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Strategies for purging CD96$^+$ stem cells in vitro and in vivo

New avenues for autologous stem cell transplantation in acute myeloid leukemia

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The persistence of leukemic stem cells (LSCs) in acute myeloid leukemia (AML) patients receiving chemotherapy may be responsible for the high frequency of relapse. The selective elimination of CD96$^+$ AML-LSCs by means of CD96-specific monoclonal antibodies may be a promising therapeutic approach and revitalize autologous hematopoietic progenitor cell transplantation.

Acute myeloid leukemia (AML) is a clonal disorder preferentially affecting the blood and bone marrow and characterized by the accumulation of abnormally differentiated myeloid cells. Despite the existence of a variety of AML subgroups, AML seems to be organized hierarchically in at least three compartments: AML leukemic stem cells (LSCs), progenitor cells and differentiated blast cells.[1] In contrast to AML blasts, which are characterized by a limited proliferative potential, AML-LSCs and progenitor cells display a high self-renewal capability and are able to initiate AML in NOD/SCID mice.[2]

Standard chemotherapeutic agents that target highly proliferating cells effectively eliminate AML blasts. However, a frequent rate of relapse is observed among AML patients treated with chemotherapy, indicating that residual leukemia initiating cells, perhaps AML-LSC, are less significantly affected. This may be due to the quiescent nature of AML-LSCs, similar to that of the normal stem cell compartment.[2]

In contrast to cytotoxic agents, innate and adaptive immune responses seem to efficiently target AML repopulating cells. Alloreactive donor-derived effector cells promote a graft vs. leukemia (GVL) effect in subjects who have undergone allogeneic stem cell transplantation, the most curative therapeutic approach for high-risk AML patients. Unfortunately, in contrast to donor-derived natural killer (NK) cells, alloreactive T cells not only eradicate malignant cells but may also promote graft vs. host disease (GvHD). Due to the treatment related mortality, the clinical outcome of AML patients subjected to the allogeneic transplantation of hematopoietic progenitor cells (HPCs) is limited as compared that of patients who received autologous HPCs.[3] The transplantation of autologous HPCs would be an option for low-risk AML patients or for subjects lacking a suitable allogeneic HPC donor, in particular if residual AML cells could selectively be removed from the graft to avoid relapse.[4] In addition, irrespective of whether autologous or allogeneic hematopoietic progenitor cell transplantation is considered, the selective eradication of residual AML-LSCs in vivo appears as a promising strategy to generally reduce the risk of relapse.

Monoclonal antibodies are potent and effective agents for the targeted therapy of human cancers. Given their antigenic specificity, monoclonal antibodies are the most powerful tool to selectively target a single cell type within mixed cell populations. Thus, immunomagnetic cell separation is a standard process for the manipulation of leukapheresis products ex vivo, e.g., for the enrichment of healthy HPCs upon the selection of CD34$^+$ cells. Moreover, monoclonal antibodies used in vivo may selectively recruit NK cells to lyse target cells by antibody-dependent cellular cytotoxicity (ADCC).[5]

In this context, the discrimination of normal HSCs and residual AML-LSCs is a stringent prerequisite. While both AML-LSCs and HSCs are characterized by the surface expression of CD34 and the lack of CD38,[1] other antigens including CD33, CD44, CD123, CD47 and CLL-1 seem to be preferentially expressed by AML-LSCs.[6] The potential use of monoclonal antibodies specific for these markers is limited by their expression on other cell types, which may result in very severe side effects. CD96 (TACTILE) has originally been detected on AML-LSCs, and its expression was confirmed on the majority of AML blasts in 30% of patients.[7,8] Importantly, the expression of CD96 on healthy HSCs is low or

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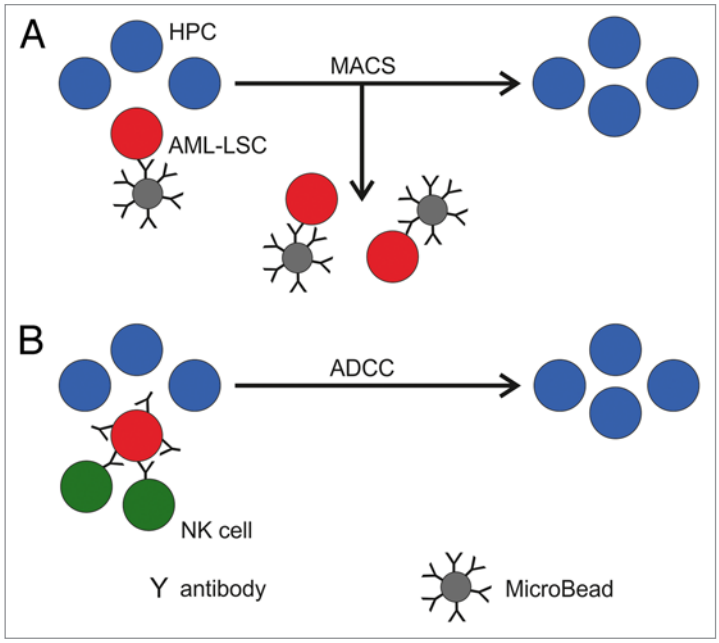

Figure 1. Strategies to purge acute myeloid leukemia stem cells in autologous stem cell grafts and patients. (**A**) The depletion of acute myeloid leukemia (AML) leukemic stem cells (LSCs, red) from autologous hematopoietic progenitor cells (HPCs, blue) grafts in vitro may be achieved by means of a MicroBead-coupled antibody targeting an AML stem cell antigen and the magnetic-activated cell sorting (MACS) technology. (**B**) Chimeric antibodies carrying a human Fc portion and binding to an AML-specific antigen may recruit autologous or allogeneic natural killer (NK) cells in patients and trigger the elimination of AML-LSCs via antibody-dependent cellular cytotoxicity (ADCC).

absent. In addition, CD96 is expressed by activated T and NK cells, where it may be involved in the adhesion between effector and tumor cells.[9] Although the physiological functions of CD96 on AML-LSCs are actually unknown, it may contribute to their adhesion to the bone marrow compartment. On the basis of the CD96 expression pattern and properties, we selected CD96 for the development of antibody-based strategies for the depletion of AML-LSCs from autologous HPC grafts ex vivo and for the elimination of $CD96^+$ cells in patients (**Fig. 1**).[10]

The mouse monoclonal antibody TH111 targeting CD96 was generated in our lab and a good manufacturing practice (GMP)-compliant protocol was developed for purging AML-LSCs from autologous HPC grafts. In combination with anti-mouse Fc MicroBeads, a more than 2-log depletion of $CD96^+$ target cells from leukapheresis products and bone marrow aspirates spiked with 1–10% of myeloid KG1a cells was achieved by magnetic-activated cell sorting (MACS). Cytofluorometric analyses and colony-forming unit (CFU) proliferation assays indicated that the total amount, viability and differentiation properties of healthy HPCs were not affected by this procedure.

To render AML-LSCs susceptible to ADCC in vivo, recombinant CD96-targeting antibodies were generated. As a CD96-binding domain, these molecules contain a single chain variable fragment (scFv) that was obtained by fusing the variable regions of the parental TH111 antibody. To enable the recruitment of human cytotoxic effectors by Fc receptor engagement, a part of the Fc of a human IgG1 optimized for the binding to Fcγ receptor IIIa (CD16a) by protein engineering was genetically fused to the CD96-specific scFv. The affinity maturation of the scFv by random mutagenesis coupled to a stringent selection procedure based on phage display led not only to a 4-fold enhanced avidity of the CD96-specific engineered antibody but also to a 2.3-fold improvement in its capacity to induce the lysis of $CD96^+$ target cells.

The engineering of HPC grafts by means of a CD96-specific antibody as well as its use in the clinics have the potential to improve the tolerability and efficacy of therapy in patients suffering from AML.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest have been disclosed.

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