Graft Purging in Autologous Bone Marrow Transplantation: A Promise Not Quite Fulfilled

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Clonogenic tumor cells contained within hematopoietic stem cell (HPC) grafts may contribute to relapse following autologous transplantation. Graft purging involves either in vivo or ex vivo HPC manipulation in order to reduce the level of tumor cell contamination. Some phase II trials suggest that patients who receive purged products may have a superior transplant outcome. Phase I trials demonstrate the feasibility of purging methods including ex vivo graft incubation with chemotherapeutic drugs, monoclonal antibodies and complement, and CD34+ cell selection. A phase II trial in follicular non-Hodgkin’s lymphoma demonstrates that patients who receive HPC products purged negative for bcl-2 gene rearrangements have a superior outcome, compared with patients who receive polymerase chain reaction (PCR)-positive products. This finding, however, has not been confirmed in a randomized trial. HPC purging has demonstrated no benefit in a phase III trial in myeloma. Phase II trials in acute myelogenous leukemia show comparable outcomes for patients who receive either purged or unpurged HPC grafts. Limitations of purging include possible progenitor cell loss, delayed engraftment, and qualitative immune defects following transplant. Data to justify routine use of HPC graft purging are insufficient. Phase I and II data support development of phase III trials of both in vivo and in vitro purging methods.

Autologous hematopoietic progenitor cell (HPC) transplantation is curative and considered the standard of care for a number of malignancies. HPC transplantation permits the use of extremely intensive, otherwise myeloablative, therapy. Unfortunately, even where the maximal feasible dose intensity is successfully delivered, disease relapse remains the primary cause of death following transplantation. While therapy-resistant systemic disease is likely a significant source for relapse, another source may be infusion of occult, clonogenic tumor cells contained within the HPC graft.[1] Bone marrow harvesting and collection of blood HPC may lead to inadvertent collection of tumor cells. The presence of these cells may be confirmed through the use of flow cytometry or molecular diagnostic technologies.[2,3] Gene-marking studies demonstrate that tumor cells infused with HPC grafts may contribute directly to relapse following transplantation. As such, graft purging has been a key area of investigation for several groups. Molecular diagnostic methods demonstrate that a daunting array of purging strategies may reduce the level of graft contamination by tumor cells. In some instances, the level of contamination may be reduced below the limits of detection. Unfortunately, the ultimate clinical impact of graft purging remains unclear. In this paper, we will review the prevalence and significance of tumor cell contamination of HPC grafts and describe some methods of graft purging. In addition, we will describe the clinical experience using purged autologous HPC grafts for three paradigmatic hematologic diseases in which autologous HPC transplantation plays a significant role: non-Hodgkin's lymphoma (NHL), multiple myeloma, and acute myelogenous leukemia (AML). Graft as the Source of Clonogenic Tumor Cells

Prevalence of HPC Graft Contamination by Tumor Cells
Molecular diagnostic studies permit the detection of tumor cell contamination of HPC grafts with a sensitivity that is 3 to 4 logs greater than histologic methods or flow cytometry.[2] Assay sensitivity may be as great as 10^-6 when disease-specific oligonucleotide probes are used for a polymerase chain reaction (PCR) analysis.[3,4] With these tools, a wide variety of tumor cell types may be detected in the HPC grafts of patients undergoing autologous transplantation, including those with multiple myeloma,[5] NHL,[6] AML,[7] neuroblastoma, Ewing's sarcoma,[8] chronic myelogenous leukemia,[9] acute lymphoblastic leukemia,[9] breast cancer,[10] and germ cell tumors.[11] Graft contamination by tumor cells may be easily detected in patients with multiple myeloma. In an analysis of blood samples from 152 patients with multiple myeloma, phenotypically abnormal CD19+ cells were identified in a majority. These cells could be detected following treatment with conventional-dose therapy without any correlation between the tumor cell number and the paraprotein level; however, they rose in number in the setting of progressive disease. In eight patients for whom PCR analysis could be used to detect disease-specific rearrangement in the complementary determining region III (CDRIII) gene, clonal rearrangements were detected in the...
In a separate study, PCR analysis demonstrated contamination by CDRIII-rearranged cells in 90% of leukopheresis products from patients with multiple myeloma.[13] PCR studies have gone even further to permit quantification of tumor cell-contamination of HPC grafts. In a study that used semiquantitative PCR to assess HPC grafts from 14 patients with multiple myeloma, the median level of graft contamination by PCR-positive cells was $10^{-4}$ (range: $10^{-3}$ to $10^{-1}$).[14] There may be differential contamination between bone marrow and blood HPC. In 13 patients with multiple myeloma who underwent both bone marrow and chemotherapy/ granulocyte colony-stimulating factor (G-CSF, Neupogen)-mobilized blood HPC collection, the median percentage of clonal cells in blood HPC products was 0.0024% (range: < 0.0002% to 0.3520%). In bone marrow products, the median was 0.74% (range: 0.20% to 6.98%).[15] Similar analysis for rearrangements in bcl-2, bcl-1, immunoglobulin heavy-chain, and T-cell receptor genes commonly detects tumor cell contamination in HPC products from patients with NHL. PCR analysis for the presence of t(14;18) in 52 patients with NHL demonstrated that 65% of the patients had evidence of the rearrangement in either their blood or bone marrow prior to blood HPC collection. HPC products from these patients were PCR-positive in 29 of 52 patients (including 4 patients whose blood and bone marrow were PCR-negative).[13] Rearranged cells may also be detected in HPC products from patients with intermediate- and highgrade NHL. Among 20 such patients, PCR analysis detected an abnormal molecular marker in HPC products from 17 of 20 patients.[6] Quantitative PCR studies demonstrate that significant numbers of clonal cells may be present in the peripheral blood and HPC products of patients with NHL. In one study, 15 of 37 patients with diffuse large-cell NHL had PCR-detectable rearrangements of CDRIII. At the time of potential blood HPC collection, the level of peripheral blood tumor contamination ranged from $10^{-2}$ to < $10^{-5}$ (median: $10^{-2}$).[16] In a separate quantification study, 26 of 28 evaluable patients had evidence of bcl-2/IgH rearrangements in either blood or bone marrow at the time of HPC collection. The number of rearranged cells ranged from 1 to approximately $10^5$ rearranged cells per million mononuclear cells.[17] Graft contamination also occurs in patients with AML.[9] In a representative study, HPC products from all 15 patients with AML1/ETO-positive AML, were PCR-positive on the first day of autologous HPC collection. Of the 11 patients who underwent a second collection procedure, all products were PCR-positive.[7]

**Gene-Marking Studies**

In the absence of direct evidence that HPC contaminants might contribute to systemic relapse, the aforementioned data would be a mere curiosity. Gene-marking techniques using transfection of HPC grafts with the neomycin-resistance gene, however, provide direct evidence that contaminating tumor cells may contribute directly to systemic relapse. Brenner and colleagues showed that the neomycin-resistance marker could be detected in leukemic blasts at the time of relapse by PCR analysis in two children who had undergone prior autologous transplantation for AML.[18] Similar studies have demonstrated that the neomycin-resistance marker gene may be detected at the time of systemic relapse in bcr-abl-positive cells in patients who have undergone autologous transplantation for chronic myelogenous leukemia[19] and in neuroblasts in patients with neuroblastoma undergoing autologous HPC transplantation.[20] The primary shortcoming of these data is that they do not indicate the frequency with which graft contamination constitutes the principle source for systemic relapse. **Methods of Graft Purging In Vivo Purging**

Purging technologies attempt to eliminate tumor cell contamination of HPC through direct or indirect manipulation of the graft. Patients may be treated in vivo with systemic chemotherapy and/or monoclonal antibody (MoAb)-based therapies in the hope of reducing the whole-body and circulating tumor cell burden. Thus, in vivo purging methods attempt to both improve systemic control of the ma lignant disease and alter the kinetics of tumor cell mobilization. The paradigm for in vivo purging is derived from data suggesting that there may be differential hematopoietic recovery with preferential mobilization of normal vs malignant hematopoiesis following intensive chemotherapy. Carella and colleagues treated 30 consecutive chronic myelogenous leukemia patients with either ICE (idarubicin [Idamycin], 8 mg/m²/d for 5 days; cytarabine, 800 mg/m²/d for 5 days; and etoposide, 150 mg/m²/d for 3 days) or mini-ICE (the same agents, but with idarubicin and cytarabine administered for only 3 days) mobilization chemotherapy. Philadelphia chromosome-negative HPC products were collected in 22 patients.[21] Other groups have confirmed these results.

- **Rituximab**-The recent availability of therapeutic MoAbs provides another potential mechanism for in vivo purging. Rituximab (Rituxan)-a humanized, chimeric MoAb directed against the B-cell surface antigen CD20-has considerable antitumor activity in patients with B-cell NHL and may produce molecular remissions in some patients with bcl-2- positive follicular NHL when used in combination with the CHOP regimen (cyclophosphamide...
Cytoxan, Neosar], doxorubicin HCl, vincristine [Oncovin], prednisone).[22] Alone or in combination with systemic chemotherapy, this agent may reduce levels of circulating bcl-2-positive cells in patients with follicular NHL.[23] In a group of 23 patients with follicular NHL, rituximab was administered to 11 patients in standard doses 1 week and 2 days, respectively, prior to autologous HPC collection. By realtime PCR analysis of bcl-2 gene rearrangements, 6 of 10 HPC products collected from patients who received rituximab were PCR-negative. In contrast, only 1 of 9 products from patients mobilized without rituximab was PCR-negative.[24] In a separate study, 28 patients with mantle cell lymphoma underwent blood HPC collection after each of two cycles of mobilization therapy combined with rituximab. Real-time PCR analysis of products obtained from 17 patients after the first cycle (cyclophosphamide, 7 g/m², and rituximab, 375 mg/m²) demonstrated that 42% of products were PCR-negative for bcl-1 gene rearrangements. Analysis of 19 patients after the second cycle of therapy (cytarabine, 2 g/m² every 12 hours for 6 days, and rituximab, 375 mg/m²) demonstrated that all products were PCR-negative.[25] When mobilization kinetics from patients with NHL who receive rituximab as an in vivo purging regimen are compared to historical controls who did not receive rituximab, the median CD34+ cell yield, colonyforming unit-granulocyte/monocyte (CFU-GM) and burst-forming unit-erythrocyte (BFU-E) appear to be comparable.[26] Other investigators, however, find a trend toward poorer mobilization and engraftment kinetics in patients who receive rituximab.[27]

**Imatinib**—Novel agents such as imatinib mesylate (Gleevec) show promise as in vivo purging agents. In a phase II trial of autologous blood HPC collection in patients with chronic myelogenous leukemia, imatinib was administered prior to G-CSF-stimulated cell collection. All 10 patients achieved a complete cytogenetic response and bcr-abl rearranged metaphases were suppressed below the level of detection by fluorescence in situ hybridization (FISH). Nine patients achieved their collection goal, and HPC products from eight patients had a normal karyotype and negative FISH studies.[28]

**Ex Vivo Purging**

HPC grafts may also be manipulated ex vivo through laboratory procedures performed after collection. These ex vivo purging methods may be used to directly remove or destroy contaminating tumor cells (negative selection), or conversely, hematopoietic progenitors cells may be selected from the graft (positive selection) and the remainder of the graft (including tumor cells) discarded.

- **Chemotherapeutic Agents**—One of the best-studied negative-selection methods involves ex vivo incubation of the HPC graft with cytotoxic chemotherapeutic drugs such as the cyclophosphamide cogeners 4-hydroperoxycyclophosphamide (4-HC) and mafosfamide or with etoposide and corticosteroids. The former two drugs are analogous agents, with mafosfamide having been used more extensively in Europe and 4-HC having been the subject of considerable study in the United States (including use in over 700 patients at Johns Hopkins Medical Center). While 4-HC largely spares primitive hematopoietic stem cells, it produces dose-dependent toxicity against tumor cells and may reduce committed progenitors such as CFU-GM by ≥ 99%. 4-HC purging may therefore cause significantly delayed neutrophil engraftment times.[30] Because direct evidence of its effectiveness is limited and was never confirmed in a prospective randomized trial, the US Food and Drug Administration (FDA) chose not to license 4-HC.[29]

- **Monoclonal Antibodies**—Antibodies-MoAbs may be used for ex vivo purging. One method involves incubation of mononuclear cellenriched HPC product with one or more disease-appropriate MoAbs and exogenous (typically rabbit) complement. Use of a single MoAb and complement may achieve approximately 3 logs of tumor cell depletion, and use of multiple antibodies may result in up to 6 logs of depletion following three cycles of purging.[31] Ball and colleagues used a similar method for purging HPC in patients with AML. HPC are concomitantly incubated with the MoAbs PM-81 (anti-CD15) with or without the addition of AML-2-23 (anti-CD14) and complement. In 138 patients transplanted between 1984 and 1997, median engraftment times for neutrophils and platelets were comparable to those seen using 4-HC-or mafosfamidepurged grafts.[32] MoAbs directed against specific malignancy-associated surface proteins may also be conjugated directed to a magnetic particle or, following incubation with the target cell, may be subsequently bound by species-specific antibodies that are in turn conjugated to a magnetic particle. Bound cells may be thereafter removed by passage through a magnetic cell separator. In 38 patients
with B-cell NHL whose bone marrow products underwent two cycles of immunomagnetic separation, median CD34+ cell recovery was 57% (range: 38% to 80%), whereas CD19+ cells were reduced by a median of 1.8 logs (range: 0.1 to 4.7 logs). In a separate study, bone marrow and blood HPC grafts from 40 patients with bcr-abl-positive acute lymphoblastic leukemia were treated ex vivo with immunomagnetic bead separation. The median recovery of CD34+ cells from both HPC sources was comparable to that in the prior study. PCR analysis of HPC grafts following purging demonstrated a median bcr-abl depletion of 2.3 logs and 1 log, respectively. At the conclusion of purging, 0 of 19 bone marrow HPC grafts and 4 of 17 blood HPC grafts previously PCR-positive for bcr-abl became negative.

Alternative forms of MoAb-based negative selection include the use of MoAbs conjugated to immunotoxins. Other novel means of ex vivo negative-selection include photodynamic therapy, adenoviral vector-based methods of tumor cell cytolysis, antisense therapy, incubation with lymphokine-activated killer cells, and incubation with interleukin (IL)-2.

- **Cell Selection Devices**
  Positive selection alternatively exploits progenitor cell expression of the cell surface marker CD34. CD34+ cells may be bound by MoAbs that are conjugated to a magnetic bead and the bound cells captured by an immunomagnetic cell collection device such as the Baxter Isolex 300i or the Miltenyi Clini-MACS. CD34+ cells may be similarly bound by biotinylated MoAbs that may be captured on an avidin column; this was the basis for the CellPro CEPRATE device. Cells that do not express CD34 antigen are discarded. Alternatively, CD34+ cells may be sorted using ultra-high-speed fluorescence-activated cell sorting.

- **Clinical Trials Using Graft Purging Non-Hodgkin’s Lymphoma**
  Interest in graft purging was dramatically stimulated by the 1991 publication of a Dana-Farber Cancer Institute trial that evaluated the course of 114 patients with follicular NHL who underwent assessment for graft contamination by malignant cells prior to autologous transplantation. PCR analysis detected disease-specific gene rearrangements in bone marrow samples from all patients prior to bone marrow purging. Bone marrow HPC from patients was purged with three cycles of incubation with MoAbs and rabbit complement. Bone marrow HPC from 57 patients remained PCR-positive for clonal gene rearrangements following purging, whereas products from 57 patients were rendered PCR-negative. Patients underwent autologous transplantation following a preparative regimen that consisted of total-body irradiation and cyclophosphamide (60 mg/kg IV daily for 2 days). At a median follow-up of 23 months, 53 of 57 patients who received PCR-negative products remained alive and free of disease, compared with only 21 of 57 patients who received PCR-positive products. The difference between groups was statistically significant. This effect was seen in patients transplanted in complete and partial remission. Using a Cox proportional-hazards regression analysis, the risk of relapse was 9.9 times greater for patients who received PCR-positive products. These data were updated in a 1999 review of outcomes for 153 patients with follicular NHL in first relapse or incomplete initial remission who underwent transplantation with the aforementioned regimen. The projected disease-free survival at 8 years was 47%, and the overall survival was 66%. A subgroup of 113 patients was evaluated by PCR for evidence of bcr-abl rearrangements. The projected disease-free survival at 8 years was 89% for patients who received PCR-negative products vs 19% for patients who received PCR-positive products. This difference was statistically significant, and in a univariate analysis, use of PCR-negative HPCs was associated with a worse rate of freedom from relapse.

- **GITMO and CUP Trials**
  Trials-In the phase II Gruppo Italiano Trapianto Midollo Osseo (GITMO) study, 92 patients with previously untreated advanced-stage follicular NHL underwent autologous HPC transplantation following intensive, chemotherapy-based in vivo purging. Patients were treated with three cycles of anthracycline-based therapy, and those who failed to achieve a complete remission received two cycles of platinum-based therapy. Patients subsequently received...
consecutive cycles of therapy with etoposide (2 g/m²), methotrexate (8 g/m²), and cyclophosphamide (7 g/m²). G-CSF-mobilized blood HPCs were collected upon recovery from cyclophosphamide. Of 126 blood HPC products collected from patients with a clonal molecular marker, 59 were PCR-negative. Although 18 of 20 patients who received PCR-negative products were in continuing complete remission at the time of the study’s publication, only 9 of 22 patients who received PCR-positive products remained in complete remission at that time. The difference between subgroups was statistically significant.[45] The European multicenter CUP (Chemotherapy, Unpurged, or Purged stem cell transplantation) trial is the sole phase III trial that examines ex vivo graft purging in patients with follicular NHL. Eighty-nine eligible patients who had achieved at least a partial remission with chemotherapy were randomized to receive either three cycles of conventional-dose chemotherapy (n = 24), transplantation with unpurged bone marrow/blood HPC (n = 33), or transplantation with purged HPC products (n = 32). Purging was performed using a combination of MoAb and complement. The transplant preparative regimen consisted of total-body irradiation and cyclophosphamide. Unfortunately, the trial was closed due to poor accrual prior to achieving the goal of 100 patients per treatment arm. At a median of 26 months following randomization, progression-free survival in the two transplant arms was superior to that in the conventional-dose therapy arm, but differences between the two transplant arms were not statistically different.[46]*

**Single-Institution Data**—There are no prospective randomized data evaluating HPC purging in patients with aggressive NHL. Fouillard and colleagues reported their single-institution experience using purged HPC transplants for patients with NHL. Sixty-four of 120 patients had aggressive NHL. Patients received either unpurged (n = 21) or purged (n = 43) bone marrow or blood HPC grafts. Grafts were purged with either mafosfamide (fixed or individually tailored doses), CD34 selection using the CEPRATE SC, or immunotoxin-based methods. Results of analysis suggest that patients whose grafts were more intensively purged with mafosfamide had a superior outcome.[47] In contrast, 20 patients with aggressive NHL who received MoAb-based immunomagnetic bead purged autologous HPC transplants were retrospectively compared with 18 similar patients who received unpurged grafts. The investigators found no difference in outcome between patient groups to suggest a benefit to purging.[33]

**Lack of Firm Conclusions**—Data from the Dana-Farber group and GITMO provide perhaps the best evidence justifying the use of purging in autologous transplantation. Both trials suggest that patients with PCR-positive vs PCR-negative HPC collections may have divergent outcomes. In both of these trials, however, it is unclear whether this finding reflects an intrinsic effect of the purging process or differences in the disease biology between patients whose HPC may be purged below the limits of detection and those in whom this is not the case. The absolute significance of HPC purging in relapse prevention seems to be further undercut by the pattern of relapse following transplantation. In a group of 99 patients who underwent purged HPC transplantation for follicular NHL, 26 of 33 patients relapsed only at sites of prior disease. This finding suggests that resistant systemic disease rather than infused tumor cells is the primary source for systemic failure in follicular NHL.[48] Ultimately, the impact of biologic variation on the capacity to purge to the point of PCR negativity and the clinical impact of HPC purging can only be determined in a sufficiently powered phase III trial. Unfortunately, the interim results from the sole phase III trial do not support the use of purging, and the trial is probably insufficiently powered to provide a definitive answer. There are no global consensus recommendations regarding the use of purged graft transplants in patients with follicular NHL. However, when an American Society for Blood and Marrow Transplantation (ASBMT) expert panel reviewed the role of HPC transplantation in patients with diffuse large B-cell lymphoma, purging was judged to be an "inadequately evaluated treatment and recommended for comparative study."[49]

**Myeloma**

In 1993, the Dana-Farber group reported on 40 chemotherapy-sensitive patients who received bone marrow HPC grafts for multiple myeloma.[50] Twenty-six received grafts purged by three cycles of treatment with monoclonal antibodies (anti-CD10, anti-CD20, and anti-PCA-1) and rabbit complement. The preparative regimen consisted of either melphalan (Alkeran, 70 mg/m² IV for 2 consecutive days) or total-body irradiation and cyclophosphamide. The median times to neutrophil recovery to 500/µL and to untransfused platelet counts greater than 25,000 were 23 and 25 days, respectively. One patient died on day 67 without evidence of engraftment. A second patient died without platelet engraftment 6 months posttransplant. Eleven patients achieved a complete remission, and 14 achieved a partial remission. At a median follow-up of 24 months, 21 patients remained alive. The median progression-free survival was 36 months. In an expanded update of these data published 2 years later, of 36 patients who underwent autologous bone marrow HPC transplants, 2 of 36 remained alive. This finding suggests that purging may be ineffective in prolonging progression-free survival.
transplantation, 14 had no evidence of progression at a median of 18 months posttransplant and 11 patients remained in complete remission at a median of 16 months posttransplant.[51] Neither study compared outcomes to those of any control group.

- **Cell-Selection Trials**-Trials-In a series of publications commencing in 1998, a multicenter group examined CD34+ selection of blood HPC grafts in patients with multiple myeloma who underwent autologous blood HPC transplantation. Prior to autologous transplantation, patients were mobilized with cyclophosphamide (2.5 g/m^2 IV), prednisone (2 mg/kg/d orally for 4 consecutive days), and G-CSF (10 μg/kg/d administered as subcutaneous injection). CD34+ cell selection was performed on blood HPC ex vivo using the CellPro SEPRATE SC column. The transplant preparative regimen consisted of busulfan (Myleran, 0.875 mg/kg orally every 6 hours for 16 doses) and cyclophosphamide (60 mg/kg IV for 2 consecutive days). In the initial report on 55 nonrandomized patients, the median progression-free survival was 14 months, whereas the 3-year actuarial progression-free survival was 29% ± 14% and the estimated overall survival was 47% ± 17%. Six toxic deaths were reported.[52]

This research group proceeded to a phase III randomized trial that included 131 patients, who received the mobilization and preparative regimens indicated above.[53] Prior to mobilization, patients were randomized to receive either unmanipulated grafts or grafts purged using CD34+ cell enrichment. The primary end point of the trial was safety; secondary end points included the level of graft contamination, infusion-related toxicities, engraftment times, and progression-free survival. A total of 67 patients were randomized to receive selected grafts, and 64 patients were randomized to receive unmanipulated grafts. Median CD34 purity in the selected grafts was 66.4%. Among the 28 patients in the CD34+ selection arm for whom PCR analysis for tumor-specific gene rearrangement could be performed, 4 had PCR-negative HPC products prior to the selection process. The selection procedure reduced the level of tumor cell contamination by a median of 3.10 logs (range: 1.5 to 6.02 logs). The investigators found statistically significant differences in neutrophil engraftment, platelet engraftment, days to platelet recovery, and the number of platelet transfusions between the two groups, with slight delays in graft recovery for the CD34+ cell-selected group. Also among patients receiving CD34+-selected grafts, peripheral blood CD4+ recovery was significantly slower through 6 months posttransplant and inversion in the CD4+/CD8+ ratio was greater through 1 year posttransplant. Actuarial estimates of progression-free and overall survival at 1 year posttransplant were equal between groups. In 2001, the group updated the results for all 190 patients who were eventually treated.[54]

Despite evidence that the CD34+ selection could deplete tumor cell contamination a median of 3.1-fold and produce products that in 54% of the analyzed patients were PCR-negative, at a median follow-up of 37 months there was no difference in actuarial overall survival (median of 202 weeks vs median not yet achieved) or progression-free survival (100 vs 104 weeks) between patients receiving selected or unmanipulated grafts. Although others have explored alternative cell-selection methods such as high-speed flow cytometry, these methods are limited by issues of delayed engraftment and have yet to be tested in a phase III trial.[55] In a recent, evidence-based review on the role of HPC transplantation in patients with multiple myeloma, the expert committee found that the use of CD34+-selected and blood HPC unmanipulated grafts were “equivalent treatments (no statistical or clinical difference between therapies).” The group also found that the use of autologous purged bone marrow HPC grafts was “not an effective treatment.”[56]

**Acute Myelogenous Leukemia**

No prospective, randomized trials have compared outcomes using purged vs unpurged HPC grafts in patients with AML. In a recent review on outcomes in patients undergoing autologous transplantation for AML, among the nine studies that included patients in first complete remission who received unpurged bone marrow grafts, leukemia-free survival ranged from 34% to 70.6% and the relapse risk ranged from 25.5% to 54%. In the 9 studies performed in patients in first complete remission using purged bone marrow, leukemia-free survival ranged from 41% to 80%, and relapse risk, from 29% to 49%.[57]

- **Retrospective Analyses of Registry Data**-The evidence supporting a benefit of purged bone marrow HPC grafts is largely garnered from retrospective comparisons of subgroups of patients evaluated using registry data or from single-institution, phase II studies. In a review
of autologous bone marrow transplantation data from the European Group for Blood and Marrow Transplantation (EBMT), the outcome for 69 patients who received purged grafts for AML in first complete remission was compared to that of 194 patients who received unmanipulated grafts.[58] Transplantation was performed at 34 different centers utilizing either total-body irradiation (n = 131) or non-total-body irradiation- based (n = 132) preparative regimens. All purging was performed using ex vivo incubation with mafosfamide- either a fixed dose of mafosfamide (n = 39) or a dose individually tailored to CFU-GM (n = 30). Overall leukemia-free survival for all patients was 39% ± 3%. The actuarial relapse risk at 4 years for patients who received purged marrow was 40% ± 6%, compared with 59% ± 4% for those who received unpurged grafts. This difference was statistically significant in a univariate analysis but failed to achieve statistical significance in a multivariate analysis. The relapse risk was lowest for the subgroup of patients who received products purged using tailored doses of mafosfamide (17% ± 7%)-a statistically significant difference from that seen in patients receiving unpurged grafts under both univariate and multivariate analysis.

Patients who received purged products had significantly delayed engraftment to neutrophils and a higher risk of both bacterial and viral infections. In comparative analysis of 1,393 patients in the EBMT registry who underwent autologous transplantation for AML in first complete remission, the 252 patients who received purged bone marrow HPC grafts produced a superior leukemia-free survival and relapse risk to that seen in the 100 patients receiving blood HPC grafts and the 1,041 who received unpurged bone marrow HPC grafts.[59] The 2-year actuarial leukemia-free survival and relapse risk for patients who received purged bone marrow grafts were 57% ± 3% and 37% ± 3%, compared with 49% ± 2% and 45% ± 2%, respectively, for patients who received unpurged bone marrow HPC grafts. Differences in the interval from initial complete remission to transplantation and use of total-body irradiation- based regimens favored the purged bone marrow group. Thus, the true significance of these findings is unclear.

**Single-Institution Data** Data-In an extended, single-institution study of 4-HC-purged autologous bone marrow transplantation for patients with AML, 50 patients in first complete remission and 25 patients in subsequent remission underwent transplantation using busulfan (1 mg/kg orally every 6 hours for 16 doses) and etoposide (60 mg/kg IV). Bone marrow grafts were purged with 4-HC (100 ?g/mL). At a median follow-up of 6.8 years, the disease-free survival and relapse risk were 70% ± 12% and 27% ± 12% for the group of patients in first complete remission. In the group of 25 patients in second or third remission, disease-free survival was an extraordinary 52% ± 20%.[60] In a subsequent publication, event-free survival for the 50 patients who underwent transplant in first complete remission was maintained at 68% at a median of 11.2 years posttransplant.[61] In the City of Hope experience, 60 patients with AML in first complete remission were enrolled at the time of their first cycle of consolidative therapy in a trial investigating autologous transplantation. Bone marrow HPC grafts were collected following recovery from intensive, high-dose cytarabine-based consolidation therapy, to exploit the in vivo purging presumed to occur after such intensive consolidation.[62] Approximately 23% of patients had a favorable karyotype, whereas at least 48% had intermediate- or unfavorable-risk cytogenetics. Sixteen patients did not proceed to transplant due to death, toxicity, or relapse. The transplant preparative regimen consisted of total-body irradiation combined with etoposide (60 mg/kg) and cyclophosphamide (75 mg/kg of ideal body weight). At a median follow-up of 37 months, analyzed on an intention-to-treat basis, the actuarial disease-free survival was 49%. Among patients who actually underwent transplantation, the actuarial disease-free survival was 61%. In an update published in 1999, longterm follow-up of these data showed that the disease-free survival curve remains stable.[63] In the last two studies cited above, with patient groups similar in composition, the extended follow-up outcomes for transplantation using highly manipulated and unmanipulated bone marrow HPC appear to be comparable. Taken as a whole, the outcome ranges for patients who receive autologous transplantation using purged vs unpurged HPC grafts seem almost superimposable. Although some of the subgroup comparisons suggest that graft purging may affect outcome in AML, without a randomized trial it is impossible to determine whether these apparent differences are due to selection bias, differences in disease biology between subgroups, or a true impact of purging.

**Conclusions** Given promising phase I and II findings, the concept of graft purging is seductive: It offers the possibility of transplanting a patient with a product that has been rendered devoid of
contaminating tumor cells. Unfortunately there is a tremendous gulf between this ideal and the reality of the clinical experience with graft purging. If purging could be performed without incurring any costs, inconvenience, or compromise in graft function, there would likely be limited resistance to its more generalized use. Enthusiasm for the theoretical benefits of graft purging, however, must be tempered by the genuine problems incurred by purging. **Ex Vivo Purging**

Potential problems appear to be the greatest for ex vivo purging methods and include the potential for CD34+ cell loss during processing, the risk of HPC infection by xenobiologic agents (especially with the use of animal-derived reagents), the expense of specialized equipment and technical training for mechanical methods of cell sorting or separation, and the need for adequate safety and validation procedures prior to clinical implementation. Ex vivo HPC manipulation may also carry small but incrementally increased risks for transplant recipients. Patients who receive 4-HC or ex vivo chemotherapy-purged bone marrow transplants may experience significant delays in engraftment. Patients who receive CD34+-selected grafts may experience reductions in CD4+ cell numbers, have a protracted inversion in the CD4+ / CD8+ ratio, and perhaps have increased transfusion requirements. New and evolving FDA guidelines regarding HPC products are likely to impose further limitations upon the use of ex vivo purging. In the future, products that undergo significant ex vivo manipulation will need to be "manufactured" in facilities that are designed and run according to the requirements of current Good Manufacturing Practices standards. These requirements will likely limit the ability to study ex vivo methods of HPC manipulation to a few large, specialized centers with the resources and expertise to build and maintain appropriate facilities. This will make it even less likely that purging will be studied in the fashion necessary to ultimately answer questions regarding its effectiveness. **In Vivo Purging**

Conceptually, in vivo purging methods seem to pose fewer a priori obstacles to further study. Inasmuch as in vivo purging may produce superior systemic control of a malignant disease, the purging process may directly benefit the patient. Evolving data regarding the use of rituximab as a means of in vivo purging illustrate the promise of this approach. The few available trials indicate that this drug may produce less contaminated HPC products with preservation of graft function. More significantly, such an approach is much more amenable to investigation in large, prospective trials. If in vivo purging is performed using FDA-approved drugs in a standardized clinical fashion, it may allow examination of this approach in a rigorous, statistically powerful method. If the strategy proves effective, it would also allow for ease of generalization. **Future Directions**

Autologous HPC transplantation is a powerful therapeutic tool that represents the standard of care for select patients with NHL, multiple myeloma, Hodgkin's disease, acute leukemia, and various other malignant diseases. HPC transplantation is unique in its ability to cytoreduce patients to a state of minimal residual disease. Posttransplant consolidation therapy is an approach that exploits the minimal residual disease state following autologous transplantation as an opportunity to eradicate any residual clonogenic tumor cells (including those infused with the graft). In effect, it may act as a posttransplant in vivo "purge." Potential posttransplant approaches include the use of biologic response modulators such as IL-2, monoclonal antibody therapy utilizing agents such as rituximab, adoptive immunotherapy with antigen-specific cytotoxic T cells or cytokine-induced killer cells, dendritic cell vaccines, posttransplant chemotherapy, or even allogeneic transplantation. Indeed, reduced-intensity allogeneic transplantation may allow extension of the graft-vs-tumor effect to patients who have undergone previous autologous transplantation while minimizing the previously limiting toxicities associated with a fully myeloablative procedure. Reduced-intensity allogeneic transplantation may be used as part of a tandem transplant process in which the initial autologous transplant serves to debulk the patient's disease while the subsequent allogeneic transplant provides a potent form of consolidative immunotherapy. This approach has been piloted in patients with high-risk Hodgkin's disease and is the basis for an ongoing trial in patients with multiple myeloma. Although a rational and compelling case can be argued on behalf of graft purging, given the limited number of mature studies, the case for routine graft purging remains largely unproven. Regulation and infrastructural requirements are likely to make it much more difficult in the future to study ex vivo graft purging. In vivo graft purging is likely to become more common, but at present, its clinical significance is still questionable. Until purging is rigorously and adequately studied in a prospective, randomized fashion, there are not enough data to justify routine purging of HPC grafts. Based upon the intriguing phase I and II data garnered to date, both in vivo and ex vivo purging should be explored further in welldesigned clinical trials.

**Disclosures:** Dr. Alvarnas is a member of the speaker's bureau for Pfizer (Vfend), and is a speaker for ESP Pharma.
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