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Editorial

Should we purge?

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Summary:

Relapse due to either residual host disease or reinfused tumor cells remains the principal cause of treatment failure after autologous stem cell transplantation. Although it is intuitively attractive to remove putative tumor cells from autologous grafts prior to transplant and more than 1000 articles have been written on the subject, there are only limited data suggesting that purging autografts has any favorable effect on relapses or disease-free survival. Certain purging techniques that remove substantial numbers of T cells or destroy progenitor cells may have adverse effects such as delayed hematopoietic or T cell reconstitution. There is a critical need for large, well-designed trials that specifically address the value of a particular purging technique on relapses and disease-free survival after autologous stem cell transplant.

Keywords: purging; autologous stem cell transplant; CD34 selection; multiple myeloma

In this issue of *Bone Marrow Transplantation*, Schiller and coworkers report data on engraftment, response rate and outcome for 55 people with drug-sensitive multiple myeloma receiving high-dose therapy followed by blood cell autotransplants of CD34-positive enriched cells (Ceprate; CellPro, Bothell, WA, USA). This study adds 18 new subjects and added follow-up of a previous report.[1] Engraftment was satisfactory in most people in the current study; however, eight subjects (15%) receiving $<2\times10^6$ $CD34^+$ cells/kg had significantly delayed platelet recovery and increased platelet transfusions compared to the 47 subjects receiving $>2\times10^6$ CD34-positive cells/kg. Response rate was 72%, but only 8% of patients had a complete response. Three-year progression-free survival of 29% is similar to reports of unmodified blood cell autotransplants.[2–5]

CD34-positive cell-enrichment was used to remove possible contaminating myeloma cells from the graft; these cells are presumed to contribute to relapse in people with multiple myeloma. CD34-positive cell-enrichment using the Ceprate column is the only FDA licensed technology in the USA for removing cancer cells from autografts; however licensing is based on reduced toxicity rather than efficacy. CD34-positive cell-enrichment is also used to decrease cancer cell contamination of autografts in women with breast cancer,[6–8] and people with non-Hodgkin lymphoma.[9] This technique results in the loss of about half of CD34-positive cells processed and a 2–3 log depletion of T and B cells[10] and cancer cells.[1,6] Loss of CD34-positive cells is usually compensated for by increasing numbers of CD34-positive cells collected. Data of immune recovery after transplants of CD34-positive cells are not reported. This could be important since, in addition to removing T and B cells, monocytes ($CD14^+$), natural-killer cells ($CD56^+$) and T helper ($CD4^+$) cells are also lost; these losses may delay immune recovery. The complete remission rate of 8% reported by Schiller and coworkers is lower than most other reports of autotransplants in multiple myeloma using unmodified grafts;[3–5] could this be from loss of immune cells or some other adverse effect of CD34-positive cell selection?

There is an on-going US randomized trial evaluating CD34-positive cell-enriched autotransplants in people with newly diagnosed multiple myeloma. This study is premature, however, since less than one-half of trial entrants will achieve a complete remission post-transplant.[3,11,12] Consequently, it will be difficult, if not impossible, to assess the benefit of removing myeloma cells from the graft in the one-half of people with less than complete remission who have residual myeloma. However, adverse effects of CD34-positive cell selection might be detected. Such an adverse outcome is not implausible; preliminary analysis of a randomized trial of conventional *vs* CD34-positive cell-enriched autotransplants in women with metastatic breast cancer showed worse event-free survival in the CD34-positive cell-enriched cohort.[8]

Blood cell autotransplants are increasingly used to treat cancer. Relapse after autotransplants usually result from residual cancer in the subject. Cancer cells infused with the autograft may also contribute to relapse. Since it is easier to deplete the graft than the subject of cancer cells, intense research activity is focused on new and better purging techniques. More than 1000 articles written about purging have been published since 1980.

Other techniques to remove cancer cells from autografts include chemicals and drugs (like 4-hydroperoxycyclophosphamide[13] and mafosphamide[14]) and anti-cancer antibodies that fix complement,[15] activate natural-killer cells or are linked to magnetic beads.[16] Antibody-related techniques are not associated with adverse outcomes. Drug and chemical

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techniques, in contrast, are associated with substantial loss of stem and committed progenitor cells resulting in delayed bone marrow recovery, especially platelet recovery.[13,14] Other less widely evaluated techniques include: CD34-positive cell enrichment using magnetic beads (Baxter Laboratories, Santa Ana, CA, USA), high-speed cell sorting (Systemix, Palo Alto, CA, USA), physical separation (density gradient), *in vitro* cultivation or cell expansion and incubation with anti-sense cDNA.

Despite intense research, there are few convincing data that infused cancer cells in the grafts cause relapses after autotransplants or that selection or purging techniques improve transplant outcome. These techniques are labor-intensive, potentially delay bone marrow recovery and increase cost. Consequently, it is important to determine if purging increases survival in randomized trials rather than using removal of cancer cells from the autograft as a surrogate.

Data from animal models clearly show that infusing cancer cells can cause cancer. Important variables include type of cancer, genetics and immune state of the recipient. Animal studies by Hagenbeek and coworkers,[17] attempting to mimic autotransplants in acute myelogenous leukemia (AML) in humans, concluded that $<10\%$ of relapses were from the graft and the remainder from residual leukemia cells in the recipient. These studies predicted that purging would be relatively ineffective in improving autotransplant outcomes in AML.

Gene marking studies in humans indicate that cancer cells from the graft can contribute to relapse after autotransplants for AML, neuroblastoma and chronic myelogenous leukemia.[18,19] Patients with a high likelihood of cancer in the graft, however, also have a high likelihood of residual cancer in them even after grafts from normal donors.

Other diseases

Several purging techniques are used in people with lymphoma.[9,15,20,21] There are no randomized trials of purging of autografts. The European Bone Marrow Transplant Group analyzed 448 patients with non-Hodgkin lymphoma receiving purged or unpurged bone marrow autotransplants.[21] Purging techniques included monoclonal antibodies with complement or magnetic beads and drugs. These analyses showed no decrease in relapse or increase in EFS for patients receiving purged grafts. These data suggest that either infusing lymphoma cells had no impact or that purging techniques were ineffective (or both).

There are few if any sensitive techniques to detect residual cancer in most people with non-Hodgkin lymphoma. Most purging studies evaluated efficacy indirectly by efficiency of removing T or B cells.[15] Polymerase chain reaction (PCR) analysis is used to evaluate efficacy of purging in people with specific cytogenetic abnormalities like the t(14;18) translocation in which bcl-2 is rearranged.[20] Detection of this rearrangement in bone marrow or blood is not, however, invariably associated with relapse.[22,23] Additionally, detecting bcl-2 rearrangement in bone marrow is more predictive of transplant outcome than detecting it in blood.[24,25]

The most widely cited data supporting efficacy of purging are those of Gribben and coworkers[20] who reported that autotransplant recipients with low-grade non-Hodgkin lymphoma whose bone marrow graft was treated with anti-B cell monoclonal antibodies and complement and who had a negative PCR for bcl-2 rearrangement had a 20% probability of relapse compared to $>85\%$ relapses in people with a positive PCR test after *in vitro* treatment. These data might mean that infusing of lymphoma cells causes relapses. However, it is also possible that people with a negative PCR have less lymphoma which may be more sensitive to drugs and radiation than people with more lymphoma. Also, unclear is whether outcome for all people receiving purged grafts was better than would have been expected following infusion of unpurged grafts.[25]

In breast cancer, bone marrow involvement by cancer cells correlates with outcome in all stages regardless of treatment.[26,27] Therefore, data that patients receiving autografts containing cancer cells have a worse outcome is not surprising.[26,27] This makes evaluating the contribution of cancer cells in the autograft difficult and points out the fallacy of using *in vitro* assays of efficacy of cancer cell removal from the graft as a surrogate for clinical effectiveness.

In AML, purging with 4-hydroperoxycyclosphosphamide or mafosphamide was evaluated in phase-2 trials; no randomized trials are reported.[13,14] Two retrospective analyses suggest 10–15% fewer relapses after purged *vs* unpurged autotransplants.[14,28] Purging in AML does not invariably translate to better survival because of increased treatment-related mortality.[13] A recent application to the FDA for licensing of 4-hydroperoxycyclosphosphamide was rejected.

Randomized trials

There are few randomized trials of purging. There are several reasons for this but the major one is the large number of subjects needed to determine efficacy. As long as most relapses result from residual cancer in the subject, definitive purging studies will be difficult, if not impossible, to perform. This problem is confounded by the fact that cancer cells in the graft do not invariably cause relapses.[22]

Cost

Costs for purging using the Ceprate device is about $7000 and double if more than two aphereses are required to achieve the desired dose of CD34-positive cells. CD34-positive cell-enrichment with the Ceprate device recovers approximately one-half of CD34-positive cells.[6–9] In not extensively pretreated subjects this may not be a problem since blood CD34-positive cell yields are usually high. Unfortunately, people with the highest likelihood of cancer contamination of their autograft may also be those with low CD34-positive cell yields in which more than two aphereses may be needed.

Post-transplant platelet recovery is directly correlated with CD34-positive cell dose.[29–31] A dose of $\geqslant 5 \times 10^6$ CD34-positive cells/kg is needed to assure recovery of platelets to $>20 \times 10^9$/l within 2 weeks in about 90% of subjects. At

lower CD34-positive cell doses, many patients have delayed platelet recovery. A single added day of platelet transfusions costs about $1300. Thus, the lower CD34-positive cell dose unavoidably infused after CD34-positive cell-enrichment could add substantial cost. These high costs require documentation of the clinical efficacy of purging. Although techniques to remove cancer cells from autografts are intuitively attractive, there is no proof of clinical benefit and a distinct possibility of harm. Surrogate markers of purging efficacy are inadequate; randomized trials are needed.

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