The Children's Oncology Group recently demonstrated that a reduction in the cumulative anthracycline dose by 25% improved survival compared with predecessor North American studies. Intrathecal chemotherapy was reduced from 7 doses to 2 in the Children's Oncology Group, with no effect on CNS relapse.[3] The Japan Pediatric Leukemia/Lymphoma Study Group eliminated high-dose cytarabine for a majority of patients, yielding a fraction of the cumulative cytarabine dose when compared with the other major ML-DS studies. For many years, the Japan Pediatric Leukemia/Lymphoma Study Group has used no intrathecal therapy without increased central nervous system relapse risk.[4] In the International European trial, ML-DS 2006, Uffmann et al report that outcomes are preserved when reducing the etoposide exposure by more than 50%.

It is critical to all international cooperative groups that we find the optimal regimen to improve survival and reduce toxicity in these vulnerable children. Taken together, the North American, Japanese, and European groups have shown that cumulative doses of daunorubicin, cytarabine, etoposide, and intrathecal treatments can each be significantly reduced while maintaining excellent survival in young children with ML-DS, although there is still no consensus on the necessity for high-dose cytarabine and intrathecal therapy. To date, each group has been successful in reducing the cumulative doses in their chosen backbone, yet it will not be easy to continue to study dose reductions that improve or maintain a 90% event-free survival rate in this rare disease. Perhaps it is now time to consider how we can layer on targeted therapies with long-term goals of eliminating, where possible, cytotoxic therapies and further improving long-term survival. It is important to remember that children with relapse ML-DS do not benefit from dose intensification and transplant.[5] In other words, although it is true that the international community of AML groups has demonstrated that dose deintensification is appropriate for most newly diagnosed children with ML-DS, dose intensification of conventional therapy is not sufficient to salvage patients with ML-DS in relapse. The identification of new and more targeted therapies will be the only effective future strategy with the potential to improve outcomes further.

The international precision medicine strategy to date in ML-DS has been the targeted reduction in toxic therapy based on the unique pharmacogenomic features of children with DS. Future precision medicine strategies now need to turn to somatic events. Various groups have reported that minimal residual disease (MRD) at the end of induction, trisomy 8, non-M7 morphology, older age at diagnosis, and relapsed disease each predicts a poor outcome with conventional therapeutic approaches. There remains some inconsistency among the prognostic factors identified in different studies,[1,3,4] but MRD is generally accepted as a poor prognostic marker that is present in up to 14% of patients.[3] MRD at the end of induction may help to identify patients at highest risk for relapse and appropriate for the clinical investigation of targeted therapies. The reported somatic mutations in ML-DS confirm that epigenetic regulators,[6,7] cell cycle check point regulators,[8] the cohesin complex, CTCF, EZH2, the ras pathway, and the Jak pathway[7] are all commonly mutated and/or targetable.[3] Nonetheless, the development of targeted small molecules and immunotherapies still lags far behind the development pace seen in other leukemias. ML-DS represents a unique biologically defined subtype of AML in a unique and vulnerable host. As each of the international cooperative groups have recently identified minimally toxic and effective backbone therapies, it is the ideal time for collaborative efforts to develop strategies to evaluate more rationally targeted therapies for these children.

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● ● ● CLINICAL TRIALS AND OBSERVATIONS

Comment on Gardner et al, page 3322

Equal opportunity CAR T cells

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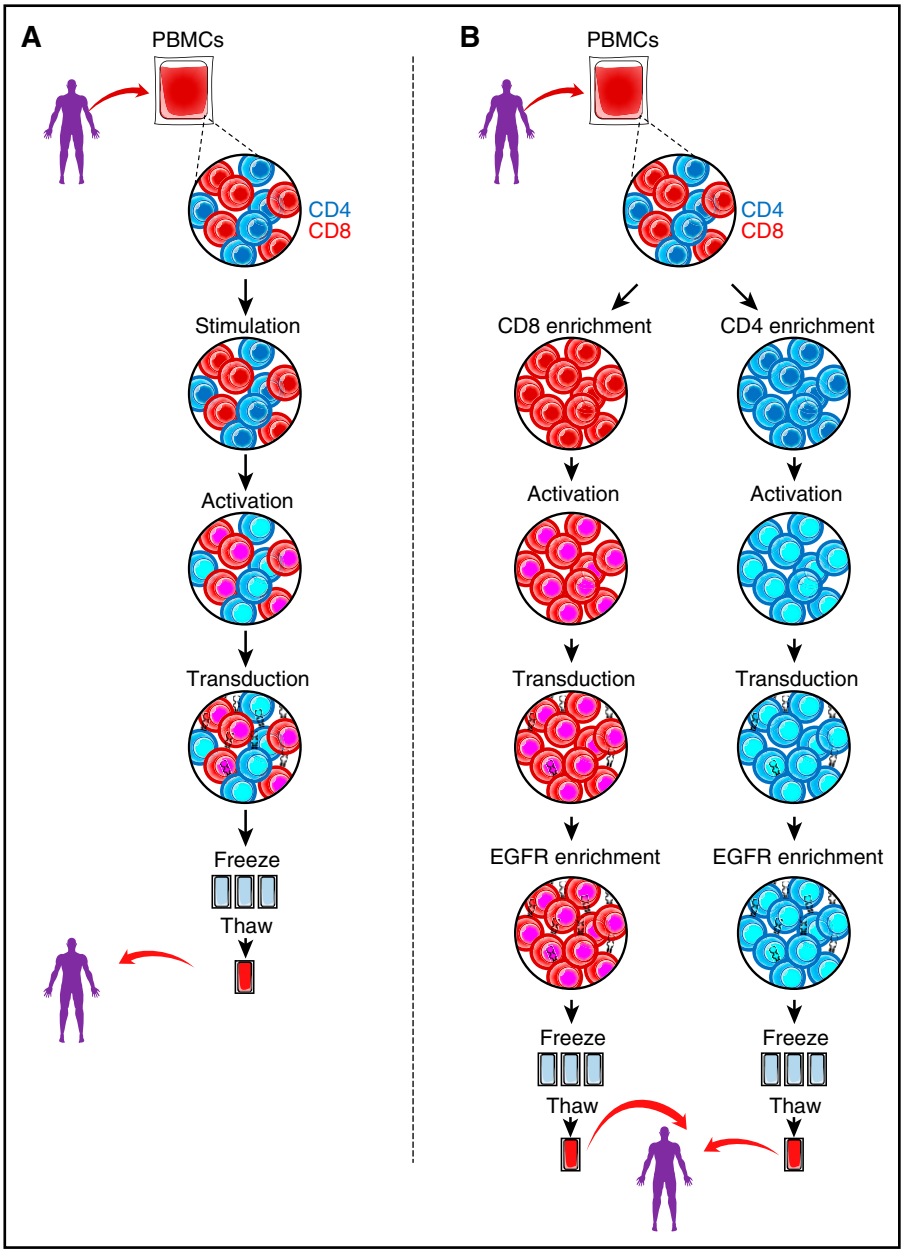
In this issue of *Blood*, Gardner et al report results of a phase 1 trial of 45 children and young adults with relapsed or refractory B-lineage acute lymphoblastic leukemia (ALL) who received a T-cell product of defined CD4/CD8 composition that was genetically modified with a CD19-4-1BB:ζ chimeric antigen receptor (CAR) lentiviral vector.[1]

The authors report successful product release in 93% of enrolled patients and an overall intent-to-treat (ITT) minimal residual disease–negative (MRD^-) remission rate of 89%. Of note, 100% of patients who received cyclophosphamide-fludarabine lymphodepletion had an MRD^- remission, further reinforcing the importance of



(A) Standard approach to manufacturing CD19 CAR T cells. After apheresis, bulk peripheral blood mononuclear cells (PBMCs) undergo stimulation and activation (with CD3/CD28 beads and cytokine supplementation) followed by transduction with a CD19 CAR vector of choice. After expansion, the bulk product is cryopreserved until ready for thaw and clinical use. (B) Manufacturing schema of PLAT-02 (defined formulation) CD19 CAR product. After apheresis, PBMCs undergo positive selection for $CD8^+$ and $CD4^+$ T cells by using immunomagnetic separation (CliniMACS device, Miltenyi Biotec). After enrichment, CD4 and CD8 T cells are separately activated with CD3/CD28 beads and then transduced with the lentiviral CD19 CAR vector expressing EGFRt in the presence of IL-7/IL-15 (CD4) and IL-15/IL-2 (CD8). The separate cell products undergo positive selection for EGFRt-expressing cells using the CliniMACS device, after which the individual products are cryopreserved until ready for thaw and clinical use.

lymphodepletion regimens that include fludarabine, as opposed to cyclophosphamide alone.[2] There were no deaths, and the adverse effect profile of the reported T-cell product was similar to or better than that in other published studies of CD19 CAR T cells, with ~23% of patients experiencing severe cytokine release syndrome (CRS) and/or reversible severe neurotoxicity attributed to the T-cell product.[3,4] With longer follow-up, the 12-month event-free survival was 50.8% and overall survival was 69.5%, which are similar to response rates reported in previous CD19 CAR T-cell studies from the University of Pennsylvania[3] and the National Cancer Institute[4] targeting ALL in pediatric patients. Of 18 relapses, 7 were associated with loss of CD19 expression, whereas loss of functional CAR T cells was a risk factor for $CD19^+$ relapse, an incidence rate similar to that reported in other studies of CD19 CAR T cells.[3,4]

In this study, the investigators used a manufacturing process that resulted in CAR T-cell products with a defined CD4/CD8 composition, uniform high-level transgene expression, and attenuated differentiation. To achieve this, the investigators isolated $CD4^+$ and $CD8^+$ T cells from apheresis products by immunomagnetic separation. After enrichment, a defined number of CD4 and CD8 selected T cells were separately stimulated with CD3/CD28 beads, transduced with the lentiviral CD19 CAR vector also expressing EGFRt, and cultured with homeostatic cytokines to limit activation-induced differentiation. CD4 cultures were supplemented with interleukin-7 (IL-7) and IL-15, and CD8 cultures were supplemented with IL-15 and IL-2. Finally, CD3/CD28 bead particles were removed, and cultures were positively selected for EGFRt-expressing cells by using the CliniMACS device. The figure illustrates the more standardized CAR T-cell manufacturing approach (panel A) whereby CAR T cells are generated from bulk populations of T cells that typically lack the aforementioned selection and enrichment steps; panel B illustrates the manufacturing process used in the Gardner et al study. The rationale for this strategy comes from preclinical studies that suggest that a 1:1 ratio of CD4 to CD8 and culture with appropriate homeostatic cytokines would ensure maximum effectiveness of both T-cell subsets and would yield a less terminally differentiated more memory-prone T-cell population with maximum killing capacity, prolonged persistence, and the ability to retain memory and self-renewal capacity.[5]

Gardner et al directly attribute the very impressive ITT MRD^- remission rates and overall survival achieved in their study to their T-cell product. A number of strategies have been explored to further enhance the efficacy of CAR T cells, such as the use of different costimulatory moieties (typically CD28 or 41BB) to provide enhanced T-cell activation and persistence[6] and modification of the spacer or hinge regions of the construct.[7,8] The impact of the cytokine milieu in which cells are manufactured and the most efficient T-cell phenotypes for CAR transduction and T-cell manufacture, as outlined in the Gardner et al article and elsewhere[9] are additional important variables.

To complicate matters, patient-specific characteristics such as age, absolute lymphocyte count (ALC), and previous therapy can also contribute to the quality of the T-cell product. Nevertheless, CD19 CAR T cells derived from different manufacturing techniques, different lentiviral or retroviral constructs, and different costimulatory moieties have had consistently excellent outcomes in studies targeting children or young adults with ALL.[1,3,4] Thus, discerning which factors are most important for CD19 CAR T-cell persistence and antitumor activity in patients is a challenge. At present, it is not possible to confirm the contention of Gardner et al and thereby justify the more complex and expensive manufacturing process they used, based on patient response rates and survival. Longer-term follow-up on more patients will be necessary to determine whether there is a difference in long-term survival between different CD19 CAR T-cell products.

Another important finding in the article by Gardner et al is the suboptimal expansion and persistence of CD19 CAR T cells in patients with MRD who have low quantities of normal and malignant $CD19^+$ B cells. As the authors point out, lack of the targeted antigen could certainly present a challenge when attempting to incorporate CAR T-cell therapy into first-line therapy or as treatment of MRD after induction or consolidation. Additional means of addressing this potential shortcoming are being explored by several groups and include targeting multiple antigens with CAR T cells or providing additional antigen as a vaccine with CAR T-cell infusion.

Although the multistep manufacturing process used in the Gardner et al study is more complex, it is notable that they report a high success rate in manufacturing with that process, and it is commendable that they report their results on ITT analysis. In some previous studies, it has proved difficult to decipher how many patients were not eligible on the basis of ALC or failure of a test culture.[10] As CD19 CAR T cells move toward licensure, it will be important to streamline the process to reduce the cost of goods and also to determine the standardized ITT response rates with all products to definitively learn whether additional manufacturing maneuvers such as those used by Gardner et al are beneficial. It is an intriguing possibility that a major benefit of the initial separation of the CD4 and CD8 T cells and their independent growth in optimized homeostatic cytokines may be in increasing the manufacturing success rate and making CD19 CAR T-cell therapy available to a greater percentage of patients.

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● ● ● HEMATOPOIESIS AND STEM CELLS

Comment on Miyawaki et al, page 3332

Human megakaryocytes: finding the root

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In this issue of *Blood*, Miyawaki et al identify the most primitive progenitor cell population that makes only megakaryocytes and platelets in adult humans and show it is expanded in myeloproliferative neoplasms such as essential thrombocythemia (ET).[1] Approximately 10^{11} platelets are produced on a daily basis in humans, but their exact journey from undifferentiated hematopoietic stem cells (HSCs) is still highly debated. Platelets have the shortest half-life of all blood components and are rapidly recruited when injury occurs, yet have long been thought to be among the cell types to be specified as the furthest from the HSCs in the hematopoietic hierarchy. For several decades, it was understood that differentiation proceeds by a series of binary fates choices, in particular with a common myeloid progenitor (CMP) downstream of HSCs that would give rise to a restricted myeloid progenitor (granulocyte-macrophage progenitor) and to a megakaryocyte-erythrocyte progenitor (MEP). Only downstream of MEPs would unilineage megakaryocyte and unilineage erythrocyte progenitors arise. Recently though, several groups have reported that megakaryocyte and platelet production may not follow this strict hierarchical branching path. Instead, committed megakaryocyte precursors could be found much earlier, either within the HSC[2-4] or the multipotent progenitor compartment.[5] An early precursor that exclusively produces human megakaryocytes in humans, however, had not been described.

Here, Miyawaki et al focused on the human myeloid progenitor compartment and used a combination of single-cell quantitative polymerase chain reaction and in vitro differentiation assays to identify a homogeneous population of cells, which fate has restricted to megakaryopoiesis. For this, they investigated single cells from the classically defined CMP, MEP, and granulocyte-macrophage progenitor