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Monoclonal antibodies to the myeloid stem cells: therapeutic implications of CMA-676, a humanized anti-CD33 antibody calicheamicin conjugate

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There are several competing models of stem cell involvement in acute myeloid leukemia (AML). At issue is whether the disease origin is in the pluripotent stem cell or whether it arises later in a more mature progenitor cell. The observation that the CD33 antigen is present on AML cells, and on normal and leukemic progenitors, suggested that one might be able to target these cells while sparing the normal stem cells. Response rates of acute myelogenous leukemia patients treated with the newly developed anti-CD33 antibody-calicheamicin conjugate suggest that at least for a proportion of patients early precursors responsible for re-establishing hematopoiesis are likely to be predominantly normal in origin. *Leukemia* (2000) **14,** 474–475.
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In the past few decades, there has been great interest in defining antigens on the surface of normal and malignant myeloid stem cells and their progeny. In early hematopoiesis, a pluripotent stem cell is thought to give rise to precursors committed to the various myeloid lineages, including precursors of granulocytes, monocytes, erythrocytes, and platelets. These early precursors, including stem cells, are marked by the CD34 antigen. The CD33 antigen, which is on progenitors and committed precursors, but not on stem cells, is also on virtually all acute myelogenous leukemia (AML) cells. This observation raised the possibility of whether CD33 leukemic cells could be targeted and then deleted along with the leukemic cell precursors, as well as normal CD33-bearing cells, while sparing the normal stem cells.

Research on which lineages are involved in the leukemic process in patients with AML was studied by evaluating females heterozygous for the X-linked gene, *G6PD*.[1–3] In normal cells, about half the cells expressed one *G6PD* type, and the other half the other type. A single *G6PD* type was found in the blast cells, demonstrating their clonal origin, and for most patients with AML, in the granulocyte/monocyte lineage, but not in other lineages. This could suggest the possibility that the precursor of this granulocyte and monocyte-restricted disease was a committed granulocyte/monocyte precursor. If not, it originated in early cells, but expansion of the precursors occurred only once they were committed to this lineage. If this were the case, then by ablating these cells, the remaining $CD33^-$ cells could be predominantly or even completely normal in origin.

This process is in contrast to chronic myelogenous leukemia, in which similar studies demonstrated that the disease originates in a pluripotent stem cell.[4,5] There is other evidence as well that for a small proportion of patients, particularly the elderly, a multipotent precursor may be involved in AML.[3] Nonetheless, in research focused on patients with disease restricted to the granulocyte/monocyte lineage,[1] the $CD33^-$ cells or $CD34^+/CD33^-$ cells of these patients were placed in long-term marrow culture and grown on an irradiated marrow stromal cell feeder layer. Selected cells were then grown in colony-forming assays and studied for their clonal or nonclonal origin. It was found that a proportion of patients grew CFU-GM that were polyclonal and thus predominantly or completely normal in origin, supporting the possibility that ablation of $CD33^-$ cells could eliminate leukemia and leukemic precursors, while allowing the remaining normal cells to establish normal hematopoiesis.

A number of investigators have challenged this notion. For example, Bonnet and Dick[6] isolated primitive $CD34^+/CD38^-$ precursors and transplanted them to immunodeficient mice. For most patient samples, they found transplanted leukemia rather than normal hematopoiesis. This finding led to a competing model of stem cell involvement in which there is an expansion of leukemic stem cells with self-renewal in the primitive pool.[7] This is in contrast to models at the other extreme in which the leukemia may arise in fairly mature precursors, and while it may arise in an early precursor, it does not expand and dominate hematopoiesis until a later stage in precursor development.

Based on these latter two models, a collaboration was established between investigators at Wyeth Ayerst (Radnor, PA, USA) and our laboratory to develop a humanized anti-CD33 antibody-calicheamicin conjugate (CMA-676) for testing whether ablation of these cells *in vivo* would allow primitive cells to establish normal hematopoiesis (Hamann *et al*, submitted). Calicheamicin is a small molecule, 1000 times more potent than doxorubicin, that should be poorly immunogenic, and when attached to antibody should not materially affect its pharmacokinetics, as seen when attaching larger molecules such as ricin. It seems to form radical intermediates intracellularly, and is a DNA minor groove binder that causes double-strand breaks and apoptosis. The conjugate was able to kill HL60 cells *in vitro*, as well as in two xenografts, and was effective in inhibiting the growth of leukemic-forming cells *in vitro*. In preclinical studies, the conjugate was able to selectively inhibit HL60 tumor cells *in vitro* as well as in an *in vivo* xenograft model, but most importantly, it was effective in inhibiting the growth of leukemic colony-forming cells *in vitro*. Moreover, other studies have demonstrated that radiolabeled anti-CD33 antibody showed very rapid access to leukemia cells in the marrow and spleen and was rapidly internalized by the target cells, suggesting a usefulness for drug delivery.[8]

A phase I trial was conducted in collaboration with Wyeth Ayerst.[9] This dose escalation study was conducted to determine whether normal hematopoiesis could be restored in patients with AML by selective ablation of cells expressing the CD33 antigen. Forty patients with refractory or advanced AML received up to three doses of CMA-676 at 2-week intervals. Elimination of morphologically detectable leukemia occurred in 20% of patients. Three patients also completely recovered

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renal toxicities were observed. The most frequent adverse events were fever and chills. In terms of nonhematopoietic toxicity, a small number of transient liver abnormalities were seen. Prolonged myelosuppression was observed in some of the patients, especially after three doses at the higher dose levels. In subsequent on-going studies, administration was limited to two doses. A dose-limiting toxicity was never reached; at the highest dose level of 9 mg/m^2, the CD33 target sites were essentially saturated. This was accepted as the dose for further study. Humoral responses to the conjugate were limited to two patients. One of these patients initially responded to CMA-676, later relapsed, and upon retreatment with CMA-676 developed an antibody response.

In an ongoing phase II trial, patients with CD33$^+$ AML in their first untreated relapse following a remission of >6 months were treated with a fixed dose.[10] Patients received a total of two i.v. infusions of 9 mg/m^2 of the conjugate. Preliminary results obtained with the 23 evaluable patients showed that 43% achieved disappearance of leukemic blasts from blood and marrow; recovery of their granulocyte count (>1500/mm^3); and were platelet transfusion independent with a platelet count >50 000/mm^3. However, a delayed platelet recovery has been seen in many of these patients. In addition to beginning to define response rate, around 38% thus far, these data have also confirmed the relatively low toxicity of this drug.

In summary, based on knowledge of stem cell development, it has been possible to develop an anti-CD33 antibody-calicheamicin conjugate, termed CMA-676. This conjugate has been shown to be well tolerated, to saturate target sites and, in some patients with AML, to eliminate leukemia *in vivo* without ablation of normal hematopoiesis. The observed response rates support the idea that early precursors responsible for re-establishing hematopoiesis are likely to be predominantly normal in origin for many patients with AML.

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