, Lewensohn-Fuchs I, Lonnqvist B, et al. Use of a semi-quantitative PCR for cytomegalovirus DNA as a basis for pre-emptive antiviral therapy in allogeneic bone marrow transplant patients. *Bone Marrow Transplant* 1996; 17:583-7.
  36. Zetterquist H, Hentschke P, Thörne A, Wernerson A, Mattsson J, Uzunel M, et al. A graft-versus-colonic cancer effect of allogeneic stem cell transplantation. *Bone Marrow Transplant* 2001; 28:1161-6.
  37. Thomas ED, Buckner CD, Banaji M, Clift RA, Fefer A, Flournoy N, et al. One hundred patients with acute leukemia treated by chemotherapy, total body irradiation, and allogeneic marrow transplantation. *Blood* 1977; 49:511-33.
  38. Ringdén O, Zwaan F, Hermans J, Gratwöhl A. European experience of bone marrow transplantation for leukemia. The Leukemia Working Party of the European Group for Bone Marrow Transplantation. *Transpl Proc* 1987; 19:2600-4.
  39. Kolb HJ, Schattenberg A, Goldman JM, Hertenstein B, Jacobsen N, Arcese W, et al. Graft-versus-leukemia effect of donor lymphocyte transfusions in marrow grafted patients. European Group for Blood and Marrow Transplantation Working Party Chronic Leukemia. *Blood* 1995; 86:2041-50.

## Non-myeloablative stem cell transplantation and targeted immunotherapy for the treatment of metastatic solid tumors

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Considering lack of specific treatment modalities against cancer, and the well-established dose-response effects in treating tumor cells *in vitro* and *in vivo*, the dogma over the past years with available anti-cancer modalities has been *the more the better*, attempting to eradicate the primary tumor by resection with or without radiation therapy and the secondary metastases with aggressive chemoradiotherapy. This dogma led to the clinical development of myeloablative chemoradiotherapy, followed by rescue with stem cell transplantation as a possible means for eradication of the maximal possible number of tumor cells. Over the years it became apparent that none of the available anti-cancer modalities or even combinations thereof could accomplish such a goal, since relapse continued to be the single major obstacle in treatment of hematologic malignancies, especially that of advanced or resistant disease.<sup>1,2</sup> On the other hand, as the intensity of the regimen used for bone marrow or blood stem cell transplantation (BMT) was escalated, procedure-related toxicity and mortality increased accordingly. Hence, although possible induction of graft-versus-tumor (GVT) effects following allogeneic bone marrow transplantation were reported in patients with solid tumors,<sup>3-5</sup> similarly to graft-versus-leukemia effects in patients with hematologic malignancies,<sup>6</sup> limited efficacy and poor outcome due to procedure-related toxicity and mortality, mostly due to acute and chronic graft-versus-host disease (GVHD), were prohibitive. Measurable response against liver metastases which disappeared transiently after cell therapy, were also documented in a patient with liver metastases that developed following myeloablative combination chemotherapy supported by autologous blood stem cell transplantation, who was subsequently administered fully matched donor lymphocytes without any additional conditioning.<sup>7</sup>

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phocytes without any additional conditioning.<sup>7</sup> The data suggest that, in analogy to graft-versus-leukemia effects, GVT effects may occur against solid tumors, as long as the host accepts donor lymphocytes. However, it became apparent that newer modalities must be introduced in order to improve the cure rate of patients with solid tumors and even those with hematologic malignancies, to reduce procedure-related complications and to improve the quality of life of successfully treated patients. For patients resistant to available chemotherapy, immunotherapy became an obvious rational alternative. Tumor cells are uniformly rejected when implanted into allogeneic recipients; hence, it seemed reasonable to assume that the mirror image may also be true, that is, that donor lymphocytes may also react against tumor cells of host origin, as long as rejection of such potentially anti-tumor effector cells can be prevented. Indeed, since alloreactive donor lymphocytes can eradicate all tumor cells in patients with hematologic malignancies following induction of host-vs-graft transplantation tolerance induced by engraftment of donor stem cells, even in patients resistant to myeloablative chemoradiotherapy, adoptive cell-mediated immunotherapy may be utilized for elimination of undesirable non-hematopoietic tumor cells of host origin. In principle, following induction of host-versus-graft transplantation tolerance, immunotherapy can be mediated by alloreactive donor lymphocytes contained in the inoculum, especially if the graft is obtained from the blood and enriched with lymphocytes following mobilization with granulocyte colony-stimulating factor (G-CSF), or when donor lymphocytes are given later as a donor lymphocyte infusion (DLI).<sup>8,9</sup>

The potential application of allogeneic cell-mediated immunotherapy for the treatment of metasta-

tic solid tumors was first suggested in the 1980s.<sup>10</sup> Following induction of host-vs-graft transplantation tolerance in female (NZBxNZW)F1 recipients (mice susceptible to develop clinical and laboratory manifestations of systemic lupus erythematosus as well as sarcoma) with BMT from BALB/c mice, donor bone marrow cells containing alloreactive lymphocytes were also responsible for complete prevention of development of spontaneous sarcoma, which developed in 24% of untreated controls and in none of the chimeras.<sup>20</sup> The data suggested induction of GVT effects following non-myeloablative conditioning prior to allogeneic stem cell transplantation independently of clinically overt GVHD. Recent successful clinical application of reduced intensity conditioning for accomplishing lymphoablation rather than myeloablation in preparation for transplantation of donor stem cells for induction of host-versus-graft transplantation tolerance as a platform for engraftment of alloreactive donor lymphocytes for induction of GVT effects,<sup>9</sup> opened the way for reconsideration of bone marrow transplantation for the treatment of metastatic solid tumors.<sup>11,12</sup> Although preliminary experience with clinical application of non-myeloablative stem cell transplantation (NST) for patients with metastatic renal cell cancer appears most promising,<sup>11</sup> many problems remain to be overcome. Since the efficacy of cell-mediated immunotherapy depends on alloreactive donor lymphocytes, except in very rare cases, GVT effects are accompanied by acute and chronic GVHD, which may be fatal or impair the quality of life of the recipient. It seems evident that cell therapy will become an effective therapeutic tool only if two conditions are met; first, targeted therapy must be accomplished to avoid insult to normal somatic cells, and second, the efficacy of GVT effects must be amplified because some tumors may grow faster than GVT effector cells can control.

*Adoptive allogeneic cell-mediated immunotherapy of metastatic breast cancer in prevention: clinical animal models*

Allogeneic cell-mediated immunotherapy was documented in a murine mammary carcinoma model (4T1) of BALB/c mice, using naive major histocompatibility (MHC)-mismatched splenocytes.<sup>13,14</sup> The frequency of alloreactive donor lymphocytes using this strategy may be sufficient for elimination of tumor cells, at the cost of GVHD, but such an approach is not feasible in clinical practice. In a subsequent study, we examined the potential of MHC compatible, minor histocompatibility antigen (MHAg) mismatched donors, mimicking the clinical

situation of HLA identical siblings or matched unrelated donors, to exert effective GVT phenomena.<sup>15</sup> Naive or immune donor cells sensitized with either tumor or normal minor mismatched splenocytes were tested for their ability to exert more effective anti-tumor activity in mice inoculated with a minimal dose of mammary carcinoma cells. BALB/c mice bearing the 4T1 tumor of BALB/c origin were given MHAg-mismatched DBA/2-derived splenocytes. GVT effects were assayed in secondary recipients of adoptively transferred lung cells derived from primary hosts previously inoculated intravenously with 4T1 cells and injected with either: 1) naive BALB/c splenocytes 2) naive DBA/2 splenocytes, 3) 4T1-immune DBA/2 splenocytes, or 4) DBA/2 splenocytes immunized with host-derived BALB/c spleen cells. Naive DBA/2 splenocytes inhibited tumor growth only slightly and hardly prolonged the survival of secondary recipients, contrasting with the results obtained using fully matched tumor/host BALB/c spleen cells. An efficient GVT reaction was demonstrated *in vitro* and *in vivo* with MHAg-mismatched DBA/2 splenocytes from mice pre-sensitized by multiple injections of irradiated tumor or BALB/c-derived spleen cells. All 30 mice adoptively inoculated with lung cells from primary hosts that had previously been treated with these pre-sensitized effector cells were tumor-free for >250 days. Secondary recipients inoculated with lung cells from mice given naive BALB/c or DBA/2 spleen cells died of metastatic tumors within 33-46 days. Pre-immunization with MHAg-mismatched tumor or spleen cells activated effector cells to induce an anti-tumor response. We suggest that cell therapy with pre-immunized effector cells may be clinically applicable in cases in which naive allogeneic cells have not been able to eradicate residual tumor cells in mammary carcinomas or other metastatic solid tumors. Taken together, our data provide the scientific background to consider the possibility of using donor lymphocytes activated against tumor antigens or host type alloantigens as a new clinical tool, both for overcoming residual disease in metastatic breast cancer and possibly other solid tumors as well as well as for the treatment of recurrent disease, especially for patients failing to benefit from all available anti-cancer modalities.

*Clinical application of NST for the treatment of metastatic solid tumors*

The Jerusalem NST approach in practice for >7 years for fully or single locus mismatched donors consists of fludarabine 30 mg/m<sup>2</sup>/day x 6 followed

by busulfan, cytoxan or total body irradiation (TBI)  $\pm$  anti-T-lymphocyte globulin (Fresenius ATG) or anti-CD52 (Campath-1H) followed by infusion of 1-2 collections of donor stem cells (mobilized blood or marrow) under cover of cyclosporin A 3 mg/kg initiated 1-4 days prior to transplantation. The dose of alkylating agents or TBI is determined by the type of disease and the bulk of host cells that need to be eliminated, ranging for busulfan from 4 to 16 mg/kg; for cytoxan from 10 (in Fanconi's anemia) 120 mg/kg; and for TBI from 200-1,200cGy. With the growing experience in patients with malignant and non-malignant indications ( $n > 330$  patients), the procedure has been well tolerated using matched siblings and matched unrelated donors (MUD). Some patients do not require blood products because complete aplasia is not produced, while there is rapid and consistent replacement of host hematopoietic cells by donor ones. The best results were obtained in patients with chronic myeloid leukemia (survival and disease-free survival 87% in first chronic phase, all in molecular remission and no disease recurrence yet observed) and in patients with non-malignant indications for BMT. Day 100 mortality was  $< 3\%$ , and was 0% among patients with non-malignant diseases. Thus, NST can be used for immunotherapy of hematologic malignancies or diseases treatable by allogeneic stem cell transplantation, even in elderly patients and those with a poor performance status. Unfortunately, acute and chronic GVHD on the one hand, and ineffective treatment of patients with advanced bulky disease on the other, still remain major problems. Nevertheless, a large number of otherwise incurable patients are alive, well, and disease-free for more than 8 years, and seem to be cured.

In the light of the above results, we are now applying NST for the treatment of patients with metastatic solid tumors. The preliminary experience is encouraging. The NST is based on the use of fludarabine and an alkylating agent, usually treosulfan instead of busulfan. The procedure is well tolerated, but, it is already clear that NST with no additional innovative improvement is unlikely to provide the final solution for patients with metastatic solid tumors, especially those with bulky disease. The new concepts we are currently investigating are following:

1. can GVT effects be maximized by using donor lymphocytes activated against tumor alloantigens?
2. can GVHD be avoided or minimized by targeting donor lymphocytes to the tumor by using

specifically immune rather than naïve donor lymphocytes?

3. can GVHD be avoided or minimized by targeting donor lymphocytes to the tumor by using antibodies or perhaps more promising bi-specific antibodies?
4. can effective GVT phenomena be mediated by alloreactive NK and NKT cells that cannot induce GVHD?

As shown by our pre-clinical animal models and very preliminary clinical experience, the answers to these challenging questions is likely to be positive; however, additional experience is required in order to determine whether these assumptions based on positive data in our murine models are also applicable in clinical practice.

## References

1. Passweg JR, Rowlings PA, Armitage JO, Gale RP, Pelz CJ, Sobocinski KA, et al. Report from the International Bone Marrow Transplant Registry and Autologous Blood and Marrow Transplant Registry - North America. *Clin Transpl* 1995; 3:117-27.
2. Gratwöhl A, Hermans J, Goldman JM, Arcese W, Carreras E, Devergie A, et al. Risk assessment for patients with chronic myeloid leukaemia before allogeneic blood or marrow transplantation. Chronic Leukemia Working Party of the European Group for Blood and Marrow Transplantation. *Lancet* 1998; 352:1087-92.
3. Ben-Yosef R, Or R, Nagler A, Slavin S. Graft-versus-tumour and graft-versus-leukaemia effect in patients with concurrent breast cancer and acute myelocytic leukaemia. *Lancet* 1996; 348:1242-3.
4. Eibl B, Schwaighofer H, Nachbaur D, Marth C, Gachter A, Knapp R, et al. Evidence for a graft-versus-tumor effect in a patient treated with marrow ablative chemotherapy and allogeneic bone marrow transplantation for breast cancer. *Blood* 1996; 88:1501-8.
5. Ueno NT, Rondon G, Mirza NQ, Geisler DK, Anderlini P, Giralt SA, et al. Allogeneic peripheral-blood progenitor-cell transplantation for poor-risk patients with metastatic breast cancer. *J Clin Oncol* 1998; 16:986-93.
6. Weiden PL, Sullivan KM, Fluornoy N, Strob R, Thomas ED. Antileukemic effect of chronic graft-versus-host disease: Contribution to improved survival after allogeneic marrow transplantation. *N Engl J Med* 1981; 304:1529-33.
7. Or R, Ackerstein A, Nagler A, Kapelushnik J, Naparstek E, Samuel S, et al. Allogeneic cell mediated immunotherapy for breast cancer after autologous stem cell transplantation: A clinical pilot study. *Cytokines Cell Mol Ther* 1998; 4:1-6.
8. Slavin S, Naparstek E, Nagler A, Ackerstein A, Samuel S, Kapelushnik J, et al. Allogeneic cell therapy with donor peripheral blood cells and recombinant human interleukin-2 to treat leukemia relapse post allogeneic bone marrow transplantation. *Blood* 1996; 87:2195-204.
9. Slavin S, Nagler A, Naparstek E, Kapelushnik Y, Aker M, Cividalli G, et al. Nonmyeloablative stem cell transplan-

- tation and cell therapy as an alternative to conventional bone marrow transplantation with lethal cytoreduction for the treatment of malignant and non-malignant hematologic diseases. *Blood* 1998; 91:756-63.
10. Moscovitch M, Slavin S. Anti-tumor effects of allogeneic bone marrow transplantation in (NZB X NZW)F1 hybrids with spontaneous lymphosarcoma. *J Immunol* 1984; 132:997-1000.
  11. 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; 343:750-8.
  12. Slavin S. Cancer immunotherapy with alloreactive lymphocytes. *N Engl J Med* 2000; 343:802-3.
  13. Morecki S, Moshel Y, Gelfand Y, Pugatsch T, Slavin S. Induction of graft vs. tumor effect in a murine model of mammary adenocarcinoma. *Int J Cancer* 1997; 71:59-63.
  14. Morecki S, Yacovlev E, Diab A, Slavin S. Allogeneic cell therapy for a murine mammary carcinoma. *Cancer Res* 1998; 58:3891-5.
  15. Morecki S, Yacovlev E, Gelfand Y, Uzi I, Slavin S. Cell therapy with preimmunized effector cells mismatched for minor histocompatible antigens in the treatment of a murine mammary carcinoma. *J Immunother* 2001; 24:114-21.

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