

Myeloid Leukemias - Current and Future Approaches to Targeted and Individualized Therapies

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Blood cancers (leukemias) in children and adolescents are the most frequent type of childhood cancer, and 25% of these are called myeloid malignancies. By far the most common myeloid diagnosis is acute myelogenous leukemia (AML). Additional rare types of myeloid cancer include myelodysplastic syndromes (MDS) and myeloproliferative syndromes (MPS), such as juvenile myelomonocytic leukemia (JMML) and chronic myelogenous leukemia (CML). In addition, children with Down Syndrome or Trisomy 21 may present as infants with a transient myeloproliferative disorder (TMD) and a distinct form of acute megakaryoblastic leukemia (AMKL). While advances have occurred in the treatment and outcome of pediatric patients with MDS and MPS, this article will focus on past successes leading to current outcomes and challenges for patients with the most common myeloid malignancy, AML.

Past Successes and Limitations of Conventional Approaches to Treatment

Chemotherapy that has proven successful for curing the majority of children diagnosed with acute lymphocytic leukemias (ALL) has not shown the same success with AML. They are both blood cancers; why do they respond so differently to treatment? Chemotherapy does indeed result in complete remission for the majority of children with either AML or ALL. However, AML typically relapses while ALL does not. One hypothesis is that the stem cells giving rise to AML are particularly resistant to chemotherapy, and they can seed the cancer again despite remission.

Chemotherapy in ALL has been successful in part by the careful matching of the intensity of the treatment with the specific subtype of ALL (risk type), with just enough drugs to effectively kill the cancer. In AML, the cancer and stem cells are generally more resistant to chemotherapy, requiring higher and higher intensities of chemotherapy.

While the majority of patients with AML are able to achieve a complete remission of their leukemia, less than 50% will be cured of their disease. As a result, curing AML has required significantly intensified courses of near myeloablative combinations of chemotherapeutic agents, possibly due to the inherent drug resistance of the AML stem cell. While this type of intensive treatment has resulted in about half of all pediatric patients surviving newly diagnosed AML, (a significant improvement over the approximately 28% survival up until the early 1990's), there are quite significant and limiting side effects of this type of therapeutic approach, leading to excessive morbidity and mortality. The strong drugs used in AML tend to destroy the normal bone marrow of the child, thus requiring bone marrow transplantation to restore the bone marrow function.

Current AML therapy is therefore based upon the use of multi-agent combinations of non-cross-resistant chemotherapeutic agents, chemotherapy dose intensification, risk-adapted use of allogeneic hematopoietic stem cell transplantation (HSCT), as well as aggressive, pre-emptive use of supportive care interventions. Risk-adapted use of HSCT has only relatively recently been introduced into the treatment of children with AML following the results of several cooperative group studies demonstrating that some children with particular types of AML do not require a HSCT for cure. In general, however, the therapeutic approaches used to treat AML are also relatively non-selective and lead to significant treatment-related toxicities.

Recent Progress in Refining AML Treatment

Advances in molecular profiling of AML has begun to separate out distinct 'risk classes,' as previously done for ALL. Molecular profiling has been recently reviewed by the Candlelighters newsletter (Fall/Winter

2006), and this involves studies of the DNA, mRNA and proteins that are diagnostic of the specific tumor type. Newly emerging improvements in subtype classification and risk stratification has slowly helped increase cure rates, while reducing toxic side effects of the treatments.

These advances in understanding of the cellular and molecular basis of AML have begun to realize significant improvements for less toxic and more effective therapies. The cellular source of AML originates from hematopoietic progenitor or stem cells that acquire genetic defects which in turn lead to decreased or abnormal cell maturation, increased growth and survival. The survival advantage conferred by these genetic changes allow leukemic cells the ability to replace normal bone marrow and peripheral blood cells, leading to anemia with pallor and fatigue, decreased platelets with easy bruising and bleeding, as well as decreased normal white blood cells resulting in susceptibility to infections.

The genetic changes that lead to AML can be both inherited as well as somatically acquired, i.e., acquired during fetal or post-natal development. The inheritance patterns and mutations leading to pediatric cancer have been reviewed in a previous Candlelighters newsletter (March 2000 and June 2000), and will not be discussed in depth here. Many of the molecular changes, particularly specific chromosome translocations (where two chromosomes exchange parts) are now recognized to contribute to the onset and progression of AML. Although different AML patients may show different chromosome abnormalities, the specific genes involved sometimes share a lot in common, suggesting a common molecular signature for AML. For example, specific exchanges seen in AML are between chromosome 8 and chromosome 21 [t (8; 21)], chromosomes 3 and 21 [t (3; 21)], chromosomes 16 and 21 [t (16; 21)] and a rearrangement within chromosome 16 called an inversion [inv (16)]. All chromosomal changes observed in AML alter the function of proteins that regulate a specific type of biological process called "chromatin structure and function". One option for experimental therapeutics is to develop drugs targeting each of the abnormal chromosomal fusion/inversion protein products (fusion proteins). However, it may prove best to target shared biological process (e.g. chromatin structure and function) that contribute to characteristic steps in AML development, and thus stop the cancer.

Many of the genetic changes seen in AML patient cells have been introduced into mice to create a 'preclinical mouse model.' The mouse models enable systematic study of each of the abnormal genes separately and together in the same mouse. These studies have shown that a single abnormal gene or protein (fusion product) is not sufficient to cause AML. Instead, multiple genetic changes are needed together before AML develops in a child. A class of proteins that has emerged as particularly important for AML is tyrosine kinase receptors (such as FLT3 and c-KIT). These proteins function as regulators of leukemia cell survival and proliferation, and mutations that activate these proteins lead to uncontrolled proliferation and cancer (AML) (see previous article on oncogenes; Candlelighters newsletter June 2000). These are being studied as potentially important therapeutic targets.

The AML Stem Cell and New Challenges for Targeted Therapy

Despite the characteristic molecular signature that differentiates AML from other cancers, AML is particularly challenging as it is most closely related to normal bone marrow cells. In other words, there is a relatively fine line between a normal bone marrow cell, and an AML cell, and finding this fine line to destroy the AML cells without destroying normal bone marrow is particularly challenging. Use of very sensitive and specific antibody markers has recently allowed the purification of AML cancer stem cells (primitive self-repopulating leukemic stem cells) apart from normal cells. These self-repopulating AML primitive cells are likely extremely rare in a patient's blood, perhaps 1% or less of the cancer cells (leukemic blasts). However, if this subset of AML cells can be destroyed, then it is anticipated that prognosis would dramatically improve.

Thus, for a leukemia dependent pathway to represent a potentially curative target and not simply one that temporarily reduces the number of leukemic cells, the altered pathway should have a specific, or at least a highly selective effect on the leukemia stem cell. Clinical trials to assess the relevance of agents directed toward these AML stem cells in order to definitively test the clinical relevance of these concepts are needed.

Targeted Therapies in Development for Pediatric AML

Based upon data from molecular profiling of AML cells described above, several pathways and targets are being currently tested in pediatric AML that are directed alone or in combination toward reversing the abnormalities in leukemia cell survival/drug resistance, proliferation, and differentiation. Some of these approaches also appear to have activity against the AML stem cell population. Promising new approaches to more selective treatments include inhibition of proliferation and survival pathways such as FLT3-ITD and c-KIT receptors and their downstream targets, like RAS, as well as transcriptional or chromatin based strategies and immunotargeted therapies with monoclonal antibodies and vaccines.

Targeting Leukemia Cell Proliferation and Survival

There are four distinct approaches that are being used to develop the therapies of the future for AML. These are: tyrosine kinases; downstream targets of the kinases; stopping the AML cell from becoming chemotherapy-resistant; reprogramming of AML cells via 'epigenetics'; AML vaccines.

Tyrosine Kinases

Laboratory studies have demonstrated the important role of cytokines, i.e., secreted proteins that increase both normal and leukemia cell survival and differentiation, and their receptors in AML. Several of these cytokine receptors, such as FLT3 and c-KIT (also called stem cell factor receptor or SCF-R) are expressed at high levels or have activating mutations that lead to increased survival, and drug resistance in AML. Furthermore, mutations in these receptors, particularly the FLT3 receptor, have been strongly correlated with poor outcome in patients with AML.

Several drug inhibitors of FLT3/ITD, such as CEP-701 (Cephalon), MLN518 (Millenium) and PKC412 (Novartis), are being tested in early clinical trials. One of these inhibitors, CEP-701 (lestaurtinib), has been tested in adults with refractory AML and was shown to decrease peripheral blood leukemic blasts but without significant reduction of leukemic blasts in the bone marrow that are critical for achieving complete remissions. Similar results have also been observed with other inhibitors of FLT3. Further preclinical work has shown increased efficacy of combining CEP-701 with cytosine arabinoside (AraC) which subsequently led to encouraging early results in a randomized phase II trial in adults with relapsed AML expressing FLT3/ITD receptors.

CEP-701 is now being tested in the Children's Oncology Group (COG) AAML06P1 trial in combination with chemotherapy (idarubicin and cytosine arabinoside) for pediatric patients with relapsed AML harboring FLT-ITD mutations. The increased expression of wild type FLT3 in AML, particularly in infant leukemia, has led to a COG clinical trial combining CEP-701 with chemotherapy in this very high risk leukemia in infants. There are plans to test the addition of FLT3 inhibitors into the COG phase III trial for patients with newly diagnosed AML expressing the FLT3/ITD mutant receptor.

Abnormalities of the c-KIT tyrosine kinase receptor have been associated with poor outcome in the subtype of core binding factor AML, which is usually associated with a better outcome than most other subtypes. Mutations of *c-KIT* involve single base pair changes leading to amino acid changes in the kinase domain and receptor autoactivation. *KIT* mutations are observed in only about 3 to 5% of AML, but in 20% to 30% of a sub-type of AML showing a molecular feature called 'core binding factors'. Thus, kinase inhibitors with activity against such *c-KIT* mutations would be expected to be potentially useful in this subset of AML. Imatinib (Gleevec) first developed for the inhibition of the bcr-abl fusion kinase and the treatment of CML, also has significant activity against c-KIT. In vitro results have demonstrated that imatinib selectively inhibits both the normal copy of c-KIT in all of cells, as well as the cancer cell's (mutant) c-KIT activity. This inhibition of c-KIT tells cells to slow down both in terms of proliferation and survival. An initial phase II study of imatinib in adult patients with refractory or recurrent AML showed disappointing results with no significant clinical responses. However, a subsequent clinical trial using imatinib in 21 adult patients with refractory AML expressing c-KIT demonstrated 5 out of 21 patients with hematologic responses, 2 of which were complete hematologic remissions in patients who had relatively low blast counts in the bone marrow or in peripheral blood. More selective and potent inhibitors of c-KIT

need to be developed and tested along with synergistic combinations with conventional chemotherapy or other targeting agents, similar to what is being tested for FLT3-ITD inhibition.

Although their names suggest that insulin growth factor-I (IGF-I) and vascular endothelial growth factor (VEGF) receptors would not play a role in AML, these receptors have been shown to be essential for bone marrow stem cell (progenitor) survival and expansion. Cell culture studies have shown that inhibition of the IGF-IR kills AraC resistant AML cells. As IGF-IR inhibitors are further studied in the laboratory and in clinical trials, a potential role of blocking this pathway alone or in combination with other targeted therapies may prove worthwhile in AML. Similarly, laboratory experiments have shown that VEGF is made by both the leukemia cells and the cells nearby helper cells (supporting stromal cells) in order to support leukemia cell survival and proliferation: VEGF. Monoclonal antibodies (e.g., Avastin or Bevacizumab) that bind and inactivate VEGF as well as small molecule inhibitors of VEGFR (e.g., SU5416 or AZD2171) have been developed to stop blood supply to the tumor (anti-angiogenic treatment), and these approaches are being tested in adults with refractory AML. Trials combining such agents with chemotherapy are in development.

The above examples demonstrate several key issues concerning the targeting of tyrosine kinase receptors in AML. First, the use of tyrosine kinase inhibitors as single agents in patients with AML is unlikely to be highly effective. However, inhibitors of tyrosine kinases appear to be able to sensitize AML cells to conventional chemotherapeutic drugs, without having significant overlapping toxicities. A cautionary note is that leukemia cell resistance to inhibitors of tyrosine kinase inhibitors has been observed.

Targeting Molecular Pathways under the Control of Tyrosine Kinase Receptors

Tyrosine kinase receptors function primarily through signaling to other molecules which in turn activate further molecules until a cascade of reactions occur that results in a change in gene expression and cell behavior. The protein, RAS, which is mutated in about 25% of AML/MDS cases, represents a central player in signaling for many different cytokine or tyrosine kinase receptors. In the absence of RAS mutations, cytokine receptor signaling can activate RAS which is the result of RAS conversion from a GDP to GTP form along with essential post-synthesis farnesylation and tethering to the inner cell membrane.

Although specific inhibitors of GTP activation of RAS have not been developed for clinical use, farnesyl transferase inhibitors (FTI) have been tested in patients with AML. These agents, though not specific for activated RAS, are believed to target the RAS pathway though they would also be expected to potentially inhibit other pathways dependent on farnesylation. Phase I studies in adults with AML reported significant clinical responses that did not appear to be dose dependent. A pediatric randomized trial of tipifarnib versus no drug in the post-allogeneic HSCT setting is being planned by the COG in patients with relapsed AML if the agent remains available.

The mTOR (mammalian Target of Rapamycin) serine/threonine kinase is another central pathway that regulates cell proliferation, cell suicide (apoptosis), as well as access to blood supply (angiogenesis) in both normal and cancer cells. mTOR is a molecule which sits at the convergence of many tyrosine kinase receptor signaling pathways. This central role in signaling and the dependence of leukemia cells on many of these pathways has made mTOR a potentially important pathway to target. Several highly selective inhibitors of mTOR have been developed, including rapamycin, CCI-779, RAD001, AP23573, and MK-8669. Although very limited data are currently available in terms of such approaches in patients with AML, combining mTOR inhibitors with conventional chemotherapy, particularly AraC containing regimens are planned in children with refractory AML.

Reversing Chemotherapy Resistance Mechanisms

In addition to the effects on drug resistance conveyed by mutations in tyrosine kinases, other molecular pathways may also lead to acquired drug resistance as well. An additional pathway to chemotherapy resistance is the up-regulation of bcl-2. Extensive work on bcl-2 in AML has established its important role

in resistance to chemotherapy in AML. The ability of inhibitors of bcl-2 to sensitize AML cells to chemotherapeutic agents has led to the development of clinical trials using bcl-2 inhibitors (e.g., antisense oligonucleotide oblimersen or Genasense). While no immediate clinical trials are being planned in pediatric AML, this pathway may become more interesting as more efficient and selective inhibitors are developed.

Most cancer cells depend on the ability to degrade proteins that are abnormal or would induce cell death to a much greater extent than do normal cells. This observation has led to a potentially novel therapeutic target called the proteasome. The proteasome is the cell's recycling plant, taking 'used' proteins, and quickly chopping them up so that the building blocks are now available for making new proteins. Malignant cells depend heavily on this process (proteasome degradation) in part because of the revved up metabolism of the cancer cell (more building blocks and new proteins needed), their increased 'free radical' production that hurts existing proteins (targets them for the recycling), and the detection of the cell of mutated and abnormal proteins (again, targeting them for recycling). By blocking this degradation system, proteasome inhibitors have been shown to increase the sensitivity of tumor cells to chemotherapeutic agents, leading to their cell death. In other words, if the cancer cell is already struggling to keep the recycling plant going and provide new building blocks, then the chemotherapy can be the double hit that pushes it to death.

The high levels of expression of the transcription and survival promoting factor, NF- κ B, along with the expression of its inhibitor, I κ B, provides a potentially important link between survival factors and proteasome inhibition. For example, the use of the proteasome inhibitor, bortezomib (PS-341 or Velcade), has been shown to decrease the degradation of I κ B and thus reduce the amount of functional NF- κ B by leading to its degradation, ultimately leading to apoptosis of AML self-repopulating or stem cells. In addition, the combination of an anthracycline and a proteasome inhibitor showed an even further increase of AML stem cell killing. Based on such data, the COG is planning to conduct a clinical trial with bortezomib in combination with chemotherapy in children with relapsed and/or refractory AML.

Epigenetically Reprogramming AML Cells

While genetic mutations clearly play an important role in the development of AML, there is growing evidence for the importance of DNA modification by the addition of methyl groups or altered DNA helix bending as a result of interactions with specific types of proteins. Such changes are termed epigenetic and, because they are potentially reversible changes, they represent potentially important targets for treatment. Several chromosomal translocations found in AML lead to fusion proteins of gene products that influence DNA structure (methylation) and chromatin function. For instance, the t(15;17), i.e., PML/RAR α fusion, found in APL or those involving the MLL gene product are able to recruit proteins to specific genes that cause their repression or silencing. When the silenced genes represent tumor suppressor genes, such changes can lead to increased growth, survival and chemotherapeutic resistance. Thus, treatments to re-program the abnormal epigenetic changes observed in AML are being actively tested in clinical trials. Several agents have already been approved by the Food and Drug Administration such as 5-azacytidine, an inhibitor of DNA methylation, in myelodysplastic syndromes as well as vorinostat, an inhibitor of histone deacetylases, in certain types of lymphoma. Although the use of such genetic reprogramming approaches has worked well in APL with the introduction of All-Trans-Retinoic Acid (ATRA), the role of DNA methylation inhibitors as well as drugs capable of altering histone and other chromatin modifying proteins in AML will require specific clinical trials.

Antibody Targeting of AML and the Development of AML Vaccines

The immune system is highly evolved to produce specific antibodies as well as T lymphocytes with exquisite specificity against target cells. From over a century ago when the German pathologist, Paul Ehrlich, dreamed of immunological 'magic bullets' to treat cancer, investigators have pursued this hope. Within the past several decades, the development of monoclonal antibodies directed against proteins expressed on the surface of tumor cells as well as the ability to manipulate anti-leukemia T cell responses have brought this hope at least in part to reality.

One example has been the successful use of monoclonal antibodies directed against the differentiation antigen, CD33, an adhesion molecule that is differentially expressed during myeloid differentiation and that is present on AML cells. Gemtuzumab ozogamicin (GO; Mylotarg™) is a humanized monoclonal antibody conjugated to the toxin, calicheamicin, that can specifically detect and bind to cells expressing CD33. GO has demonstrated significant activity in AML and was the first toxin/monoclonal antibody fusion approved by the Food and Drug Association (FDA). Initial clinical trials in adults and children with relapsed or primary refractory disease AML showed response rates of approximately 30 to 35% when GO was used as a single agent. Of note, the response in children with primary refractory disease was the same as that for patients with relapsed disease who had achieved a prior remission. These results suggested that resistance to conventional chemotherapeutic drugs could be circumvented with this approach.

A COG phase I trial using GO in combination with either mitoxantrone plus high dose AraC or with Cappizzi II chemotherapy (high dose AraC plus L-asparaginase) established the dosing of these combinations and showed considerable responses of between 40% to 50% complete remissions or complete remissions without full platelet recovery in patients with refractory AML. In addition, the toxicities of these combination regimens were noted to be primarily those usually seen in patients with AML as well as those involving the liver and pancreas. Based on these results, the current COG Phase III trial is testing in a randomized fashion whether the addition of GO to induction and intensification courses of treatment, results in an improved outcome for pediatric patients with newly diagnosed AML.

The role of AML vaccines for the treatment of patients is still in its beginning phases. Several animal models have successfully shown that AML cells are able to evade the host immune system by decreasing the expression of critical cell surface proteins. When the leukemia cells are genetically manipulated to be forced to express such molecules, the host immune system is then able to recognize and kill them along with other leukemia cells that do not express these immunostimulatory receptors. Such studies have helped to establish the basis for developing AML vaccines to be used in patients. Studies employing these approaches are being done in adults with AML. Other approaches use proteins that are highly expressed in AML, such as the Wilms tumor gene protein (WT1) or that are uniquely expressed, such as fusion proteins formed from chromosomal translocations. These proteins are added to host dendritic cells which are potent stimulators of T lymphocytes and then patients immunized with the whole cell vaccine. A key element of all these vaccine approaches is that they are most likely to be effective when there is minimal leukemia in the patient, i.e., after the patient has been treated and obtained apparent remission. Because most of the treatments we currently use are immunosuppressive, they may limit the utility of vaccine approaches. This further stresses the need to develop less immunosuppressive and effective anti-leukemic targeting treatments.

Another approach to stimulate anti-leukemic immune responses is to take advantage of the graft-versus-leukemia reaction that can occur during a HSCT. By exploiting incompatibility of natural killer (NK) cell receptors on donor lymphocytes with proteins on host cells that are bound to these receptors, several studies have suggested that increased graft-versus-leukemia can be enhanced without increasing the risk of graft-versus-host disease. This type of NK receptor mismatching is being testing in a COG trial for patients with high risk, newly diagnosed AML.

Reality Check and Future Challenges

AML remains an incredibly challenging disorder to cure in part because of the many different sub-types and forms of AML (heterogeneity), its intrinsic and acquired resistance to conventional therapies and its origins in hematopoietic stem cells that closely resemble normal bone marrow stem cells. Nevertheless, real progress has been made from a disease that was uniformly fatal prior to 1970 to one that today is cured in 50% of children and adolescents. However, despite this progress, the primary cause of death in patients with AML is still progressive, refractory leukemia. Dose escalation of conventional therapies along with risk stratification has been the primary approaches leading to our current cure and toxicity outcomes. While these approaches have clearly improved outcomes, only half of patients are being cured and further dose escalation is really not practical or desirable.

To escape this 'Catch 22' dilemma, more individualized and tumor targeted approaches are being developed that will hopefully increase anti-leukemic activity without adding significant toxicity. Some of these agents, such as antibody-directed therapies and tyrosine kinase inhibitors, are currently in Phase II and III clinical trials. The results of these important trials should determine whether these approaches will be integrated into future regimens, thus establishing new standards for AML therapy.

A goal of these approaches should one day be to eliminate the need for conventional cytotoxic chemotherapeutic agents that are associated with significant short and long term adverse late effects. While such a goal may appear impossible now, it should be quite achievable by applying creative, integrative and collaborative laboratory and clinical investigation. Genomic, gene expression or proteomic signatures along with epigenetic patterns of both leukemia and normal host cells should lead to the identification of additional and critical pathways for specific therapeutic targeting that will include the leukemia stem cell population.

But the laboratory based identification of new targets and approaches to modulate their activity still require predictive preclinical models and more efficient and definitive ways to perform and interpret clinical trials. These preclinical models and actual clinical trials will need to reflect the growing recognition of heterogeneity of the various types of AML as well as individual patient differences. The historical approach of using one or two chemotherapy hammers to hit all different types of tumors has been similar to using a sledge hammer on a tiny finishing nail. As AML is split into a number of carefully and molecularly defined sub-groups, the tools are being refined that are more appropriate to each AML subtype. Thus, these challenges will force close collaboration with international colleagues as well as defining more creative funding sources from government, pharmaceutical and community sources. To effectively pursue solutions to these challenges, we need to streamline regulatory road blocks.

We should also not lose sight of the fact that about 85% of children with cancer live in developing countries, where early diagnosis is often difficult and standards of treatment and supportive care may not always be optimal. An important spin-off and consequence of developing targeted therapies is that their effectiveness should not be compromised by severe toxicities requiring extensive supportive care. An additional hope is therefore that effective targeted treatments for AML should greatly benefit all children regardless of where they live.

Figure. Schematic of possible cellular and molecular targets in AML (adapted from Arceci, R.J. and Cripe, T, Pediatric Clinics of North America, 49: 1339-1368; 2002).

