

Second malignancies after allogeneic hematopoietic stem cell transplantation: new insight and current problems

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Abstract With increased number of patients surviving on the long term, late effect after allogeneic hematopoietic stem cell transplantation have become of major clinical importance. Among these late effect, second malignancies have increasingly been recognized in the recent years. It has been usual to divide the problem of secondary malignancies following hematopoietic stem cell transplantation into three groups, i.e. leukemia, lymphoma and solid tumors. Recent clinical and biological data on these three types of malignancies, occurring after allogeneic stem cell transplantation, are summarized in this review. We will focus here only on second malignancies after *allogeneic* stem cell transplantation with particular emphasis on recent development on the pathogenesis, and early diagnosis, and treatment of these transplant-related complications. © 2002 Elsevier Science Ltd

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INTRODUCTION

Secondary malignancies are a well recognized complication in patients with Hodgkin's disease (HD) or non-Hodgkin's lymphoma treated with chemotherapy or combined modality treatment. However, it was only in the early 1990s that the risk secondary malignant diseases was described after allogeneic bone marrow transplantation (BMT). Since its introduction in the early 1970s, the number of patients who undergo BMT for a variety of malignant and non-malignant disorders has increased steadily. Improvement in survival after BMT has resulted in a need to assess issues related to long-term complications, such as malignant neoplasm. Experiments in the 1960s and 1970s in murine models suggested, that a graft-versus-host reaction after allogeneic spleen cell transplantation trigger the development of Epstein-Barr virus (EBV)-induced lymphomas. Finally, marrow transplant studies in rhesus monkeys and dogs in the 1970s and 1980s showed a significant increase in the incidence of malignancies relative to controls in animals irradiated with lethal doses of TBI and infused with allogeneic marrow cells. Thus, it should not be surprising that new malignancies occur in patients

after allogeneic hematopoietic stem cell transplantation, where one or several of these risk factors are present.

According to the Seattle group, it has been usual to divide the problem of secondary malignancies following hematopoietic stem cell transplantation into three groups, i.e. leukemia, lymphoma and solid tumors. Recent clinical and biological data on these three types of malignancies, occurring after allogeneic stem cell transplantation, will be summarized in this review. Some aspects on second malignancies after hematopoietic stem cell transplantation have already been extensively reviewed,¹ in particular the critical problem of myelodysplastic syndrome (MDS) and acute leukemia following autologous hematopoietic stem cell transplantation.² In this review we will focus only on second malignancies after *allogeneic* stem cell transplantation with particular emphasis on recent development on the pathogenesis, and early diagnosis, and treatment of these transplant-related complications.

Chronologically lymphoma, leukemia, and solid tumors have a typical time course. Most lymphomas occur within the first months of transplant, while solid tumors and leukemia are mostly diagnosed years after transplant. Time course and relative risk of the different categories of post-transplant malignancies are summarized in Fig. 1.

POST-TRANSPLANT LYMPHOPROLIFERATIVE DISORDERS AND LYMPHOMAS

Most cases of lymphoproliferative disorders (PTLD) after hematopoietic stem cell transplantation have been observed in allogeneic recipients. Most of these PTLD are best classified as B-cell PTLD rather than non-Hodgkin's lymphoma.³⁻⁸ In addition, some T-cell PTLD have been reported. Thirdly, lymphomas with clinical and biological characteristics typical for non-Hodgkin's or Hodgkin's lymphoma as seen in non-transplanted patients have occurred following stem cell transplantation.

B-cell post-transplant lymphoproliferative disorders

Incidence

B-cell PTLD are clinically and morphologically heterogeneous; usually they are associated with T-cell dysfunction and the presence of EBV. B-cell PTLD were first described as a distinct entity in the late 1970s in kidney allograft recipients, and have been observed with almost any organ transplant.⁴ B-cell PTLD generally develop early after transplant. The mean interval from transplantation to the development of B-cell PTLD lies between 5 and 6 months, with most being diagnosed within 3 months. It appears that patients transplanted for congenital immunodeficiencies are at a particularly high risk for PTLD, presumably due to the underlying immunodeficiency and the use of T-cell depletion of the donor graft generally used for these diseases (see risk factors). The diagnostic criteria may differ from study to study. For example, a nonlethal 'infectious mononucleosis-like' syndrome may resolve spontaneously, and acute-onset extensive disease may be diagnosed only at autopsy. In the largest series involving 18 014 patients who underwent allogeneic bone marrow transplantation at 235 centers worldwide PTLD developed in 78 recipients with 64 cases occurring less than a year after

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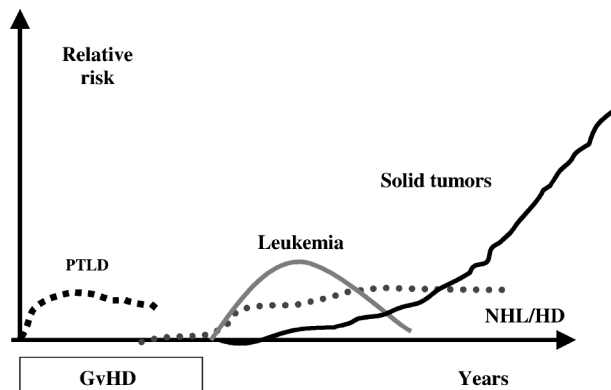


Fig. 1 Scheme of time course and relative risk of second malignancies after allogeneic stem cell transplantation.

transplantation.⁹ The cumulative incidence of PTLD was $1.0 \pm 0.3\%$ at 10 years. Incidence was highest 1–5 months post-transplant (120 cases/10 000/year) followed by a steep decline to less than 5/10 000/year among 1 year-plus survivors.

Clinical features

The most frequent presenting findings of PTLD are fever, lymphadenopathy and occasionally tonsillar enlargement. Intra-abdominal lymphadenopathy, splenomegaly or hepatomegaly may cause non-specific symptoms such as abdominal pain, vomiting or diarrhea. As with other lymphoproliferative disorders that develop in immunodeficient patients, extra-hematopoietic organ involvement including lungs, kidneys, and the central nervous system (CNS) is frequent. CNS involvement is of particular clinical concern since it has been associated with a dismal prognosis. With the exception of organomegaly, presenting symptoms are often non-specific. The differential diagnosis should include PTLD a priori in high-risk situations such as in recipients of T-cell depleted or HLA-non-identical transplants. A high index of clinical suspicion along with the use of aggressive diagnostic work-up involving imaging studies and biopsies increased the number of B-cell PTLD diagnosed during life, in the 1990s.

Today, the use of quantitative polymerase chain reaction (PCR) of EBV DNA has dramatically change diagnostic criteria. Using EBV viral load, patients are now frequently diagnosed with 'PTLD' while they present only isolated fever with (or even without) low tumor burden and a monoclonal gamma-globulin. This became important in the late 1990s since powerful therapeutic tools have been developed. It soon appeared that early diagnosis can be established and the effect of therapy can now be monitored by quantitative PCR of the EBV DNA (see pathogenesis and treatment).

Pathology

B-cell PTLD occurring after allogeneic hematopoietic stem cell transplantation are almost always of donor origin, and associated with EBV-genomic DNA integration. Biopsies reveal monomorphic or polymorphic, diffuse large-cell lymphoma of B-cell origin. However, while the morphology of B-cell PTLD occurring after solid organ transplantation has been described extensively, few studies have examined in detail the histologic features of PTLD in hematopoietic stem cell recipients.^{10–14}

Those reports show that whereas some PTLD after stem cell transplantation are histopathologically similar to the polymorphic PTLD described in solid organ transplant recipients, as many as half of the cases after stem cell transplantation show aggressive features of immunoblastic lymphoma.⁷ Also, in contrast to PTLD after organ transplantation, most B-cell PTLD occurring after stem cell transplantation are oligoclonal or monoclonal, as determined by analysis of immunoglobulin gene (Ig) rearrangements and fused termini of episomal EBV DNA,^{7,15–18} although some discrepancies between these two methods (tumors appearing monoclonal on the basis of EBV genomic analysis and polyclonal by analysis of Ig gene rearrangement) have been observed.^{16,19}

PTLD express the full array of latent EBV antigens, including EBNA-1, -2, -3, -4, -5, and -6, and LMP1.^{7,20–24} Karyotypic analyses have identified non-consistent cytogenetic abnormalities, more frequently in monoclonal lesions of more aggressive histology. However, with the exception of two cases of B-cell PTLD developing in heart transplant recipients, the characteristic translocation of Burkitt's lymphoma has not been observed in lymphoproliferative disorders developing after marrow (or solid organ) transplantation.²⁵ In a study, Orazi and coworkers attempted to correlate morphology with clonality (based on Ig chain gene rearrangement and immunohistochemistry), proliferative activity as measured by immunostaining for the proliferating cell nuclear antigen (PCNA), and presence of p53 over-expression.¹⁴ The reported cases included seven polymorphic B-cell lymphomas and three immunoblastic lymphomas. Ig heavy chain gene rearrangement analysis revealed B-cell clonality in three of seven polymorphic lymphomas and in all three immunoblastic lymphomas. The EBV genome, the expression of the EBV latent membrane protein, or both, were found in all ten cases. High proliferative activity as assessed by the expression of the PCNA antigen was found in all cases, and five specimens were p53+.

Risk factors

B-cell PTLD were the first post-transplant malignancies for which risk factors were identified. In 1989, Witherspoon and colleagues showed in multivariate analysis that treatment of acute graft-versus-host disease (GvHD) with either antithymocyte globulin or monoclonal anti-CD3 antibody, total body irradiation, T-cell depletion of donor marrow, and HLA non-identity between donor and recipient were risk factors for PTLD.²⁶ A survey by the Minneapolis group showed the following factors to be associated with an increased risk of B-cell PTLD: T-cell depletion of the graft (relative risk (RR) = 11.9), HLA mismatch (RR = 8.9), use of antithymocyte globulin for acute GVHD prophylaxis (RR = 5.9) or in the preparative regimen (RR = 3.1) and primary immune deficiency disease (RR = 2.5). The cumulative risk of developing a B-cell PTLD in patients with primary immune deficiency who received a T-cell depleted HLA-mismatched transplant was $64.8\% \pm 17.7\%$ at 4 years, compared to $0.9 \pm 0.2\%$ ($P < 0.001$) in patients who received an HLA-matched transplant with no in vitro manipulation of the graft.²⁷ The role of HLA-mismatching in the pathogenesis of B-cell PTLD is not entirely clear but may consist in chronic antigenic stimulation, or delayed immune reconstitution. In unrelated transplants, the National Marrow Donor Program (NMDP) reported an incidence of PTLD of

2% overall, 5% in patients receiving a T-cell depleted marrow and 1% for those receiving a T-repleted graft.²⁸ Available data suggest, however, that the risk is not uniform but depends on the method of T-cell depletion, and the type of additional immunosuppression used in the post-transplantation period. While in patients transplanted with marrow depleted of T-cells with specific monoclonal antibodies the incidence of EBV positive PTLD ranged from 11 to 25%, the incidence was <1% with techniques removing both T and B lymphocytes (e.g. soybean agglutinin or Campath-1), possibly reflecting the 2 to 3 log reduction in B lymphocytes associated with these procedures.^{7,9,29} However, when additional posttransplant immunosuppression with steroids and antithymocyte globulin was given following HLA-matched or mismatched related transplants or transplants from unrelated donors using soybean agglutination/E-rosetting for T-cell depletion, the incidence of PTLD increased to 6–18%. Even in the absence of in vitro T-cell depletion, the use of intensive in vivo immunosuppressive prophylaxis or therapy of GVHD, especially with anti-T-cell agents such as OKT3 antibody or antithymocyte globulin is associated with the development of B-cell PTLD.

The largest survey performed under the hospice of the NIH USA, including 18 014 patients from the International Bone Marrow Transplant Registry (IBMTR), the Fred Hutchinson Cancer Research Center (FHCRC) allowed detailed analysis on risk factors.⁹ In multivariate analyses, risk of early-onset PTLD (less than a 1 year) was strongly associated ($P < 0.0001$) with unrelated or HLA mismatched related donor (RR = 4.1), T-cell depletion of donor marrow (RR = 12.7), and use of antithymocyte globulin (RR = 6.4) or anti-CD3 monoclonal antibody (RR = 43.2) for prophylaxis or treatment of acute GvHD. There was a weaker association with the occurrence of acute GvHD grades II-IV (RR = 1.9, $P = 0.02$) and with conditioning regimens which included radiation (RR = 2.9, $P = 0.02$). Methods of T-cell depletion that selectively targeted T cells or T + NK cells were associated with markedly higher risks of PTLD than methods that removed both T and B cells, such as the CAMPATH-1 monoclonal antibody or elutriation ($P = 0.009$).

Finally, it should be underlined that patients who now received transplants after non-myeloablative conditioning using highly immunosuppressive drugs seem to be a novel population at risk for PTLD³⁰ (GS, unpublished data), and clearly need careful monitoring of EBV DNA load.

Pathogenesis

B-cell PTLD are thought to develop because of a combination of depressed EBV-specific cellular immunity and the inherent transforming capacities of EBV. EBV is a ubiquitous herpes virus that infects 95% of individuals by adulthood. The virus persists as a latent infection in certain epithelial cells, where reactivation and replication may occur intermittently, and in B lymphocytes.³¹ EBV type A and type B have been defined on the basis of sequence divergence in the EBNA-2 gene. In a series of 27 solid organ transplant recipients who developed PTLD, type A EBV was present in 24 of 27 cases (89%) by PCR amplification of EBNA-2 and EBNA-3c regions. In addition, there was polymorphism at the EBER locus documenting the presence of four different type A EBV strains. None of the

27 cases harbored type B EBV.³² Whether the same applies to marrow transplant recipients remains to be determined.

Among the 80–100 EBV-encoded proteins, the latent membrane protein 1 (LMP-1) plays an essential role in B-cell immortalization. LMP-1 has recently been shown to induce the expression of bcl-2, which inhibits programmed death of the infected cells. LMP-1 is also considered an oncogene because of its ability to transform rodent fibroblasts. Deletions near the 3' end of the LMP-1 gene, in a region that affects the half-life of the LMP-1 protein, have been reported in some EBV-related lymphoproliferative disorders.^{33,34} More recently, the St Jude group reported a patient who developed lymphoma after marrow transplantation and who received donor-derived, EBV-specific CTLs but died with progressive disease. The tumor cells proved substantially less sensitive to cytolysis than the EBV-transformed B-cell line used for CTL generation. The major cytolytic activity of the donor CTL was directed against 2 HLA-A11-restricted epitopes in the viral EBNA-3B antigen. Sequence analysis of this gene in the tumor virus revealed a 245-base pair deletion, which removed these 2 CTL epitopes. Hence, the viral antigen in the tumor had mutated in a way that allowed escape from CTLs. Analysis of EBV polymorphisms demonstrated that before CTL infusion, more than one virus was present, including a virus with wild-type EBNA-3B. After CTL infusion, only the virus with the EBNA-3B deletion could be detected, suggesting that the infused CTLs had selected a resistant strain in vivo.³⁵

Infection of B cells by EBV also induces high levels of IL-1, IL-5, IL-6, IL-10, CD23, and TNF. The cellular IL-10 and the EBV-induced BCRF1, a homolog of IL-10, act as autocrine growth factors, stimulating the proliferation of EBV-transformed B cells and inhibiting their susceptibility to apoptosis. Much of the initial work investigating anti-EBV cellular responses was performed in patients with acute infectious mononucleosis.⁷ Early in the course of the disease, NK cells and cytotoxic and suppressor T-cells reactive against EBV emerge. Using standard assays of cell mediated cytolysis, Crawford et al. found that in recipients of unmodified marrow, 7 of 10 patients studied had defective killing of autologous targets at 3 months post-transplant, but all were normal by 6 months.³⁶

In a study, investigators at Memorial Sloan-Kettering Cancer Center explored whether deficiencies of EBV-specific cellular immunity contribute to EBV-PTLD susceptibility.³⁷ They performed limiting dilution analysis to quantify anti-EBV specific cytotoxic T-lymphocyte precursors (CTLp) frequencies in 26 recipients of unmodified or T-cell depleted grafts from EBV-seropositive donors. At 3 months, only 5 of the 26 patients had EBV CTLp frequencies in the normal range of seropositive controls, while at 6 months, 9 of 13 patients were within the normal range. This time interval corresponds to the period in which B-cell PTLD are observed. The same investigators showed that EBV-specific cytotoxic T-lymphocytes home preferentially to and induce selective regression of autologous EBV-induced B-cell lymphoproliferative lesions in xenografted SCID mice.³⁸ These studies have led to clinical trials (see below) on the role of EBV-specific T-lymphocytes in controlling EBV-induced B-cell proliferation. Rather definitive proof has been provided by the St Jude group using adoptive transfer of gene-modified EBV-specific T-lymphocytes.³⁹ Preliminary clinical results showed that adoptive transfer of EBV-specific

cytotoxic T-lymphocytes offered effective therapy for B-cell PTLD.⁴⁰ The investigators showed long-term persistence of gene-marked EBV-specific cytotoxic T-lymphocytes *in vivo*. These cells not only restored cellular immunity against EBV, but also provided a population of CTLs that responded to *in vivo* or *ex vivo* challenge with the virus for as long as 18 months.

Finally it was recently demonstrated in the xenografted SCID mouse model that NK cells may also play a role in the genesis of PTLD. Baiocchi and coworkers,⁴¹ used SCID mouse engrafted with human leukocytes to evaluate the use of human cytokines in the prevention of EBV-LPD *in vivo*. Daily low-dose IL-2 therapy could prevent EBV-LPD in SCID mouse, but protection was lost if murine natural killer (NK) cells were depleted. They demonstrated that combined therapy with human GM-CSF and low-dose IL-2 was capable of preventing EBV-LPD in SCID mic in the absence of murine NK cells. Lymphocyte depletion experiments showed that human NK cells, CD8(+) T cells, and monocytes were each required for the protective effects of GM-CSF and IL-2 combination therapy. This treatment resulted in a marked expansion of human CD3(+)CD8(+) lymphocytes *in vivo*. Using HLA tetramers complexed with EBV immunodominant peptides, a subset of these lymphocytes was found to be EBV-specific. These data establish that combined GM-CSF and low-dose IL-2 therapy can prevent the immune deficiencies that lead to fatal EBV-LPD in xenografted-SCID mouse depleted of murine NK cells, and they point to a critical role for several human cellular subsets in mediating this protective effect.

Measuring EBV load and monitoring EBV-specific immune response after transplantation in the 2000s

As stated previously the diagnosis of PTLD in the 2000s has dramatically change since the introduction of effective and highly sensitive techniques to measure EBV load and to estimate early EBV-specific immune reconstitution after allogeneic stem cell transplantation.

In early 2000 these types of techniques were first use to guide the prophylactic infusion of EBV-specific cytotoxic T-cells. For example in a study the Huddinge group developed a semi-quantitative polymerase chain reaction assay to monitor the blood EBV-DNA in 9 patients receiving allogeneic bone marrow transplants.⁴² Four of 5 recipients of HLA-mismatched T-cell-depleted grafts showed a 4- to 5-log increase of EBV-DNA within 1 to 3 months after BMT. Administration of 2 to 4 infusions of 10^7 EBV-specific cytotoxic T-lymphocytes (CTLs)/m² starting from the time of maximal virus load resulted in a 2- to 3-log decrease of virus titers in 3 patients. One patient, who received a T-cell culture lacking a major EBV-specific component, progressed to fatal EBV-positive lymphoma. Administration of EBV-CTLs before the onset of the EBV-DNA peak resulted in stabilization of the virus titers within 2 to 3 logs above the normal levels in the fifth patient. A moderate increase of virus titers was also detected in 3 of 4 patients receiving non manipulated HLA-matched grafts, whereas 1 patient with Wiskott-Aldrich syndrome reached a 5-log increase of EBV-DNA load within 70 days after BMT. This suggest that a rapid increase of circulating EBV-DNA occurs in the absence of EBV-specific T-cell precursors or in the presence of

congenital immune defects that prevent the reestablishment of virus-specific immunity.

The next step was the development of truly quantitative PCR techniques that allows frequent monitoring of the DNA load to predict the development of PTLD. This was first applied to solid organ transplant recipients. After lung transplantation, Stevens et al.⁴³ studied recipients with and without PTLD. In PTLD patients, 78% of tested whole blood samples were above the cut-off value of quantitative competitive polymerase chain reaction (Q-PCR) (greater than 2000 EBV DNA copies per ml blood), with the majority of patients having high viral loads before and at PTLD diagnosis. Especially in a primary EBV-infected patient and in patients with conversion of immunosuppressive treatment, rapid increases in peripheral blood EBV DNA load diagnosed and predicted PTLD. In non-PTLD transplantation recipients, only 3.4% of the whole blood samples was above the cut-off value ($P < .0001$) despite heavy immune suppression and cytomegalovirus (CMV)-related disease. After allogeneic stem cell transplantation (SCT), the utility of such a monitoring was confirmed by the Rotterdam's group. Van Esser and coworkers^{44,45} retrospectively monitored 85 EBV-seropositive recipients of a T-cell-depleted (TCD) allo-SCT and 65 EBV-seropositive recipients of an unmanipulated allogeneic SCT. Viral reactivation (more than 50 EBV genome equivalents [gEq]/ml) was monitored frequently by quantitative real-time plasma polymerase chain reaction until day 180 after SCT. Probabilities of developing viral reactivation were high after both unmanipulated and TCD-allogeneic SCT (31% +/- 6% versus 65% +/- 7%, respectively). A high CD34(+) cell number of the graft appeared as a novel significant predictor ($P = 0.001$) for EBV reactivation. Recurrent reactivation was observed more frequently in recipients of a TCD graft, and EBV-LPD occurred only after TCD-SCT. High-risk status, TCD, and use of antithymocyte globulin were predictive for developing EBV-LPD. Plasma EBV DNA quantitatively predicted EBV-LPD. The positive and negative predictive values of a viral load of 1000 gEq/ml were, respectively, 39% and 100% after TCD.

Finally recent developments in immunology allow now the monitoring of EBV-specific immune response after transplantation. EBV-specific T-lymphocytes can now be monitored through the tetramers technology that allows detection of minutes number of antigen-specific T-cells. This technique was first applied to healthy individuals following primary infection and was then applied to SCT recipients. In a study, Marshall and coworkers⁴⁶ used HLA class I tetramers to investigate the reestablishment of the EBV-specific CD8 T-cell repertoire in patients following allogeneic SCT. CD8(+) T cells specific for lytic and latent cycle-derived EBV peptides rapidly repopulated the periphery of matched sibling allogeneic SCT patients. The relative frequencies of T-cells specific for different EBV peptides in transplantation recipients closely reflected those of their respective donors. Investigation of patients at monthly intervals following unmanipulated allo-PBSCT demonstrated that the frequency of EBV-specific T cells correlates with the number of EBV genome copies in the peripheral blood and that expansion of EBV-specific T-cell populations occurs even in the setting of immunosuppressive therapy. In contrast, patients undergoing T-cell-depleted or unrelated cord blood transplantation have undetectable EBV-specific T cells,

even in the presence of Epstein-Barr viremia. Thus, the protective shield provided by EBV-specific CD8 T cells seems to be rapidly established following unmanipulated matched sibling allogeneic peripheral SCT and this study demonstrates that HLA class I tetramers complexed with viral peptides can provide direct and rapid assessment of pathogen-specific immunity in this patient populations.

Prophylaxis and treatment

Since various recognized risk factors such as initial diagnosis (primary immune deficiency syndrome) or type of donor (HLA-non-identical) cannot be changed, and others (e.g. GvHD prophylaxis) are considered an integral part of the overall treatment regimen, it has been proposed to use early identification of EBV-associated PTLD as an indication for therapy rather than apply true prophylaxis. The St Jude group used both the outgrowth of transformed B lymphocytes *ex vivo* and detection of EBV DNA by a PCR method as tools to detect EBV-PTLD before clinical disease developed.⁴⁷ Semi-quantitative, and now quantitative PCR assay for EBV DNA in peripheral blood is used easily to assist in the detection of PTLD and in monitoring the effect of therapy.^{40,42-45,48-50}

Complete regression of B-cell PTLD has been reported in 40% of patients following reduction or discontinuation of immunosuppressive drugs, particularly in renal transplant recipients (dialysis was re-instituted if the kidney was rejected).⁵¹ Immunosuppression is intrinsic to marrow transplantation, and discontinuation of immunosuppression is likely to result in flares of GvHD and a further delay in recovery of T-cell mediated immunity. EBV-transformed B cells contain a circular viral DNA that is not susceptible to inhibition with thymidine kinase (TK) inhibitors. Nevertheless, anecdotal reports suggest tumor regression with either acyclovir and ganciclovir therapy.⁵² Chemotherapy and irradiation generally have not proven useful, although in a recent series of cardiac transplant recipients, among 19 consecutive patients with PTLD, 6 of 8 treated with aggressive chemotherapy (ProMACE-CytaBOM) are surviving in complete remission, at a median follow-up of 38 months. Surgical resection has proven effective when the PTLD was limited to single sites in solid organ transplant recipients.⁵³

Three approaches have shown promise in the treatment of B-cell PTLD in marrow transplant recipients: α interferon, B-cell specific monoclonal antibodies and cellular therapy.

A combination of α interferon and intravenous immunoglobulin was first reported in 1988 by the Minneapolis group to be effective in B-cell PTLD. Remissions were maintained in several patients.⁵⁴ In a recent update, three of seven patients receiving α interferon achieved a complete remission (Gross TG and Filipovich AH). However, experience of other groups with interferon gave far from satisfactory results, and now, with highly efficient therapy with Rituximab or cellular therapy, it is unlikely that this therapy will still be used.

Two anti-B-cell antibodies (anti-CD21 and anti-CD24) were used in a multi-center trial.⁵⁵⁻⁵⁷ Among 19 marrow transplant recipients, 10 had a complete remission and 6 survived at a median follow-up of 20 months. The survivors in this series all were patients with oligoclonal diseases. Studies in a SCID mouse model⁵⁸ show that following initial remission, with such an approach 30 to 50% of mice relapsed within 30-70 days, providing a very strong indication that persistence of residual

B cells can provoke a second tumor in the absence of efficient cytotoxic T cells. Anti-CD21 and CD24 antibodies used in these studies are no longer available for clinical use. Based on *in vitro* data showing an antitumor effect of anti-IL6 antibody in neutralizing the IL-6-dependent proliferative loop,⁵⁹ the same authors tested this antibody in patients with PTLD.⁶⁰ Twelve recipient of transplanted organ were treated and complete remission was obtained in 5. No data on the use of this antibody after SCT are yet available.

Most recently the efficacy of anti-CD20 monoclonal antibody (Rituximab) in the treatment of PTLD after SCT has been reported and seems to be highly effective in this setting (see references, and Hospital St Louis, unpublished data). Rituximab efficacy was first reported in 3 patients by Dr Heslop's group.⁶¹ In a French survey, Milpied and co-workers⁶² reported 20 complete responses occurring in 32 patients. Finally, in children, Faye and coworkers⁶³ investigated tolerance and efficacy of humanized anti-CD20 monoclonal antibody (rituximab) as first-line treatment in 12 children with B-cell PTLD. At diagnosis, eight patients had tumoral involvement. The other four patients had fever, associated with raised EBV viral load and monoclonal gammopathy. Rituximab was given at the dose of 375 mg/m² once a week by intravenous infusion (1-9 infusions). Only 1/48 infusions was associated with a grade 2 clinical adverse event. Eight out of 12 (66%) patients responded to the treatment and were in complete remission. All patients without tumoral involvement responded to the treatment. A rapid decrease in fever within 1 week was observed in all responders. Non-responders did not show any clinical response during the first week. Currently many group consider pre-emptive treatment based on increased EBV load in high risk patient. Whether this approach is safe and efficacious is however still need further longitudinal multi-center studies.

In 1994 Papadopoulos and coworkers first reported therapeutic efficacy of the infusion of donor leukocytes in five patients who developed a B-cell PTLD following T-cell depleted allogeneic marrow transplantation.¹⁹ Non irradiated donor leukocytes were infused at doses calculated to provide 1.0×10^6 CD3+ T cells/kg of body weight. All five patients had complete pathological or clinical responses. Three of the five patients developed chronic GvHD and two died of respiratory failure with no evidence of lymphoma at autopsy. Subsequently Rooney et al.⁴⁰ reported on the use of gene-marked EBV-specific T lymphocytes to control or prevent B-cell PTLD in 10 patients. Three of the patients had shown signs of EBV reactivation, with or without overt lymphoproliferation, and seven received T-cell infusions as prophylaxis. In the three patients with EBV reactivation, EBV DNA concentrations which had increased 1000-fold or more, returned to control levels within 3-4 weeks of immunotherapy. In a recent update, the Sloan-Kettering Center reported data on 15 patients with eradication of B-cell PTLD in 14; GvHD occurred in 6 among the 12 evaluable patients.⁶⁴ The St Jude group described the prophylactic use of EBV-specific T-cell clones in 25 patients, none of which developed PTLD. Among six patients who either refused CTL therapy or were ineligible for treatment, two developed lymphomas that were successfully treated with CTL.⁶⁵ Bordignon's group most recently reported on the use of HSV-TK gene transfer in donor lymphocytes infused to control B-cell PTLD in two patients. One of these

patients subsequently developed GvHD that was successfully treated with ganciclovir by way of activating the HSV-TK suicide gene.⁶⁶

Thus, promising approaches have been developed for the treatment of B-cell PTLD in high-risk marrow⁶⁷ and solid organ transplant recipients.⁶⁸ However, the numbers of patients treated are still limited. Also, the use of cellular therapy may induce GvHD if non-EBV-specific CTL are used and still requires high-level biotechnology laboratories to provide either EBV-specific CTL clones or HSV-TK transduced T lymphocytes. The most readily available and most efficacious treatment seems to be Rituximab that needs, however, further studies with larger number of patients before being considered as the gold standard treatment in allogeneic SCT recipients.

T-cell lymphoproliferative disorders

Besides the well defined B-cell PTLD, an entity of T-cell proliferative disorders without EBV association have been reported both after solid organ and marrow transplantation. After solid organ transplantation these disorders have occurred predominantly at extra-nodal sites and were monoclonal.^{69,70} After marrow transplantation only few such cases have been reported;⁷¹ four occurred late after transplant and may be included in the late-onset lymphoma category (see below). None of the cases was associated with HTLV1, HIV or HHV6 infection.

Late-onset lymphoma

Some cases of late occurring lymphomas have been reported in the literature.^{5,72-78} At least some have been linked to EBV infection (just as early onset PTLD) and some were associated with T-cell depletion of the graft. These cases presented like ordinary non-Hodgkin lymphoma with lymph node enlargement with or without general symptoms; one of these patients has been reported to be disease free following chemotherapy. At Hôpital Saint Louis in Paris such a late occurrence of EBV-related Hodgkin disease in donor cells was observed in a patient transplanted 8 years before for chronic myelogenous leukemia.⁷³ Ongoing studies seem to support the notion that these late-occurring lymphomas represent an entity distinct from the early occurring B-cell PTLD. This was strongly supported by two NIH-sponsored studies performed by the IBMTR and the FHCRC. Among more than 18 000 patients 14 late onset lymphoma were reported.⁹ The only risk factor identified for late-onset PTLD was extensive chronic GVHD (RR = 4.0, $P = 0.01$). Furthermore in another study the same group reported that SCT recipient may also prone to develop HD. The risk of HD was evaluated among 18 531 persons receiving allogeneic BMT between 1964 and 1992 at 254 centers.⁷⁹ There were 8 reported cases of HD; each confirmed by histologic review. Risk of HD was increased compared to the general population with an observed-to-expected incidence ratio (O/E) of 6.3 (95% confidence interval (CI) 2.7-12). Mixed cellularity subtype predominated (5 of 8 cases, 83%). Five of 6 evaluable cases contained EBV genome, as demonstrated by EBER1 probes. HD differed from PTLD by later onset (> 2.5 years), lack of established risk factors, such as T-cell depletion of donor bone marrow and HLA disparity, and relatively good prognosis. Although based on small numbers, patients with HD were more likely than matched controls to have acute graft-versus-host disease (GvHD) II-IV

and/or therapy for chronic GvHD ($P = 0.002$). Thus this finding of an increased incidence of HD after SCT, in particular mixed cellularity type, adds support to current theories which link over-stimulation of cell-mediated immunity and exposure to EBV with various subtypes of HD. The long latency and lack of association with usual PTLD risk factors is noteworthy and should be explored further for possible insights into pathogenesis.

SOLID TUMORS

Observations in animal models suggested that post-transplant (or post-irradiation) solid tumors occurred with considerable delay, ranging from 7.5 to 15 (median 11.5) years in X-irradiated and 4 to 15 (median 8) years in rhesus monkeys irradiated with fission neutrons.⁸⁰ The time interval in gamma-irradiated dogs was 1.6 to 10.5 (median 8) years.⁸¹ Extrapolation to humans with a longer expected life span would suggest that solid tumors might develop a decade or more after transplantation. This appears to be born out by the actual data.^{1,3,82} Indeed, malignant solid tumors may be viewed as the sole really late malignant complication of allogeneic SCT. While second leukemia occurred with a median elapsed time of 6.7 months and PTLD with a median of 2.5 months, the median elapsed time from SCT to solid tumors lies between 5 to 6 years.

Initial reports, generally on small numbers of patients who had undergone allogeneic (or syngeneic) marrow transplantation, documented the development of some adenocarcinomas of the rectum, brain tumors (glioblastomas), particularly in patients who had also received cranial irradiation (1800-2400 cGy) before transplantation, squamous cell carcinomas of the skin, and cancers of the oropharyngeal mucosa.^{83,84} In the first larger series, analyzing results in 2145 patients transplanted from 1970-1987 in Seattle, Witherspoon et al.²⁶ found 35 new malignancies, including 13 solid tumors, i.e. glioblastomas, melanomas, squamous cell carcinomas, adenocarcinoma, hepatoma, and basal cell carcinoma. These solid tumors were diagnosed between 2.5 months and 14 years (median 4.6 years) after transplantation. While TBI was a significant risk factor when all malignancies were considered, only the use of antithymocyte globulin as an immunosuppressive agent was identified as a significant risk factor for solid tumors. Subsequent analysis of the results in patients with aplastic anemia transplanted in Seattle and at Hôpital Saint Louis in Paris as well as reports from other European centers showed that irradiation, in particular total lymphoid or thoraco-abdominal irradiation was a significant risk factor (as compared to conditioning regimens that did not involve irradiation) for the development of solid tumors.⁸⁵ A combined analysis of results in 700 patients with aplastic anemia transplanted at the Fred Hutchinson Cancer Research Center or Hôpital Saint Louis suggested that in addition to irradiation (RR 3.9) treatment of chronic GvHD with azathioprine (RR 7.5) and older age (RR 1.1) increased the risk of a post-transplant malignancy.⁸⁶ Not surprisingly the highest incidence of malignancy was observed in patients in whom the etiology of marrow failure was Fanconi anemia (Kaplan-Meier estimate at 15 years approximately 40%). Of note, however,

no hematological malignancies (MDS, etc.) were observed in either idiopathic or Fanconi-associated aplastic anemia, an indication that the transplanted (allogeneic) stem cells were able to develop and differentiate normally in the patient's marrow microenvironment. Bhatia and colleagues²⁷ summarized the Minneapolis results: among 2150 patients, 15 developed a solid tumor (8 in 1400 allogeneic and 7 in 750 autologous transplant recipients) for a cumulative probability of 5.6% at 13 years. Again, irradiation was the major risk factor (RR 6; $P = 0.008$).

In a collaborative study, Curtis et al.⁸⁷ analyzed results in 19 220 patients (97.2% allogeneic, 2.8% syngeneic recipients) transplanted between 1964 and 1992 at 235 centers. There were 80 solid tumors for an observed/expected (O/E) ratio of 2.7 ($P < 0.001$). In patients surviving at least 10 years after transplantation, the risk was increased 8fold. The cumulative incidence of tumors was 2.2% at 10 years and 6.7% at 15 years. The risk was increased significantly for melanoma (O/E 5.0), cancers of the buccal cavity (11.1), liver (7.5), CNS (7.6), thyroid (6.6), bone (13.4), and connective tissue (8.0). The risk was highest for the youngest patients and declined with age (P for trend < 0.001). Most striking was the link of squamous cell carcinoma with chronic GVHD and male gender. The underlying diagnosis was important insofar as the risk of solid tumors was higher for patients with leukemia and lower in patients with lymphoma or aplastic anemia. The risk associated with TBI declined if irradiation was given with a fractionation regimen but increased with the total cumulative dose administered. This analysis strongly suggests that reduced doses of TBI, the omission of limited field irradiation, and the prevention of GVHD, in particular chronic GVHD, should reduce the risk of post-transplant solid tumors.

Since the risk was highest in youngest patients, the NIH/IBMTR and FHCRC research group then analyzed a cohort of children transplanted for leukemia.⁸⁸ A cohort of 3182 children diagnosed with acute leukemia before the age of 17 years who received allogeneic SCT between 1964 and 1992 at 235 centers was studied. Observed second cancers were compared with expected cancers in an age- and sex-matched general population. Risk factors were evaluated using Poisson regression. Cumulative risk of solid cancers increased sharply to 11.0% (95%, 2.3% to 19.8%) at 15 years and was highest among children at ages younger than 5 years at transplantation. Thyroid and brain cancers ($n = 14$) accounted for most of the strong age trend; many of these patients received cranial irradiation before BMT. Multivariate analyses showed increased solid tumor risks associated with high-dose total-body irradiation (RR 5.3) and younger age at transplantation (RR 5.3), whereas chronic GVHD was associated with a decreased risk (RR 0.2).

Finally, since in the first NIH/IBMTR and FHCRC study relatively few patients surviving more than 10 years post-SCT were included, we have continued surveillance of these and other SCT survivors to determine whether solid cancer risk changed beyond 10 years after transplantation.⁸⁹ The preliminary results of these survey could be summarized as follows. New cancers were searched in 28 884 allogeneic SCT recipients. 23 543 patients were transplanted from 1964-1994 by 271 IBMTR teams with follow-up through 1995; 5341 patients were transplanted 1969-1996 at FHCRC and followed through during

1996. 6530 patients had survived 5 years post-transplant, 1927; 10 years; 367, 15 years; and 59, 20 years. Transplantation was done predominantly for leukemia (acute and chronic myelogenous leukemia, acute lymphoblastic leukemia, 74%), aplastic anemia (10%), lymphoma (5%) and myelodysplastic syndromes (5%). Average age at transplantation was 27 years (range < 1 -72 years). 67% of patients received total body irradiation (TBI) as part of their preparative regimen. The cumulative incidence of invasive solid cancers for all patients was $2.2\% \pm 0.4\%$ at 10 years, $5.0\% \pm 1.2\%$ at 15 years, and $8.1\% \pm 3.1\%$ at 20 years. Compared to an age- and sex-matched general population, transplant recipients were at significantly higher risk of developing new invasive solid cancers (observed second cancers = 161; O/E = 2.3; 95%CI 1.93, 2.64). Risk increased with time since transplantation; the O/E ratio was 4.8 (3.2, 6.8) among 10 year survivors. Sites with significantly ($P < 0.05$) increased risks of second cancers after transplantation were oral cavity (O/E = 11.6), salivary glands (O/E = 14.2), liver (O/E = 6.9), skin (O/E = 4.2), brain (O/E = 6.0), thyroid (O/E = 6.3) and bone/connective tissue (O/E = 8.4). A new finding in this study, not seen in previous reports, is a significantly increased risk of breast cancer among 10-year survivors (O/E = 3.3 with 5 observed cases). Univariate analyses of transplant related variables suggests that conditioning with TBI may increase the risk of subsequent cancers of the salivary, brain, thyroid, breast and bone/connective tissue and melanoma of the skin. Excess risk of solid cancers diminishes with increasing age at transplantation. These data indicate BMT survivors face increasing risks of solid cancers with time after transplantation, supporting lifelong surveillance.

In conclusion, these data, mainly coming from this latter largest study group, suggest the following.

- First cancer incidence continue to increase with prolonged follow-up without evidence of any plateau phase.
- Second; irradiation is the strongest risk factor but while chemotherapy alone must be used in patients with aplastic anemia, other disease many do need irradiation within the conditioning regimen and recent data do suggest that solid cancers do occur after non-irradiation based conditioning regimen
- Third; chronic GVHD and/or its treatment is strongly associated with the occurrence of squamous cell carcinoma
- Fourth; these and ongoing studies try to identify cancer-specific risk factors (see Table 1)
- Fifth; biological data are needed to study in more depth oncogenetic process in SCT recipients.

Concerning this latter point, recent studies at the hospital Saint Louis try to identify if viral infection could play a role in the development of solid tumors after SCT.⁹⁰ Since Human herpes virus 8 (HHV8), EBV and papillomaviruses (HPV) sequences have been found in squamous cell carcinoma (SCC) occurring in organ transplant recipients and since the tumor suppressor gene p53 has been strongly linked to the occurrence of SCC in the non-immune-compromised population. These oncogenetic pathways were studied in 8 SCT patients with SCC

Table 1 Risk factors of second malignancies according to tumor type

Risk factor	Second malignancies
TBI	Melanoma, Thyroid, CNS tumor
Limited field irradiation	SCC, head and neck
T-cell depletion	Melanoma, PTLD
Chronic GvHD	SCC, head and neck, skin
HLA-mismatch	PTLD
ATG, OKT3	PTLD
Acute GvHD	PTLD
Oncogenic viruses	PTLD, SCC head and neck

TBI, total body irradiation; CNS, central nervous system; SCC, squamous cell carcinoma; GvHD, graft-versus-host disease; ATG, anti-thymocyte globulin; PTLD, post-transplant proliferative disorder.

in whom we searched for HHV8, EBV, varicella-zoster virus, adenovirus and HPV sequences from DNA extracted from selected areas of SCC. We also looked for p53 expression in those specimens as well as the presence of anti-p53 antibodies in the serum of these patients at the onset of SCC. In 1 patient we found the presence of both HHV8 and EBV sequences and in another patient HPV-16 sequences. All 5 tumors that could be studied disclosed evidence of p53 accumulation but none of the 8 patients had anti-p53 antibodies in the sera. Thus, SCC developing in marrow transplant recipients seem to occur via a multi-step process. Genetic predisposition may be present, as in patients with Fanconi's anemia. Transplant related factors, such as irradiation, and chronic-GvHD do also have a role. In this paper we thus added two more potent risk factors: p53 alteration(s) and in some cases a role of oncogenic viruses. In another paper we studied HPV infection and anogenital condyloma (a pre-malignant tumor) in bone marrow transplant recipients.⁹¹ Anogenital lesions associated with human HPV infection have been described in renal transplant recipients but not after SCT. HPV types 16 and 18 are strongly linked to the malignant transformation. In a series of 238 patients with allogeneic SCT, three had anogenital lesions. We looked for HPV in DNA extracted from embedded tissue to study HPV genotypes, p53 expression, and ploidy. In two patients, HPV sequences were detected. One of them, with giant condyloma, had HPV type 18 and two aneuploid clones, but p53 expression was not found. Thus, as in solid organ transplant recipients, anogenital condyloma may develop after SCT. Because the oncoprotein of HPV is able to bind and to degrade p53, it may lead to genetic instability, and subsequently to malignant transformation.

- Sixth; most patients who underwent allogeneic SCT had received substantial amount of chemotherapy and radiation before transplant. The role of those pre-transplant treatments on the occurrence of solid tumors clearly needs evaluation. Such a study is currently done by the NIH/IBBMTR and FHCRC group.

However, a rather small survey on the role of pre-transplant treatment has already been done by Bhatia and coworkers⁹² which results are the following: 2129 patients who had

undergone BMT for hematological malignancies at the City of Hope National Medical Center between 1976 and 1998 were studied. A retrospective cohort and nested case-control study design were used to evaluate the role of pre-transplantation therapeutic exposures and transplant conditioning regimens. Twenty-nine patients developed solid cancers after BMT, which represents a two-fold increase in risk compared with a comparable normal population. The estimated cumulative probability for development of a solid cancer was 6.1% +/- 1.6% at 10 years. The risk was significantly elevated for liver cancer (standardized incidence ratio [SIR], 27.7, cancer of the oral cavity (SIR, 17.4), and cervical cancer (SIR, 13.3). Each of the two patients with liver cancer had a history of chronic hepatitis C infection. All six patients with squamous cell carcinoma of the skin had chronic graft-versus-host disease. The risk was significantly higher for survivors who were younger than 34 years of age at time of BMT (SIR, 5.3). Cancers of the thyroid gland, liver, and oral cavity occurred primarily among patients who received total-body irradiation. This largely confirms previous results. However, no significant factor emerged from the nested case-control study used to evaluate the role of pre-transplantation therapeutic exposures.

- Seventh; how to treat patients with second solid tumors after SCT? There is very few data to answer this critical and practical question. Our experience at the hospital Saint Louis could be summarized as follows (G Socie; unpublished information 2002): patients with SCC of the head and neck (mostly aplastic and Fanconi anemia) have in our experience a bad prognosis using conventional therapy with surgery and/or radiotherapy. With this exception patients with solid tumors could generally be treated safely with conventional treatment.

The only published series we are aware of about the treatment of solid tumors after SCT has recently been published by the Basel group.⁹³ In this study, the authors retrospectively evaluated treatment and outcome of patients who developed solid tumors. From August 1974 to July 1996, six solid tumors were observed in five of 387 patients 2 to 13 years after SCT, corresponding to a probability of developing a second solid tumor of 9% (95 CI, 1-17%) at 15 years: these comprised endometrial carcinoma, carcinoma of the thyroid gland, cervical carcinoma, sarcoma of the small intestine, osteosarcoma of the tibia and ovarian carcinoma. All five patients were treated as intensively as they would be without a history of SCT. At last follow-up four of the five patients were alive and without signs of tumor. The authors postulate that second solid tumors after SCT should be treated as de novo tumors. Early detection based on consequent clinical follow-up of the transplant patients might explain the relatively good outcome.

MYELODYSPLASTIC SYNDROME AND ACUTE LEUKEMIA

Already in the early 1970s, Fialkow et al.⁹⁴ and Thomas et al.⁹⁵ reported on two patients with acute lymphoblastic leukemia given TBI and transplanted with marrow from an HLA-identical sibling donor who within approximately 2-4 months experienced what appeared to be a relapse of their original disease.

Further studies, however, using cytogenetic and enzyme analysis, revealed that the leukemic cells were donor-derived. Both donors continued to be healthy. Several similar cases, including patients with AML, ALL and CML, were subsequently reported from other institutions.⁸³ Conditioning regimens in those patients consisted of chemotherapy only or chemotherapy plus TBI, and the diagnosis of 'recurrent leukemia in donor cells' was made 6 months to more than 3 years after transplantation. The mechanism that would lead to leukemia in previously healthy transplanted cells was not clear. Several hypotheses have been proposed: donor cells may have been transformed by antigenic stimulation through host tissue, as observed in murine models of marrow transplantation. However, if this was the case, one would expect a higher frequency of this event. Alternatively, the recipient lymphohematopoietic environment in which the original leukemia had developed might trigger a similar development in donor cells. Further, fusion of normal cells with leukemic cells still residing in the recipient or transfection of an etiologic agent (virus/oncogene) might have transformed donor cells. While these possibilities are conjectural, the clinical observations are of interest in the context of leukemogenesis in general (reviewed in¹).

More recent studies have employed refined molecular biology tools (e.g. variable number tandem repeat [VNTR] analysis) to determine the origin of cells - normal or abnormal - in patients posttransplant. As determined by microsatellite analysis, disease reappearance in donor-derived cells is infrequent.⁹⁶

MDS in particular, has occurred extremely infrequently after allogeneic transplantation (even in patients with Fanconi anemia in whom MDS develops frequently if not transplanted with normal cells), an observation that provides indirect support for the notion that MDS after autologous transplantation is related to pre-transplant factors rather than the transplant itself.

CONCLUSION

Although quiet infrequent, second malignancies after allogeneic SCT has emerged as a significant complication. PTLD is clearly the second malignancy type where most advances in the biology and treatment have been made in the recent years. The development of highly-immunosuppressive but non-myeloablative conditioning before transplantation from HLA-identical sibling and unrelated donors makes likely that the incidence of PTLD will raise in this patient population. Solid tumors incidence rates show alarming slope with increasing incidence with increased follow-up. Similarities of solid tumor rates and tumor type with those occurring after treatment of HD may indeed predict that solid tumors incidence may reach 15–20% in very long-term survivors in the forthcoming years. For solid tumors the only practical attitude is to closely follow long-term survivors on an (at least) annual basis to allow early diagnosis and treatment. Finally, although extremely rare donor cells leukemia provide a unique opportunity to study leukemogenesis. In any case of late appearing leukemia in long-term survivors the question of donor versus recipient origin should be asked.

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