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New Developments in the Therapy of Acute Myelocytic Leukemia

Norbert C. Gorin* (Chair), Elihu Estey, Richard J. Jones, Hyam I. Levitsky, Ivan Borrello, and Shimon Slavin

Current conventional treatment for patients with acute myelogenous leukemia results in a high percentage of clinical responses in most patients. However, a high percentage of patients still remain refractory to primary therapy or relapse later. This review examines the search for new agents and new modes of therapy. In Section I, Dr. Estey discusses new agents directed at various targets, such as CD33, angiogenesis, inappropriate methylation (suppressor) genes, cell cycle checkpoints, proteosomes, multidrug resistance (MDR) gene, mitochondrial apoptotic pathway. He also reviews preliminary results of phase I trials with the nucleoside analog troxacitabine and liposomal anthracyclin and suggests new strategies for trials of new agents.

In Section II, Dr. Jones revisits differentiation therapy and presents results of preclinical and clinical studies that demonstrate that a variety of clinically applicable cell cycle inhibitors (interferon, phenylbutyrate, vitamin D, retinoids, bryostatin-1) preferentially augment growth factor-mediated induction of myeloid leukemia terminal differentiation, as well as blocks growth factors’ effects on leukemia proliferation. The combination of cell cycle inhibition plus myeloid growth factors may offer a potential treatment for resistant myeloid leukemias.

In Section III, Drs. Levitsky and Borrello address the question of tumor vaccination in AML and shows that, although tumor rejection antigens in AML have not been formally identified to date, a growing number of attractive candidates are ripe for testing with defined antigen-specific vaccine strategies. Interestingly, the ability to drive leukemic blasts to differentiate into competent antigen presenting cells such as dendritic cells may be exploited in the creation of cellular vaccines. Ultimately, the successful development of active immunotherapy for AML will require integration with dose-intensive chemotherapy, necessitating a more complete understanding of host immune reconstitution.

In Section IV, Dr. Slavin reviews the concept of delivering non-myeloablative stem cell transplantation (NST) and delayed lymphocyte infusion (DLI) to increase tolerance in particular in high risk and older patients, and take advantage of the graft-versus-leukemia (GVL) effect.

All these approaches hold promise in reducing morbidity and mortality and differ from the older concepts aiming at delivering the highest possible doses of chemotherapy and/or total body irradiation to reach maximum leukemia cell kill, whatever the toxicity to the patient.

In the past two decades, clinical research aimed at improving the cure rate in acute myelocytic leukemia (AML) has focussed primarily on increasing cytotoxic drug delivery to patients to maximize tumor cell kill. Both autologous and allogeneic stem cell transplantation, when feasible, have been considered mainly as tools enabling the use of otherwise myeloablative therapies, although the contribution of the graft-versus-leukemia (GVL) effect was recognized as important as early as 1990. While these approaches have indeed resulted in an increased cure rate, they also have been associated with significant early and late toxicity, including procedure-related death and secondary malignancies. They also have been costly. Most significantly, considering those patients who are not in selected good-prognosis subgroups, the majority of AML patients are not cured.

New concepts are emerging for the treatment of AML, some of which may emerge as new de novo strat-
gies, other as complements of more classical approaches. In this review we will consider successively the search for new agents with new modes of action, the potential of differentiation therapy, prospects for anti-leukemic vaccines and the current results of non-myeloablative stem cell transplantation.

I. NEW AGENTS AND NEW TARGETS FOR THE TREATMENT OF AML

Elihu Estey, M.D.*

The need to find new agents for treatment of AML is widely appreciated. Recent discoveries of novel targets (Table 1) may make drug development both more rational and more successful.

Agents Targeting Cell Surface Antigens

Mylotarg (CMA676)

CD33 is an appealing target for therapy because it is expressed in 90% of cases of AML and is absent from normal hematopoietic stem cells and non-hematopoietic tissue. Mylotarg, formerly known as CMA 676, is a combination of humanized anti-CD33 antibody and the anthracycline antibiotic calicheamicin.1 The drug was recently approved by the FDA based on a multicenter phase II trial that enrolled 142 patients with CD33 positive-AML in untreated first relapse.2 The median patient age was 61 years, and the median first CR duration was 11 months (minimum 3 months). Patients with organ dysfunction, poor performance status, or AML secondary to myelodysplastic syndrome (MDS) or treatment for prior cancer were ineligible. The CR rate was 16% (95% CI 11–23%). Another 13% (95% CI 8–20%) had “CRP”, with the “P” denoting that the platelet count remained below 100,000, although platelet transfusions were not required. Survival of CRp patients to date appears similar to that seen in CR patients and longer than that seen in non-responding patients. Interestingly, first CR duration seems to have less effect on response to mylotarg than on response to cytosine arabinoside (ara-C), and CRp was very uncommon in ara-C-treated patients (Table 2). A multivariate analysis comparing response to mylotarg in the phase II trial to that seen following ara-C-containing regimens in 120 M.D. Anderson patients with AML in untreated first relapse indicated that, after accounting for age and cytogenetics, the response rate was higher with mylotarg than with ara-C if the duration of first CR was 6–9 months; response was considerably higher with ara-C if the first CR was longer. Mylotarg appears to produce considerably less non-hematologic toxicity than conventional regimens, encouraging attempts to combine it with other active agents. Because its anti-leukemic effect appears inversely correlated with efflux of Di (OC22), a specific substrate for MDR1/P-glycoprotein, mylotarg could also be combined with agents that inhibit the function of this protein. Because there has been no correlation between CD33 expression and response, trials of mylotarg even in CD33 “negative” AML might be of interest. Trials in patients in remission and as part of a preparative regimen prior to transplantation would also seem indicated.

HuM195 and Anti-CD45

HuM195 is a humanized mouse monoclonal antibody (M195) to CD33 that illustrates the potential utility of monoclonal antibodies in the treatment of minimal residual AML. Specifically, Jurcic et al have reported that “native” HuM195 produced PCR negativity in 9/20 patients with acute promyelocytic leukemia (APL) in whom PCR positivity persisted in hematologic CR after one induction course of ATRA with or without chemotherapy.

Table 1. Targets for new anti-AML compounds.

<table>
<thead>
<tr>
<th>Target</th>
<th>Relevant Compounds</th>
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<tbody>
<tr>
<td>CD33</td>
<td>Mylotarg, HuM195 +/- 131I, 90Y, 213Bi</td>
</tr>
<tr>
<td>CD45</td>
<td>131I-Anti-CD45</td>
</tr>
<tr>
<td>MDR1/PGP</td>
<td>Cyclosporine, PSC 833</td>
</tr>
<tr>
<td>Angiogenesis and/or VEGF</td>
<td>Thalidomide, SU5416, Anti-VEGF antibodies</td>
</tr>
<tr>
<td>Hypermethylated chromatin</td>
<td>Decitabine</td>
</tr>
<tr>
<td>Histone deacetylase</td>
<td>Phenybutyrate trichostatin A, trapoxin</td>
</tr>
<tr>
<td>Bcl-2</td>
<td>Bcl-2 antisense</td>
</tr>
<tr>
<td>S-phase checkpoint</td>
<td>UCN 01</td>
</tr>
<tr>
<td>20S proteasome</td>
<td>PS-341</td>
</tr>
<tr>
<td>Tyrosine kinase</td>
<td>STI 571</td>
</tr>
<tr>
<td>(c-kit receptor)</td>
<td>BMS-214662, RH115777</td>
</tr>
</tbody>
</table>

Table 2. Comparison of CR + CRp rates with mylotarg and high-dose ara-C.

<table>
<thead>
<tr>
<th>First CR Duration</th>
<th>3 months – &lt; 1 year</th>
<th>&gt; 1 year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ara-C</td>
<td>95</td>
<td>71</td>
</tr>
<tr>
<td>Mylotarg</td>
<td>80</td>
<td>62</td>
</tr>
</tbody>
</table>

1 ara-C ≥ 0.5 gm/m²/dose +/- fludarabine or anthracyclines as given to M.D. Anderson patients with AML in untreated first relapse 1990-1999. Patients selected to meet eligibility criteria of mylotarg protocol.

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The comparable rate in patients continued on ATRA alone was 7/34. Whereas all HuM195-treated patients became PCR negative after one further course of chemotherapy, three courses of similar chemotherapy were required in patients not given HuM195.3

HuM195 has been attached to both α and β particle-emitting radioisotopes (Table 3). The latter, e.g. 131I, 90Y, produce prolonged myelosuppression; thus their principal value may be as myeloablative transplant preparative regimens. 131I has also been conjugated with anti CD45 for use, together with cyclophosphamide (CY) and total body irradiation (TBI), as a preparative regimen. CD45 is the most broadly expressed hematopoietic antigen, but is limited to the hematopoietic system. A phase I trial conducted by Matthews et al identified the maximum tolerated dose (MTD) of 131I, given as the 131I anti-CD45 compound, as 10.5 Gy delivered to the liver (the normal organ receiving the highest radiation absorbed dose). This translates into 24 Gy delivered to the marrow, in addition to the TBI dose.4 This result is noteworthy because TBI increments of as little as 3.75 Gy have been reported to decrease relapse rate in AML in first CR. Preliminary data suggest that patients with AML in remission receiving 131I anti-CD45 + CY + TBI have better outcome than historical patients who received CY + TBI. 90Y, which has been conjugated to HuM195, has a shorter half-life than 131I, reducing the time necessary to clear radiation before stem cell infusion, thus potentially making it preferable to 131I for myeloa blation. Future studies will likely further define the relative benefits of 90Y HuM195, 131I anti-CD45 + CY + TBI and other new preparative regimens, e.g. those containing intravenous busulfan or mylotarg.

The ratio of radiation delivered to the marrow, spleen, and liver relative to the whole body is 1,000- to 10,000-fold higher when HuM195 is conjugated to a particle emitter (e.g. 212Bi, 211Bi, 213At, 9-11) rather than to β emitters such as 131I, or 90Y. This reflects the shorter path length of the former. The resultant predicted lower rate of non-specific toxicity appears to have been borne out in a phase I trial of 212Bi HuM195 conducted by Jurcic et al.5 These data suggest that therapy with α particles may be preferable outside the transplant setting.

Mdr1/P-Glycoprotein as a Target

New agents assume particular interest when capable of directly affecting factors associated with poor prognosis (Table 4). The presence of the multidrug resistance protein MDR1/PGP with attendant high rates of efflux of drugs that bind it (e.g. daunorubicin, mitox antrone, probably mylotarg) unfavorably affects outcome in AML.6 This has prompted clinical trials of drugs, such as cyclosporine (CSA) and PSC 833, that inhibit MDR/PGP1.

Cyclosporine

The greatest success using MDR1/PGP-reversing agents was observed in a trial of 226 patients with relapsed/refractory AML by List et al.7 Patients randomized to CSA together with high-dose ara-C and continuous infusion daunorubicin had slightly higher CR rates (40% vs. 33%) and strikingly better outcome in CR (relative risk of relapse free survival .56, with 2-year actuarial rates of 34% vs 9%). This effect on CR and its duration translated into a significant improvement in survival. Daunorubicin levels were higher in the CSA group, probably due to CSA’s ability to inhibit hepatic metabolism of daunorubicin, but higher daunorubicin levels were only associated with better outcome if patients received CSA. CSA produced superior results in both MDR1 negative and positive disease although the difference between CSA and no CSA was greater in MDR1-positive patients. The Pediatric Oncology Group (POG) recently (4/2000) closed a 650-patient study in which children in first CR were randomized to receive 2 cycles of mitoxantrone and etoposide with or without CSA. Preliminary analysis suggests a 10% improvement in event-free survival in the CSA arm (H.

Table 3. Radioisotope-monoclonal antibody conjugates in clinical trials.

<table>
<thead>
<tr>
<th>Isotope</th>
<th>Type Particle</th>
<th>Antibody</th>
<th>Future Role</th>
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<tbody>
<tr>
<td>131I</td>
<td>β</td>
<td>HuM195, anti-CD45</td>
<td>Myeloablation prior to transplant</td>
</tr>
<tr>
<td>90Y</td>
<td>β</td>
<td>HuM195</td>
<td>Myeloablation prior to transplant</td>
</tr>
<tr>
<td>213Bi</td>
<td>α</td>
<td>HuM195</td>
<td>Treatment of minimal residual disease</td>
</tr>
</tbody>
</table>

Table 4. Published randomized trials of MDR1/PGP modulation.

<table>
<thead>
<tr>
<th>Trial/Pts</th>
<th>Modulating agent/dose</th>
<th>Chemotherapy</th>
<th>Patients</th>
<th>Principal Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>List et al7</td>
<td>CSA/16 mg/kg/day</td>
<td>Ara-C, Daunorubicin (cont. infusion)</td>
<td>226</td>
<td>CSA improved survival</td>
</tr>
<tr>
<td>Liu Yin et al21</td>
<td>CSA/5-10 mg/kg/day</td>
<td>Daunorubicin (bolus), Ara-C, Etoposide</td>
<td>235</td>
<td>Survival worse with CSA in pts over age 60</td>
</tr>
<tr>
<td>Greenberg et al26</td>
<td>PSC 833 10 mg/kg/day</td>
<td>Mitoxantrone, Etoposide, Ara-C</td>
<td>113</td>
<td>No effect of PSC 833</td>
</tr>
<tr>
<td>Baer et al27</td>
<td>PSC 833 10 mg/kg/day</td>
<td>Daunorubicin, Ara-C, Etoposide</td>
<td>120</td>
<td>Higher early death rate with PSC</td>
</tr>
</tbody>
</table>
expression. In a CALGB trial9 randomizing 120 newly (i.e. +/-PSC 833) appeared independent of MDR1/PGP xantrone, etoposide, and ara-C. The CR rates in both arms found no advantage for patients given PSC 833 + mitoxantrone, etoposide, and ara-C. The CR rates in both arms (i.e. +/-PSC 833) appeared independent of MDR1/PGP expression. In a CALGB trial9 randomizing 120 newly diagnosed patients ≥ 60 years of age to receive or not receive PSC 833 together with ara-C, daunorubicin, and etoposide chemotherapy, CR rates were 31% in the PSC arm and 45% in the standard therapy arm. The lower CR rate resulted chiefly from an increase in early deaths (35% vs 16% by day 30), which appeared to reflect multiple toxicities and led to early termination of the study. Explanations for the poor results observed to date with PSC 833 in comparison with the CSA trial by List et al have included differences in doses and type of chemotherapy, less compliance with the use of PSC 833, and the possibility that CSA has effects other than inhibition of MDR1/PGP. None of these explanations appear entirely satisfactory. Neither the ECOG nor the CALGB trials assessed whether administration of PSC 833 inhibited MDR1/PGP function in blasts obtained from patients given the drug in vivo. Therefore, the potential value of PSC 833 in the subset of patients in whom the drug effectively inhibits MDR1/PGP remains unknown.

Newer agents affecting MDR1/PGP
LY-335979 is an agent that, unlike CSA and PSC 833, does not affect hepatic excretion of drugs, VX-710 and VX-853 are agents that inhibit both MDR1/PGP and MRP1-mediated efflux, although the prognostic significance of the latter has been questioned.6

Anti-Angiogenesis Therapy
Suggestions of increased angiogenesis in AML10 together with reports of the activity of the anti-angiogenesis agent thalidomide in multiple myeloma have sparked interest in treatment of AML with angiogenesis inhibitors. An ongoing M.D. Anderson trial randomizing patients with newly-diagnosed AML, RAEB-t, or RAEB and abnormal karyotypes (except inv (16), t(8;21), and t(15;17)) to liposomal daunorubicin plus ara-C with or without thalidomide (400-600 mg daily) showed no difference in early CR (CR achieved on the first course and within 50 days of starting that course) rates in each arm (44%). Higher pre-treatment plasma levels of vascular endothelial growth factor (VEGF) appear prognostically unfavorable, but marrow microvascular density (MVD) is not predictive of response. These data suggest that any effect of VEGF is not mediated by an effect on angiogenesis. It remains possible that a subset of patients can be identified in whom higher MVD is unfavorable and unaffected by thalidomide. Such patients might be targets for trials of naturally occurring angiogenesis inhibitors (angiostatin, endostatin, platelet factor-4), antibodies to VEGF or fibroblast growth factor, or drugs that inhibit endothelial cell proliferation, such as SU5416. It is also plausible that anti-angiogenesis therapy may require a relatively long time to be effective. Under these circumstances, trials of such therapy might be more profitably undertaken in patients in CR than in patients with active AML.

Agents That Remodel Chromatin

Hypomethylating agents
Hypermethylation of CpG-rich regions of the genome is a physiologic mechanism of gene inactivation that is usurped by leukemic cells, which use it to silence tumor-suppressor genes and related proteins.11 Decitabine (DAC) is chemically related to 5 azacytidine but in vitro is a more potent inducer of demethylation. DAC doses of 100–200 mg/m2 daily X 5 induce CR in chronic myeloid leukemia (CML) myeloid blast phase12 and produce drug levels of 1.4–4.0 mM. Dose-response experiments using multiple cell lines, as well as specimens from patients with AML, indicate that decitabine concentrations of 0.05–0.8 mM induce demethylation and reactivation of expression of genes such as p15, versican, and the estrogen receptor. These data suggest that low doses of the drug (e.g. 5–20 mg/m2 daily) will produce hypomethylation without the myelosuppression that occurs at higher doses. This might permit DAC to be infused for a longer time (e.g. for 14 days), with the potential of increasing demethylation and thereby inducing differentiation. A phase I-II trial of low-dose DAC has been begun at M.D. Anderson.

Histone deacetylase inhibitors
The silencing of transcription associated with hypermethylation may be mediated by histone deacetylase (HADC).13 Removal of acetyl groups by HDAC allows histones to bind more tightly to DNA, thus preventing gene transcription. The pathogenesis of APL and of t(8;21) AML is believed related to inappropriate recruitment of HDAC complexes by the PML-RARα and
AML1-ETO fusion proteins respectively. These observations have heightened interest in the clinical application of HDAC inhibitors. The most striking success has been reported with phenylbutyrate in the treatment of a patient with ATRA-refractory APL, whose response was associated with an increase in histone acetylation. Inhibitors that are more specific for HDAC include trichostatin A and trapoxin. The pathogenesis of other types of AML, in particular those involving abnormalities of the MLL gene on chromosome 11q23, may involve inappropriate histone acetylase (HAT) activity, and it is likely that inhibitors of this enzyme will be developed for clinical use. Finally, because the effects of methylation may be mediated by an HDAC complex, combinations of hypomethylating agents (e.g. DAC) and HDAC inhibitors might be explored; in vitro synergy exists between these two classes of agents.

**Modulators of Apoptotic Pathways**

*Agents targeting Bcl-2*

Bcl-2-like proteins have been hypothesized to prevent apoptosis, perhaps by the retention of cytochrome c in mitochondria. This family is, therefore, a rational target for modulation. A direct approach to inhibit bcl-2 is through use of bcl-2 antisense. Promising results have been reported in lymphoma. Studies of bcl-2 antisense combined with traditional chemotherapy are planned in AML. Were this to be of interest it would encourage the development of anti-sense constructs to other anti-apoptotic proteins.

*Abrogation of cell cycle checkpoints*

Cells may respond to chemotherapy (e.g. by ara-C)-induced DNA damage by arresting progress through the cell cycle, thus limiting further damage and subsequent apoptosis. UCN-01 is a protein kinase C inhibitor that abrogates both the S- and G2-phase cell cycle checkpoints, preventing cell cycle arrest. While exposure of clonogenic human AML cells to low ara-C concentrations had little effect on clonogenicity, addition of otherwise innocuous concentrations of UCN-01 to the ara-C led to substantial reductions in clonogenicity. The impact of UCN-01 on the efficacy of ara-C at concentrations used clinically is being determined in an ongoing trial.

*Arsenic trioxide*

Although best known for its use in the treatment of APL, arsenic trioxide (As2O3) has also been described to exert anti-apoptotic effects in non-APL AML cell lines at clinically achievable concentrations. As2O3 also induces hyperacetylation of histones, indicating the difficulty in categorizing some agents as “modulators of apoptosis” rather than “chromatin remodeling agents.” These in vitro results have prompted ECOG trials of As2O3 in non-APL AML.

**Proteasome inhibitors**

The 20S proteasome is an ATP-dependent multicatalytic protease. Increased proteasome-mediated degradation of proteins (e.g. p53, p21, p27, IkB) that lead to apoptosis and/or cell cycle arrest may be mediated by alterations in the activity or expression of the enzyme Cdc34 in leukemic cells. PS-341 is a specific inhibitor of the 20S proteasome that is in phase I trial in AML. A goal of the trial is to determine whether 20S proteasome activity is inhibited at the MTD.

**Inhibition of Tyrosine Kinase**

Although best known for its activity in CML, STI 571, an inhibitor of the tyrosine kinase (TK) characteristic of bcr-abl-positive CML, has also been reported to inhibit the TK associated with the c-kit receptor in an AML cell line. Inhibition occurred at STI concentrations that are similar to those that inhibit bcr-abl TK and was accompanied by inhibition of proliferation. c-kit expression is common in AML, suggesting that STI 571, probably combined with chemotherapy, may find application in c-kit positive AML.

**Miscellaneous Agents**

*Troxacinabine*

Troxacinabine is the first L- (as opposed to β-D) nucleoside analog shown to have anticancer activity and, unlike ara-C, is not a substrate for the deactivating enzyme deoxycytidine deaminase. In a phase I trial, Giles et al found mucositis, rash, and a painful hand-foot syndrome the limiting side effects and observed a CR rate of 2/10 at the MTD of 8 mg/m^2^ daily for 5 days. It remains to be seen whether the same prognostic factors will predict response to both troxacinabine and ara-C, but the preliminary data have led to trials combining troxacinabine with ara-C, idarubicin, and topotecan.

*Farnesyltransferase inhibitors*

Although RAS mutations (chiefly in N-RAS) are only inconsistently detectable in AML, it has been proposed that RAS may be activated and lead to abnormal proliferation not only through mutation, but through other genetic alterations that occur. Such alternate mechanisms of RAS activation might explain the general failure to find a relationship between RAS mutation and prognosis. Under such circumstances inhibition of RAS function might be therapeutic even in patients in whom RAS is not mutated. To function, RAS must move from the cytoplasm to the cell membrane; this process requires
transfer of a farnesyl group to RAS. Several farnesyl transferase inhibitors (FTIs) have been developed and entered clinical trial in AML; these include BMS-214662 and R115777.

**New Statistical Strategies for Trials of New Agents**

The number of potential new therapies to test is large relative to the number of available patients with AML. In the conventional phase II paradigm, a small number of drugs is investigated, with each given to 14–50 patients. The agents to be investigated are chosen based on pre-clinical rationale. A problem with this approach is that experience suggests that it is often difficult to identify which, if any, new therapies will be useful without first performing a clinical trial. 2CdA in hairy cell leukemia or interferon in CML are examples of therapies that have proven successful, although the explanation for their success remains elusive. Accordingly, a case can be made for an approach that studies a larger number of therapies with relatively small sample sizes. These considerations have motivated the development of formal randomized Bayesian “selection” designs, which use clinical trial data from < 10–20 patients, rather than informal assessments of the strength of pre-clinical rationale, to select which agents to pursue in larger confirmatory trials. The same general formulation allows monitoring of both response and toxicity in phase I-II hybrid trials. The number of patients available for trials of new agents can also be increased by extending eligibility to untreated patients with particularly poor prognoses. The National Cooperative Cancer Network (NCCN), a consortium of prominent academic centers has recommended such an approach.

**II. Differentiation Therapy Revisited**

Richard J. Jones, M.D. *

Understanding the biological basis for the resistance to cytotoxic anti-leukemic chemotherapy is essential to improving the outcome of patients with AML. Anti-apoptotic signals are a major, and possibly the most important, cause of resistance to cytotoxic anti-leukemic agents.\(^1,2\) Since induction of apoptosis is the final event following all cytotoxic cellular damage, it is likely that over-expression of anti-apoptotic signals generates pan-resistance to cytotoxic anti-leukemic agents regardless of their specific intracellular targets.\(^1\) Thus, it should not be surprising that unfavorable leukemias are resistant to multiple different anti-leukemic regimens. However, a number of biologic anti-cancer strategies appear capable of overcoming or circumventing anti-apoptotic signals and may therefore be useful in pan-resistant leukemias.

Immunotherapy is one approach that should be truly non-cross-resistant with cytotoxic anticancer therapy. Although apoptosis induced by cytotoxic agents is inhibited by anti-apoptotic signals such as bcl-2 and bcr-abl, it has been shown that immunologic cell-mediated apoptosis is not.\(^3,4\) Thus, it appears that immunotherapy can be effective in pan-resistant leukemias.

Differentiation therapy leading to cellular senescence of leukemic progenitors is another approach that may be effective in resistant leukemias. A variety of agents with different presumed mechanisms of action demonstrate in vitro differentiating activity against a broad range of tumor cell types\(^5\) (Table 5). All-trans-retinoic acid (ATRA) for acute promyelocytic leukemia (APL) is the prime example of successful clinical differentiation therapy. Although APL is considered a favorable AML subtype, the long-term leukemia-free survival of patients with APL is less than 20% when treated with standard cytarabine/anthracycline-based anti-leukemia chemotherapy alone.\(^6\) APL cells are actually relatively resistant to the induction of apoptosis, as well as to standard cytotoxic anti-leukemic agents such as cytarabine and daunorubicin in vitro.\(^1,2\) However, the addition of ATRA to standard anti-leukemia regimens improved the long-term leukemia-free survival of patients with APL to greater than 60%.\(^6\) Thus, ATRA-mediated induction of terminal differentiation is able to convert drug-resistant APL into a favorable AML subtype.

The consistent chromosomal translocation of APL, t(15;17), fuses the retinoic acid receptor alpha (RAR\(\alpha\)) gene from chromosome 17 to the promylocytic leukemia (PML) gene on chromosome 15 yielding the fusion protein PML/RAR\(\alpha\), which is responsible for the sensitivity of APL to ATRA. However, outside of APL, ATRA and other differentiating agents have displayed little clinical activity\(^5\) (Table 5). ATRA is also ineffective in the

<table>
<thead>
<tr>
<th>Agent</th>
<th>In Vitro Activity</th>
<th>Clinical Activity</th>
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<tbody>
<tr>
<td>Retinoids (ATRA)</td>
<td>Broad</td>
<td>APL</td>
</tr>
<tr>
<td>Interferons</td>
<td>Leukemia</td>
<td>–</td>
</tr>
<tr>
<td>Vitamin D(_3)</td>
<td>Broad</td>
<td>–</td>
</tr>
<tr>
<td>Butyrates</td>
<td>Broad</td>
<td>–</td>
</tr>
<tr>
<td>Polar-planar compound (HMBA, DMSO)</td>
<td>Broad</td>
<td>–</td>
</tr>
<tr>
<td>Protein Kinase C Activators</td>
<td>Leukemia</td>
<td>–</td>
</tr>
<tr>
<td>(Phorbol esters, Bryostatin-1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amifostine</td>
<td>–</td>
<td>? MDS</td>
</tr>
<tr>
<td>Cytotoxic Drugs (Ara-C, Hydroxyurea, 5-Azacytidine)</td>
<td>Leukemia</td>
<td>? MDS</td>
</tr>
<tr>
<td>Growth factors/Cytokines</td>
<td>Leukemia</td>
<td>MDS, CML</td>
</tr>
</tbody>
</table>

Abbreviations: HMBA, hexamethylene bisacetamide; DMSO, dimethylsulfoxide; ATRA, all-trans retinoic acid; APL, acute promyelocytic leukemia; MDS, myelodysplastic syndrome

[\(^*\) Johns Hopkins Oncology Center, The Bunting-Blaustein Cancer Res. Bldg., 1650 Orleans Street, Room 207, Baltimore MD 21231]
t(11;17) APL variant, where the promyelocytic leukemia zinc finger (PLZF) gene is fused to RARα. Understanding the reasons for the relative ineffectiveness of most differentiation agents has been confounded by the limited understanding of the mechanism of action of most differentiating agents.

Many pharmacologic differentiating agents, including phorbol esters, phenylbutyrate, and retinoids, share a common biologic activity in that they inhibit of cell cycling. It has long been recognized that signals that stimulate cell cycle progression usually produce a block in differentiation, while interventions that produce a cell cycle arrest sensitize cells to the induction of differentiation. Therefore, inhibition of the cell cycle may be a prerequisite step in initiating terminal differentiation.

Growth factors are biologic differentiating agents, and growth factor-mediated induction of terminal differentiation appears to have anti-leukemic activity against CML in vitro and clinically. However, growth factors also have other competing effects on tumor cell growth, including stimulating tumor cell proliferation and survival. A variety of cytotoxic agents have also been shown to augment cytokine-mediated tumor cell differentiation. Cytotoxic agents induce cell cycle arrest at G₁ and/or G₂, and this could be the mechanism by which they augment differentiation. Bcr-abl gene rearrangement delays G₂-M progression, possibly also explaining how this chimeric gene augments growth-factor mediated differentiation.

In order to determine whether inhibition of the cell cycle enhances growth factor-mediated differentiation, a variety of clinically applicable agents that inhibit cell cycling, have different mechanisms of action, and have been previously studied as differentiating agents were studied in two AML cells lines, U937 and HL60, that express functional GM-CSF receptors. Phenylbutyrate (a histone deacetylase inhibitor) and interferon block the cell cycle at G₁. Hydroxyurea blocks the cell cycle in S phase. Protein kinase C activators, such as phorbol esters and bryostatin-1, induce cell cycle arrest at G₂ (as well as G₀). In vitro studies, hydroxyurea (≥ 100 µg/ml), phenylbutyrate (≥ 1.5 mM), and bryostatin-1 (≥ 10⁻⁸ M) inhibited leukemic growth, caused a cell cycle block, and induced differentiation by morphology, cell surface antigen expression, and loss of clonogenicity. These agents produced no evidence of differentiation at doses that are not cytostatic. GM-CSF (200 units/ml) alone induced a small amount of differentiation of the leukemic cell lines. However, there was marked differentiation of the AML cell lines after just 5 days in GM-CSF combined with the cytostatic agents.

To study whether pharmacologic differentiating agents and cell cycle inhibition are sufficient, or primarily permissive, for differentiation, we repeated the studies with the clinically applicable cell cycle inhibitors in the leukemia cell lines with and without the addition of neutralizing antibodies to growth factors. Neutralizing antibodies to GM-CSF and IL-3 completely inhibited the differentiating activity of bryostatin-1, demonstrated by both clonogenic assay (reversing CFU-L growth inhibition) and analysis of cell surface antigen expression (inhibition of differentiation antigen expression). Thus, cell cycle inhibition alone was unable to induce differentiation; full induction of terminal differentiation required lineage-specific growth factors. Of note, the cytostatic agents with or without GM-CSF did not inhibit normal hematopoietic progenitors.

Other clinically applicable cell cycle inhibitors, including vitamin D₃ and retinoids, also appear to augment growth factor-mediated differentiation of tumor cells. We have begun to study the effect of bryostatin-1 plus GM-CSF on clinical specimens from patients with myeloid malignancies. In vitro, this combination induces terminal differentiation of malignant hematopoietic progenitors from patients with CML, MDS, and AML. We also found that interferon-α induces a G₁ block and augments GM-CSF-mediated terminal differentiation of CML progenitors in vitro. In addition, this approach (lineage-specific growth factors + cell cycle inhibition) has potential clinical applicability beyond myeloid leukemias. The combination of IL-6 and clinically applicable cell cycle inhibitors, including interferon and bryostatin-1, has marked activity against multiple myeloma in vitro.

We have begun to study differentiation therapy with growth factors combined with cell cycle inhibition in clinical trials. A trial in CML (interferon + GM-CSF) has been open for over 18 months, and a trial of bryostatin-1 + GM-CSF in AML and advanced MDS is about to begin. The interferon + GM-CSF trial in CML has accrued 30 patients (18 patients have been entered in the past 6 months). Of the 12 patients followed for at least 6 months, 10 have entered a major (< 35% Philadelphia chromosome (Ph)+ cells) cytogenetic remission. Seven patients have been followed for 9 months and the mean Ph+ is 5% (range 0–25%), with four patients in a complete cytogenetic remission. Only four patients have been followed for 12 months, and three are 100% Ph- and RT-PCR⁺; one of these patients is Ph⁻ and RT-PCR⁻ at 18 months.

Thus, agents that have previously been termed “differentiating agents” may be insufficient alone in promoting terminal differentiation but are likely to facilitate differentiation by inhibiting the cell cycle. We have found that full induction of terminal differentiation after induction of cell cycle arrest requires lineage-specific growth factors. Conversely, growth factors alone are sufficient for the induction of differentiation, but have multiple other competing effects on the growth of leukemias, including
stimulating their proliferation and enhancing their survival. The net effect of growth factors on a tumor cell population is determined by the balance of their pleiotropic effects on tumor self-renewal, survival, and differentiation. The preferential enhancement of tumor self-renewal and/or survival may hasten tumor progression, while a predominant or selective induction of tumor cell differentiation may exhaust the neoplastic clone. A variety of clinically applicable cell cycle inhibitors (interferon, bryostatin-1, phenylbutyrate, vitamin D, retinoids, hydroxyurea) preferentially augment the differentiation effects of growth factors on myeloid leukemias, and also block the effects of growth factors on leukemia cell proliferation. In addition, strategies that induce differentiation of leukemia cells could lead to eradication of the malignant clone by inducing tumor cell terminal differentiation as well as by enhancing immunologic anti-tumor activity via the production of leukemia-specific antigen presenting cells (APC). It is now clear that CML and AML progenitors can be differentiated into functional APC in vitro by cytokines.17 Interestingly, cell cycle inhibitors such as phorbol esters18 and interferon-α19 can augment the ability of CD34+ cells to differentiate into functional APC in vitro.

III. Harnessing Antitumor Immunity in the Treatment of AML: Prospects for Anti-Leukemic Vaccines

Hyam I. Levitsky, M.D.,* and Ivan Borrello, M.D.

A growing body of evidence suggests that immune-mediated events may aid in the tumor cell kill of patients with hematologic malignancies. The reduced relapse rates observed in the allogeneic transplant setting compared to autologous BMT1,2 and the clinical benefits achieved with donor lymphocyte infusions serve to highlight the potential of the immune system to impact favorably on residual leukemia. Unfortunately, the lack of tumor specificity of the allogeneic immune response is associated with an increase in the morbidity and mortality of graft-versus-host disease (GVHD) and its treatment. These clinical observations, together with recent advances in basic immunology, have provided an impetus for developing novel strategies seeking to enhance tumor-specific immunity through active immunization and/or adoptive immunotherapy in the treatment of AML.

Active Immunotherapy—Preclinical Models, Immunologic Mechanism, and Early Phase Clinical Trials

Recent advances in the identification of factors regulating immune responses have led to a renewed interest in tumor vaccines. The overall goal of this approach is to generate an active systemic immune response in the cancer-bearing host that is capable of specifically rejecting disseminated cancer cells, and to provide long-lived immunologic memory against relapse. One hurdle in the development of this approach is the identification of appropriate antigens to target. For most malignancies, including AML, dominant tumor rejection antigens have yet to be defined. Therefore one general approach has been to utilize autologous tumor cells as a source of antigen. Although injection of irradiated tumor cells alone often results in some degree of measurable anti-tumor immunity, current tumor cell-based vaccine strategies seek to enhance the immunogenicity of the vaccinating tumor cells by modifying them to express immunomodulatory molecules such as cytokines or co-stimulatory molecules through ex vivo gene transfer prior to injection.3 A number of such strategies have now been explored in rodent models, including vaccination with tumors cells engineered to express IL-2, IL-4, IL-7, IL-12, gamma interferon, lymphotactin, G-CSF, GM-CSF, B7-1, and B7-2. Although these diverse strategies differ substantially in the nature of the immune responses that are raised, several tumor cell-based vaccine approaches have demonstrated the ability to generate systemic, tumor-specific immune responses capable of protecting mice against a subsequent tumor challenge (i.e. immunologic memory) as well as eradicating a small pre-established systemic tumor burden when administered as a therapeutic vaccine.

Examination of the immunologic mechanisms that mediate tumor rejection in these systems has demonstrated the requirement for tumor-specific T-cell activation (frequently involving both CD4+ and CD8+ T cells), as well as the critical role of augmented tumor antigen presentation, either by host APCs4 or by the tumor itself.5 Mechanistically, one of the most potent tumor cell-based vaccines contains irradiated tumor cells engineered to secrete high local concentrations of GM-CSF. It protected mice best against a non-immunogenic melanoma when compared to vaccination with the tumor cells transfected to express 10 other cytokines and cell surface molecules.6 GM-CSF-based tumor vaccines have shown significant activity in mouse models of melanoma, colon, lung, renal cell, and prostate cancer, as well as lymphoma, and acute leukemia. The paracrine production of GM-CSF results in the local recruitment of bone marrow-derived APCs at the vaccine site, including macrophages and dendritic cells that process tumor antigen from
the irradiated tumor cells injected in the vaccine. These APCs then migrate to the draining lymph nodes where they present both MHC class I and class II restricted peptides derived from the tumor antigens to tumor specific CD8+ and CD4+ T cells, respectively. These cells traffic systemically and regulate the effector phase of the antitumor response at distant sites of tumor. Although most of the mechanistic studies of tumor cell based vaccines have focused on the induction of T cell-mediated immunity, GM-CSF-producing tumor cells have also been shown to elicit potent antibody responses and to recruit tumoricidal macrophages and eosinophils at the site of the rejecting tumor. Ultimately, however, the induction of this multipronged immunologic attack requires the priming of tumor-specific T cells that regulate these downstream effector pathways and provide antigen specificity.

These preclinical studies of GM-CSF-producing tumor vaccines formed the basis for several clinical trials in the treatment of patients with solid tumor malignancies. Phase I/II studies have been completed in patients with metastatic renal cell carcinoma, prostate cancer, melanoma, and more recently in lung as well as pancreatic cancer. Immunologic activity was demonstrated in each of these studies by conversion to delayed type hypersensitivity (DTH) positivity against irradiated autologous tumor, induction of novel antibody responses, and/or activation of tumor-specific T cell responses as measured in vitro. Furthermore, although these trials were not powered for detecting clinical endpoints, anecdotal regression of measurable disease was reported in some patients.

**The Treatment of AML Represents an Ideal Setting for the Evaluation of Active Immunotherapy with Autologous Tumor Cell Vaccines**

Hematologic malignancies such as AML offer an ideal setting for the evaluation of active immunotherapy for several reasons. One of the major hurdles in bringing autologous tumor cell-based vaccine therapy to practice is obtaining adequate numbers of cells to be used for vaccination. Both mouse studies and preliminary clinical data demonstrate that there is a minimum number of tumor cells (“antigen dose”) required in the vaccine injection to achieve optimal priming. The difficulty in obtaining adequate numbers of autologous tumor cells has proven to be an important logistical limitation in the execution of the initial dose finding studies in the treatment of patients with solid tumors. In contrast, for most patients presenting with AML it is feasible to harvest large quantities of autologous tumor cells with relative ease from the marrow or by leukopheresis, prior to initiating induction therapy. From our own experience, between $10^8$ and $10^{10}$ AML blasts can be collected from most patients presenting with de novo AML, providing ample material for multiple immunizations. Most centers currently treating AML have the infrastructure that is required for collecting and storing viable autologous tumor cells. Such tumor cell populations do not require in vitro propagation prior to use and are more likely to represent the full antigenic profile of a heterogeneous systemic tumor.

This strategy is further simplified by recent evidence demonstrating that cytokine producing tumor cell-based vaccines (such as GM-CSF/tumor vaccines) do not necessarily require individualized gene transfer in the formulation of a given patient’s vaccine. This is because it is not necessary for the autologous tumor cells (i.e. the antigen source) to be directly producing the cytokine, as long as there is a sustained, “paracrine” production of cytokine in the vicinity of the injected tumor cells. This observation has opened the possibility of using a “bystander” approach, in which a generic, “off the shelf” cytokine gene-transduced allogeneic cell line is mixed together with irradiated autologous tumor cells prior to injection. We have recently reported the creation and characterization of such a reagent, using the human erythroleukemia cell line K562, engineered by simple plasmid transfection to secrete very large amounts of human GM-CSF. This line, which fails to express HLA class I or II antigens, is less likely to prime strong allo-responses that might divert immune recognition away from the antigens expressed by the immunizing autologous leukemic blasts. Using this simplified strategy, collecting and banking tumor cells and preparing a vaccine formulation with a bystander source of cytokine is within the technical capabilities of most centers currently treating AML.

A second form of “tumor cell-based” vaccine that may be uniquely suited for the treatment of myeloid leukemias is based upon the observation that myeloid leukemic progenitors, like normal hematopoietic stem cells, can be induced to differentiate in vitro into cells with many of the features of normal dendritic cells (DCs). Such cell populations have been shown by chromosomal analysis to be derived from the leukemic lineage and are potent APCs in vitro. Indeed, repeated stimulation of polyclonal T cell populations with leukemia-derived DCs has been reported to amplify T cell populations that can recognize unmodified leukemic blasts in vitro. In addition to their obvious potential for stimulating leukemia-specific T cells for adoptive immunotherapy, such cells may be potent immunogens in vivo. This offers the possibility of DC-based vaccine strategies without the requirement for antigen loading. One theoretical concern with this approach, however, is that the spectrum of tumor antigen expression may be substantially altered upon differentiation of leukemic blasts into DCs. An alternative approach circumventing this concern would be to pulse autologous DCs (generated perhaps from remission blood samples) with a lysate of unmodified leuke-
mic blasts stored at the time of presentation. Ultimately, a direct comparison of these DC-based formulations may be required to assess which induces the best response to antigens most represented in the patient’s unmodified tumor.

**Antigen-Specific Vaccine Strategies**

In the setting where potential tumor antigens are known, a number of vaccine strategies have been developed that can initiate systemic immune responses capable of mediating tumor rejection. Such strategies include immunization with protein plus adjuvant, antigenic peptide vaccines, naked DNA vaccines, recombinant viruses (such as pox viruses or adenovirus) encoding antigen, recombinant bacteria (e.g., *Listeria monocytogenes*), and immunization with antigen loaded dendritic cells.

As with most malignancies, tumor rejection antigens have yet to be formally identified in AML. In spite of this uncertainty, a few intriguing candidates are being explored. Although not truly tumor specific, proteinase 3, a primary neutrophilic granule protein, is markedly overexpressed in myeloid leukemias and has the interesting immunologic attribute of being the target auto-antigen in Wegener’s granulomatosis. By using algorithms based on HLA class I peptide-binding motifs, peptides from proteinase 3 have been identified that bind well to common HLA alleles, and have been used to stimulate T cell populations leading to the direct amplification of proteinase 3 specific cytolytic T cells that recognize and kill unmodified myeloid leukemia cells in an HLA- and antigen-specific fashion. Such candidate peptides are being explored both for their ability to amplify leukemia-specific T cell populations for adoptive therapy as well as being used directly in vaccines. Another interesting tool that candidate peptide antigens provide is a means to monitor the frequency of leukemia antigen-specific T cells in the course of therapy, by using tetrameric HLA molecules loaded with the peptide of interest. These “tetramers” bind to the unique cell receptors of T cells that are specific for the antigen/HLA complex being studied and can be used with flow cytometry to provide “real time” monitoring of evolving immune responses during therapy.

A second intriguing candidate leukemia antigen is the Wilms’ tumor gene encoded transcription factor (WT1), which is also markedly overexpressed in AML and CML (as well as in several solid tumors). WT1 is a candidate protein contributing to leukemogenesis, potentially making tumor escape by antigen loss more difficult with this target. Similar to the approach described above, an HLA A201-restricted epitope has been identified from WT1, and T cells specific for this epitope have been generated and shown to kill leukemia cell lines and inhibit colony formation by transformed CD34+ progenitor cells from CML samples, while not affecting normal CD34+ progenitors.

One category of potential antigens in AML are those derived from fusion proteins arising as a result of gene rearrangements that are the hallmarks of specific subtypes of the disease. These targets are attractive in that they represent true tumor-specific (but not necessarily patient-specific) antigens. Furthermore, for certain of these gene products, tumor escape through antigen loss in response to selective immunologic pressure may not be possible if the fusion protein is required for transformation. In spite of these desirable features, one must bear in mind that the antigenicity of a fusion protein is largely influenced by the small number of amino acids that immediately flank the fusion site (the upstream and downstream sequences are unmutated segments of normal self proteins). The novel fusion protein must be cleaved appropriately by proteases to generate candidate antigenic peptides, and those peptides flanking the fusion site must fit the binding motif of a given patient’s HLA antigens. Statistically, it therefore seems unlikely that a particular transformation characteristic of a subtype of AML will provide a “universal antigenic” target that is useful in the majority of patients presenting with the disease.

Finally, as in the case of human melanoma, novel tumor antigens will continue to be discovered through the generation of tumor-specific T cell lines that are then used as probes for the molecular identification of the antigens that they recognize. Similarly, antibodies in patient sera are being used to screen leukemia cDNA expressed by phage display (serologic expression cloning, “SEREX”) to define antigens recognized by the humoral response. Although these strategies represent the most labor-intensive approach to the selection of antigens, the use of such methodologies may be more likely to isolate immunodominant tumor antigens, since identification requires that an in vivo immune response can indeed be generated against the antigen.

**Issues of Tumor Burden, Tolerance, and Chemotherapy-Induced Immunosuppression**

Both mouse models and early phase clinical trials of cancer vaccines suggest that the efficacy of therapeutic immunization appears to be limited to patients with relatively small tumor burdens. Recently, increasing evidence supports the contention that tumor growing in vivo impairs the responsiveness of the immune system to vaccination. There are two mechanisms by which this may occur: 1) induction of tumor-antigen specific tolerance, and 2) tumor-mediated global immunosuppression. Utilizing T cell receptor (TCR) transgenic mice specific for a model tumor antigen, we have recently obtained direct evidence supporting the existence of tumor antigen-specific tolerance that develops with tumor progression.
these studies, early in the course of tumor progression, tumor-specific T cells show clear evidence of encountering tumor antigen and even become partially activated. In spite of this recognition, this population quickly becomes hyporesponsive to subsequent antigenic stimulation, resulting in a markedly blunted response to attempted vaccination. The capacity to regain antigen-specific T cell responsiveness upon reduction of the tumor burden to a state of minimal residual disease with cytoreductive therapies has yet to be determined.

A second means by which tumors may render the immune system unresponsive is illustrated by the global immunosuppression often found in patients with advanced malignancies. Although the mechanisms involved are still not well defined, numerous clinical studies of cancer patients have documented impaired immune function manifested by decreased delayed-type hypersensitivity responses to recall antigens, decreased lymphocyte lytic function, and decreased lymphocyte proliferative responses.23,24 These changes have been reported in patients with renal carcinoma, colon cancer, melanoma, follicular lymphoma, as well as CML and AML.24 Importantly, remission induction (either by surgical debulking for solid tumors or chemotherapy for leukemia) appears to lead to reversal of these alterations and restoration of immune responsiveness.

Because of the initial responsiveness of most hematologic malignancies to chemotherapy, tumor vaccines will be best utilized by integrating them into a setting of minimal residual disease following remission induction. Significantly, although residual AML cells that survive induction/consolidation are typically multi-drug resistant, it has been demonstrated that tumors that are resistant to drug-induced apoptosis remain susceptible to T cell-mediated killing,26 implying that this approach exploits two non-crossresistant forms of therapy.

One concern with the combined use of chemotherapy and vaccine strategies that require an immunocompetent host is the immunosuppression generated by commonly used anti-neoplastic agents. While it is clear that the use of many cytotoxic drugs such as cyclophosphamide leads to suppression of cell-mediated immune responses, other drugs such as the anthracycline doxorubicin have been reported to possess immunostimulatory activities in animal cancer models.27,28 In fact, in one such model, an analysis of nine commonly used anti-neoplastic agents combined with GM-CSF-transduced tumor cell vaccines demonstrated improvement in the cure rate achieved with drug-vaccine combinations over that obtained with either modality alone.29 In this study, the sequence and intervals of drug/vaccine administration proved to be critical parameters effecting outcome. To date, a detailed examination of phenotypic and functional immunologic recovery following standard therapy for AML has not been reported. Clearly, this issue will require careful study for the successful integration of tumor vaccines into the treatment diseases such as AML.

Cancer Vaccines as an Adjunct to Bone Marrow Transplantation

Myeloablative chemo-radiation therapy and bone marrow transplantation are highly effective in reducing the residual tumor burden following remission induction in the treatment of AML. Nevertheless the ultimate success of these therapies remains limited due to substantial rates of tumor relapse (particularly following autotransplants) and the morbidity and mortality of GVHD following allotransplants. Accordingly, efforts to augment tumorspecific immunity in the context of bone marrow transplantation may provide a means to diminish relapse rates without a concomitant increase in toxicity. Unfortunately, hematologic reconstitution following bone marrow or PBSC transplantation is accompanied by a state of global immunosuppression that only gradually recovers to a near normal state of immune function over time.30 Immunosuppression is more severe and recovery more delayed following allogeneic transplantation.

Although the time to achieving a complete recovery of immune function is prolonged following BMT, there is evidence that responsiveness to some forms of vaccination returns fairly early after engraftment. While many clinical reviews on post-transplant immunization do not advocate immunization within the first year of transplantation, responses to vaccination given in the first few months have been reported. In fact, in an analysis of alternate strategies of vaccination against hepatitis B in autologous transplant (ABMT) patients, the seroconversion rate (measured after engraftment) in response to a single immunization given in the pretransplant period (mean 21.1 days pretransplant) was comparable to the reported response rate for healthy volunteers (63% vs. 71%).31 Interestingly, a small subset of the patients in this study (10 patients) received their first vaccination 30-60 days after transplant, with six patients seroconverting, suggesting that responses to this vaccine formulation are possible even quite early after engraftment. Response to vaccination in the early post-transplant period has also reported in a study of lymphoma patients immunized with tumor idiotypic protein conjugated to KLH. Antibody responses to idiotypic and to KLH were detectable as early as 1 month post ABMT. Cellular responses were also detected to KLH, although such responses to idiotypic protein were only seen in patients immunized ten months or more after transplant.

We have established a syngeneic mouse BMT model for the integration of GM-CSF-based tumor cell vaccines into the ABMT setting.32 In this model, functional responsiveness to vaccination (as measured by the ability
to cure an established tumor burden) occurred surprisingly early after transplant—long before reconstitution of normal numbers of peripheral T cells. In fact, a greater percentage of mice were rendered tumor free when vaccinated in the post transplant setting as compared to vaccination of non-transplanted animals that received the same tumor challenge. Mature T cells accompanying the graft participated substantially in this response. Interestingly, in this minimal residual disease model, tumor-specific T cells were found to undergo a massive clonal expansion and activation in the early post-transplant period, which precipitously declined in close temporal association with the development of macroscopic relapse. Vaccination with irradiated GM-CSF-producing tumor cells during immune reconstitution substantially decreased the incidence of tumor relapse and was accompanied by the persistence of an expanded population of activated tumor-specific T-cells. These findings suggest that a “graft-versus-tumor effect” also occurs in the ABMT setting but that it is not sustained. Repeated immunizations during immune reconstitution may serve to maintain the increased precursor frequency and activation state of tumor-specific T cells that is required to prevent relapse.

Efforts to augment the graft-versus-tumor effect through vaccination in the allogeneic setting are also being explored. One concern with this strategy using autologous tumor cell-based vaccines is the potential to exacerbate GVHD, since the vaccine could prime the transplant recipient against minor antigen differences between donor and host. In fact, recent studies using mouse models of MHC-matched, minor antigen-mismatched transplants have demonstrated that tumor cell-based vaccination did indeed exacerbate GVHD when donors were immunized, presumably as a result of priming minor antigen specific T cells that accompany the graft. Presumably T cell tolerance to the minor antigen differences between donor and host was established early in the post transplant period when vaccination occurred. Whether the development of tolerance to tumor antigens follows similar kinetics when tumor is present throughout immune reconstitution remains to be determined. These findings also suggest that it may be possible to achieve reciprocal donor/host tolerance with a non-myeloablative allogeneic transplant and subsequently utilize donor lymphocyte infusions plus vaccination to mediate an anti-tumor effect (see Section IV).

Systemic Immunomodulation as an Adjunct to Immunization

It can be argued that bone marrow transplantation represents an extreme (albeit uncontrolled) example of systemic immunomodulation. Many of the immunologic events that occur during the period of immune reconstitution likely contribute, either positively or negatively, to the ability to generate and sustain tumor-specific immunity in response to vaccination. These include the creation of lymphoid space, disruption of homeostatic mechanisms that normally limit the magnitude of an active immune response, regeneration of the T cell repertoire from both thymic-dependent and post-thymic pathways, skewing of the nascent T cell repertoire toward recognition of antigens encountered during regeneration of the T cell pools, repopulation of the peripheral tissues with new APC populations, and activation of APCs through encounter with microbial products as a result of preparative regimen related tissue damage. As new immune-based therapeutic reagents are developed, it is becoming increasingly possible to selectively manipulate components of these pathways in a more controlled fashion in both the transplant and the non-transplant setting. For example, APC repopulation can be markedly influenced by systemic administration of flt-3 ligand, whereas APC activation may be targeted by the CD40-signaling pathway. Similarly, the magnitude and character of the T cell response to immunization may be influenced by the provision of cytokines (e.g. IL-2, IL-12, IL-15), as well as the manipulation of co-stimulatory pathways, such as through inhibition of CTLA-4 signaling. Finally, the ability to stimulate thymic output in adults following chemotherapy is likely to have a profound impact on vaccine efficacy. Although the optimal growth factor(s) necessary to support this process remain undefined, systemic IL-7 appears to favorably impact on the extent and rate of T cell repopulation in animal models.

Conclusions

Oncologists treating hematologic malignancies have long been aware of the potential of the anti-tumor immune response. The striking remissions occasionally observed in post-transplant lymphomas upon withdrawal of immunosuppression and the dramatic responses of relapsed leukemia to donor lymphocyte infusions demonstrate that this system has a significant impact on treatment outcome. In the development of novel therapeutics, picking the right clinical setting to test and apply a given treatment strategy profoundly influences the likelihood of success. In this light, the progress made in the treatment of acute myelogenous leukemia has provided the immunologist with a remarkable opportunity to improve overall tumor-free survival through the selective elimination
of a relatively small residual leukemic stem cell population—a task for which the immune system appears to be ideally suited. The tools to manipulate immune responses are becoming increasingly available. Reasonable vaccine candidates have been identified, and more will soon follow. The time is ripe for cancer vaccines assume a greater role in the treatment of AML.

IV. TREATMENT OF MYELOID LEUKEMIAS WITH NON-MYELOABLATIVE STEM CELL TRANSPLANTATION: ACCOMPLISHMENTS AND FUTURE GOALS

Shimon Slavin, M.D.*

Recently a strategy—non-myeloablative stem cell transplantation (NST)—was proposed that focused on the use of BMT as a platform for adoptive allogeneic cell therapy with alloreactive donor lymphocytes present in the graft, or using donor lymphocyte infusion (DLI) given post grafting to react against residual malignant cells of host origin.2 The use of BMT as a basis for optimal immunotherapy by allogeneic lymphocytes rather than as a method for administration of maximally tolerated doses of chemotherapy and total body irradiation (TBI) offers many advantages that will be reviewed below. Avoiding or minimizing procedure-related toxicity and mortality as well as late complications may help to improve disease-free survival while using better tolerated procedures in hematologic malignancies (see Table 6).

Table 6. The principles and rationale for non-myeloablative stem cell transplantation (NST) in acute myelocytic leukemia (AML) and myelodysplastic syndromes (MDS).

- Maximal chemoradiotherapy may not be sufficient for eradication of disease in AML/MDS.
- Host-versus-graft tolerance may be accomplished without myeloablation by a window of immunosuppression allowing engraftment of donor stem cells.
- Following engraftment, alloreactive donor lymphocytes may be effective against AML and MDS, despite resistance of tumor cells to chemoradiotherapy.
- Graft-versus-leukemia (GVL) effects may be induced by alloreactive donor T cells in tolerant mixed chimeras; hence, myeloablative conditioning may not be mandatory.
- Following NST, donor lymphocyte infusion (DLI) may eliminate residual or recurrent leukemia.
- NST may be applied after failure of earlier myeloablative BMT or subsequent to autologous BMT for optimal tumor debulking.
- NST may offer an easier, safer and more effective option for inducing GVL effects especially at the stage of minimal disease.

NST in Preclinical Animal Models

The potential of allogeneic BMT to eliminate leukemia through immune-mediated GVL effects has been suggested ever since the earliest days of experimental BMT. In contrast to the dogma that myeloablative chemoradiotherapy may be mandatory for eradication of leukemia, mice inoculated with a lethal challenge of murine B-cell leukemia (BCL1) responded to BMT across MHC following non-myeloablative conditioning with total lymphoid irradiation (TLI), independently of GVHD.2 These observations suggested that non-myeloablative conditioning might be a successful alternative to myeloablative chemoradiotherapy to treat hematologic malignancies, mediated in part by GVL effects inducible in hosts tolerant to donor alloantigens. Although GVL effects normally occur in parallel with acute and/or chronic GVHD, GVL effects independent of clinically overt GVHD have been observed in experimental animals and man, suggesting that the two phenomena are at least partially separable.1,3

Interestingly, as was originally documented in mice, resistance to GVHD increased as the time interval from BMT to DLI increased.4,5 These observations support the efficacy and safety of graded increments of DLI after prolonged time intervals after BMT for treatment of MRD or prevention of relapse.5,6

The Seattle group has used a canine model to suggest that immunosuppression without myeloablation may be sufficient to induce durable engraftment of donor hematopoietic cells, host-versus-graft tolerance, and effective GVL effects independent of GVHD in mixed chimeras.7 These murine and canine experiments supported the hypothesis that marrow grafts could create their own space and that myelosuppressive therapy was not necessary to establish allogeneic engraftment. They further suggested indirectly that TBI may also be replaced with immunosuppressive agents.7

The protective effects against GVHD induced in mixed chimeras may reflect downregulation of alloreactive donor lymphocytes due to the continuous presence of host hematopoietic cells. Mixed chimeras, regardless of the conditioning protocol, show a reduced incidence of GVHD as compared to chimeras conditioned with myeloablative chemoradiotherapy, which are immediately reconstituted with 100% donor hematopoietic cells. It appears that a delicate balance exists between immunohematopoietic cells of host and donor origin as a consequence of hematopoietic competition on the one hand, and downregulatory anergy or veto effects on the other.8 Hence, the higher the inoculum of bone marrow cells, the higher the proportion of donor cells following non-myeloablative conditioning.9

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Clinical Experience Using NST Focusing on AML and MDS

Elimination of all tumor cells in patients with resistant or relapsing AML or patients with MDS is unlikely to be achieved by maximally tolerated chemoradiotherapy alone. DLI has potential efficacy even in some cases resistant to all available anti-cancer modalities, and it therefore appears that NST may replace myeloablative chemotherapy for these patients.10-14 Adoptive allogeneic cell-mediated immunotherapy may be induced by allogeneic lymphocytes or part of the transplant or supplemented with DLI administered late following NST, especially for the treatment of minimal residual disease. This working hypothesis has resulted in the development of new protocols, focusing on immunosuppression rather than myeloablative therapy for engraftment of donor stem cells. In the present report, we have collected data from several BMT centers pioneering the clinical application of NST, focusing on patients with AML and MDS. The large variability of protocols despite the limited number of patients investigated reiterates the need for prospective cooperative studies in order to confirm the potential indications and benefits of NST for each disease category. As recently reported,10-15 non-myeloablative approaches can be roughly divided into three categories: 1) reduced-intensity NST regimens prior to allogeneic stem cell transplantation focusing on pre-grafting immunosuppression; 2) pre-grafting combined with post-grafting immunosuppression directed at both host and donor immunocompetent cells, and 3) high-dose autologous stem cell transplantation for tumor debulking followed by NST to eradicate minimal residual disease by adoptive allogeneic cell therapy. In all these settings, NST serves as the platform for subsequent adoptive immunotherapy of the underlying malignancy using allogeneic donor lymphocytes.

Focusing on Pretransplant Immunosuppression

The first NST protocol was designed to achieve acute immunosuppression for prevention of rejection of donor blood stem cells, by using anti-T lymphocyte globulin (ATG) and incremental doses of cyclophosphamide. Consistent engraftment was observed at cyclophosphamide doses ≥ 50 mg/kg/day x 2 (Slavin et al, manuscript in preparation).

Two centers—the M.D. Anderson Cancer Center in Houston, Texas, and the Hadassah Hospital in Jerusalem—have pioneered the clinical application of purine analogues, 2-deoxyadenosine (2CDA) and fludarabine in NST because of their intense immunosuppressive effects, as manifested by CD4+ and CD8+ lymphopenia, occurrence of opportunistic infections and occurrence of transfusion-associated GVHD following administration of unirradiated random blood products.16,17 Fludarabine proved an effective and well-tolerated immunosuppressive agent, which could be administered safely to infants and elderly individuals, with the exception of patients with renal insufficiency. In Jerusalem, fludarabine was used at 30 mg/m2/day x 6 in combination with busulfan 4 mg/kg/day x 2 (orally or more recently also intravenously), given in conjunction with ATG (Fresenius) 5–10 mg/kg/day x 4. Equally effective alternative protocols, mostly for other disease categories, involved the use of a similar dose of fludarabine with low dose cyclophosphamide 60 mg/kg/day x 2 or one single low dose TBI 200 cGy without ATG. Following lymphoablation, peripheral blood stem cells (PBSC), obtained from an HLA-matched sibling or matched unrelated donor (MUD) pre-treated with G-CSF (5 mg/kg bid for 5 days), or bone marrow cells were infused to induce transplantation tolerance to donor stem cells and lymphocytes. Durable engraftment of donor T cells was mandatory to induce GVL effects to eliminate residual malignant cells of host origin. A transient stage of mixed chimerism was frequently observed prior to elimination of the entire immune system of the patient. Low-dose cyclosporine (CSA) (3 mg/kg/day) was initiated one day prior to transplantation and discontinued within 1–3 months, in the absence of GVHD to facilitate development of GVL effects against malignant host cells. NST could also be used to induce a graft versus tumor response for other diseases.13,18

In Jerusalem a total of 132 patients (age range 3–64 years) with observation periods > 6 months were enrolled in NST trials over the past 5 years. The clinical details of the 40 patients with AML or MDS are shown in Tables 7 and 8. Twenty-one patients with different non-malignant and genetic diseases were also allografted using NST. All patients are available for analysis of toxicity, engraftment and GVHD. Of the 132 patients evaluable for engraftment, 128 received PBSC or marrow cells from a fully matched sibling (n = 112) or MUD (n = 16), and all engrafted. Four other patients were allografted with partially mismatched sibling cells, and one rejected the graft (overall engraftment rate of 99.2%). Some patients had a detectable transient stage of mixed chimerism but most patients converted to 100% donor cells, either spontaneously or after discontinuation of CSA, or when indicated, following additional DLI administered after discontinuation of CSA. Fludarabine-based NST regimens were better tolerated in comparison with standard myeloablative regimens. Following NST, the patient’s well being was excellent, and for many patients in good performance status, much of the conditioning could be administered on an outpatient basis. Among the first patients analyzed, one-third did not develop complete aplasia, 10% did not develop granulocytopenia below 0.5 x 10^9/L and approximately 10% required neither blood nor platelet transfusions throughout the posttransplant course.
Following NST most patients were eating a normal diet. Mucositis was absent or very mild. GVHD was the single major problem, with an overall incidence of 47%. GVHD was the primary cause of death in 14% of the patients. Nonetheless, the majority of the patients (57%) developed no or only Grade I GVHD while on CSA. GVHD was frequently activated when CSA was discontinued too soon, within one month of transplantation, or when DLI was attempted too soon. Day 100 mortality was 4% and the overall probability of relapse for the whole group reached 23%. To date, with a follow-up of up of nearly 5 years, the probability of survival and disease-free survival were 58% and 42%, respectively. Of 16 patients treated with DLI for recurrent disease, with or without prior chemotherapy, 11 responded.

In Jerusalem, the same fludarabine/busulfan/ATG protocol was used for 16 patients receiving transplants from fully matched unrelated donors,19 of whom four had AML. The series is too small for a detailed analysis of MUD transplants for AML except to indicate that it may

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### Table 7. NST for patients with AML receiving grafts from matched sibling or matched unrelated donors.

<table>
<thead>
<tr>
<th>Total No.</th>
<th>Donors</th>
<th>Conditioning</th>
<th>Status (No.)</th>
<th>Engraftment</th>
<th>Alive &amp; Disease-free</th>
<th>Center</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>Sibling</td>
<td>Fludarabine 30 mg/m²/d x 6&lt;br&gt;Busulfan 4 mg/kg/d x 2&lt;br&gt;ATG (Fresenius) 5-10 mg/kg x 4</td>
<td>First CR (10)&lt;br&gt;Second CR (7)&lt;br&gt;Relapse (3)</td>
<td>20/20</td>
<td>12/20</td>
<td>Jerusalem, Israel</td>
</tr>
<tr>
<td>4</td>
<td>Sibling</td>
<td>Fludarabine 30 mg/m²/d x 6&lt;br&gt;TBI 200cGy</td>
<td>Relapse (4)</td>
<td>4/4</td>
<td>2/4</td>
<td>Jerusalem, Israel</td>
</tr>
<tr>
<td>4</td>
<td>Sibling</td>
<td>Fludarabine 30 mg/m²/d x 6&lt;br&gt;Busulfan 4 mg/kg/d x 2&lt;br&gt;ATG (Fresenius) 5-10 mg/kg x 4</td>
<td>Relapse after autoBMT</td>
<td>4/4</td>
<td>2/4</td>
<td>Jerusalem, Israel</td>
</tr>
<tr>
<td>4</td>
<td>MUD</td>
<td>Fludarabine 30 mg/m²/d x 6&lt;br&gt;Busulfan 4 mg/kg/d x 2&lt;br&gt;ATG (Fresenius) 5-10 mg/kg x 4</td>
<td>First CR (2)&lt;br&gt;Second CR (1)&lt;br&gt;Relapse (1)</td>
<td>4/4</td>
<td>3/4</td>
<td>Jerusalem, Israel</td>
</tr>
<tr>
<td>29</td>
<td>Sibling</td>
<td>Fludarabine 30 mg/m²/d x 4&lt;br&gt;Ara-C 2 mg/m² x 4&lt;br&gt;Idarubicin 12 mg/m²/d x 3&lt;br&gt;or 2 CDA 12 mg/m²/d x 5&lt;br&gt;Ara-C 1 g/m²/d x 5</td>
<td>First CR (6) ³&lt;br&gt;Second CR (20)&lt;br&gt;Relapse (21)</td>
<td>20/29</td>
<td>9/29</td>
<td>Houston, Texas, USA</td>
</tr>
<tr>
<td>16</td>
<td>Sibling</td>
<td>Fludarabine 30 mg/m²/d x 4&lt;br&gt;Melphalan 140 mg/m²/d&lt;br&gt;Or 180 mg/m²/d</td>
<td>First CR (1) ³&lt;br&gt;Second CR (1)&lt;br&gt;Relapse (16)</td>
<td>14/16</td>
<td>5/16</td>
<td>Houston, Texas, USA</td>
</tr>
<tr>
<td>13</td>
<td>MUD</td>
<td>Fludarabine 25 mg/m²/d x 5&lt;br&gt;Melphalan 180 mg/m²/d</td>
<td>&gt; Second CR (3)&lt;br&gt;Relapse (10)</td>
<td>12/13</td>
<td>5/13</td>
<td>Houston, Texas, USA</td>
</tr>
<tr>
<td>4</td>
<td>Sibling</td>
<td>Fludarabine 30 mg/m²/d x 6&lt;br&gt;Busulfan 4 mg/kg/d x 2&lt;br&gt;ATG (Fresenius) 5-10 mg/kg x 4</td>
<td>First CR (3)&lt;br&gt;Second CR (1)</td>
<td>4/4</td>
<td>4/4</td>
<td>Brno, CzechRepublic</td>
</tr>
<tr>
<td>11</td>
<td>Sibling</td>
<td>Fludarabine 30 mg/m²/d x 5&lt;br&gt;Busulfan 4 mg/kg/d x 2&lt;br&gt;or 3.3 mg/kg/d IU</td>
<td>First &amp; Second CR (6)&lt;br&gt;Advanced (5)</td>
<td>11/11</td>
<td>7/11</td>
<td>Dresden, Germany</td>
</tr>
<tr>
<td>16</td>
<td>MUD</td>
<td>Fludarabine 30 mg/m²/d x 5&lt;br&gt;Busulfan 3.3 mg/kg/d x 2&lt;br&gt;ATG (Merieux) 2.5 mg/kg/d x 4</td>
<td>First CR (5)&lt;br&gt;Advanced (11)</td>
<td>16/16</td>
<td>7/16</td>
<td>Dresden, Germany</td>
</tr>
<tr>
<td>7</td>
<td>MUD</td>
<td>Fludarabine 30 mg/m²/d x 3&lt;br&gt;TBI 200 cGy</td>
<td>Unavailable</td>
<td>7/7</td>
<td>6/7</td>
<td>Leipzig, Germany</td>
</tr>
<tr>
<td>5</td>
<td>Sibling</td>
<td>Fludarabine 30 mg/m²/d x 3&lt;br&gt;Busulfan 4 mg/kg/d 2&lt;br&gt;Cyclophosphamide 350 mg/m²/d x 3</td>
<td>Unavailable</td>
<td>5/5</td>
<td>4/5</td>
<td>Mexico City, Mexico</td>
</tr>
<tr>
<td>3</td>
<td>Sibling</td>
<td>Fludarabine 30 mg/m²/d x 5&lt;br&gt;Busulfan 4 mg/kg/d x 2</td>
<td>Unavailable</td>
<td>3/3</td>
<td>3/3</td>
<td>Hammersmith, London, UK</td>
</tr>
</tbody>
</table>

Abbreviations: ATG, antithymocyte globulin; MUD, matched unrelated donors; 2 CDA, 2 chlorodeoxyadenosine
Table 8. NST for patients with MDS receiving matched sibling or matched unrelated donor (MUD) grafts.

<table>
<thead>
<tr>
<th>Total No.</th>
<th>Donors</th>
<th>Conditioning</th>
<th>Status (No.)</th>
<th>Engraftment</th>
<th>Alive &amp; Disease-free</th>
<th>Center</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>Sibling</td>
<td>Fludarabine/Bu/ATG</td>
<td>RAEB (3)</td>
<td>3/3</td>
<td>3/3</td>
<td>Jerusalem, Israel</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Second RAEB (5)</td>
<td>5/5</td>
<td>2/5</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Sibling</td>
<td>Flag/Ida or 2CDA/araC</td>
<td>First CR (2)</td>
<td>3/4</td>
<td>2/4</td>
<td>Houston, Texas, USA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Relapse (2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Sibling</td>
<td>Fludarabine /melphalan*</td>
<td>Relapse (4)</td>
<td>4/4</td>
<td>1/4</td>
<td>Houston, Texas, USA</td>
</tr>
<tr>
<td>4</td>
<td>MUD</td>
<td>Fludarabine/melphalan*</td>
<td>Relapse (4)</td>
<td>4/4</td>
<td>1/4</td>
<td>Houston, Texas, USA</td>
</tr>
<tr>
<td>5</td>
<td>MUD</td>
<td>Fludarabine/Bu/ATG</td>
<td>RAEB-T (3)</td>
<td>3/5</td>
<td>1/5</td>
<td>Dresden, Germany</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>RAEB (1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Third PR (1)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* 140 or 180 mg/m²

Abbreviations: Bu, busulfan; ATG, antithymocyte globulin; Flag/Ida, fludarabine, cytosine arabinoside, G-CSF, and idarubicin; RAEB-T, refractory anemia with excess blasts in transformation

be feasible. In general, NST was much better tolerated compared with conventional myeloablative protocols. All patients maintained oral intake throughout the procedure and there were no episodes of sepsis, pulmonary toxicity or any serious veno-occlusive disease of the liver. Acute GVHD > Grade II was observed in three patients: Grade III in two; Grade IV in one patient. No patient developed chronic GVHD during a median follow-up period of 12 (range 3–25) months. The actuarial Kaplan-Meier survival and disease-free survival for the entire group at 12 months were 75% and 70%, respectively. Three of the four (75%) patients treated with MUD-NST are alive and disease free with 100% donor chimerism. Three of these patients responded with disappearance of host type cells and are currently disease-free.

Protocols developed at the M.D. Anderson Cancer Center were based on immunosuppression with either cladribine (2CDA, 60 mg/m²) or fludarabine (90–120 mg/ m²) in conjunction with non-myeloablative doses of chemotherapy to allow engraftment of HLA-compatible hematopoietic progenitor cells in patients with CML or recurrent AML as well as for lymphoid malignancies.

Some of the regimens were extremely well tolerated and allowed consistent engraftment with cells from matched sibling donors (Table 7). Unfortunately, no data was available using similar protocols for AML or MDS, to evaluate the efficacy for these diseases. Giralt et al reported on several protocols combining either 2CDA and cytosine arabinoside (ara-C) 1 g/m²/day for 5 consecutive days; or fludarabine and ara-C 2 g/m²/day for 5 consecutive days and idarubicin for 3 days supported by G-CSF starting from day –1 for the treatment of patients with advanced AML treated with matched sibling allografts (n = 29) or MDS (n = 4) (Tables 7,8). Alternatively, fludarabine was combined with melphalan 140 or 180 mg/m² for patients grafted with cells from matched siblings (n = 16). Fludarabine was combined with melphalan 180 mg/m² for patients with AML (n = 13) or MDS (n = 4) grafted from matched unrelated donors (Tables 7,8). Most patients with refractory relapse had rapid recurrence and progression of their disease, but 56% of patients with chemotherapy sensitive disease remained in continuous remission over one year. The rate of relapse was higher for patients with no GVHD in comparison with patients with GVHD (p = 0.0002). Specific outcome for patients with AML and MDS is shown in Tables 7 and 8. Taken together, the M.D. Anderson experience confirms that NST may serve as a replacement for myeloablative BMT, especially for older patients and those with co-morbidities that preclude high-dose chemoradiotherapy.

The use of NST for patients with AML in other centers is still limited. Mayer et al from Brno, the Czech Republic, were the first to confirm the efficacy of the Jerusalem NST protocol in a series of 16 patients, of whom four had AML and one MDS, with an age range of 43–55 years. NST was well-tolerated and very limited GVHD was noted in two patients (one skin and one liver). All four patients with AML are alive and well (range 54–891 days) (personal communication). Data from two Mexican institutions, Puebla and Monterrey, describe a total of 22 patients, five with AML, age range of 14–62 (median 46) years, treated with a modified fludarabine-based NST including busulfan and cyclophosphamide (Table 7) (personal communication). Data from two Mexican institutions, Puebla and Monterrey, describe a total of 22 patients, five with AML, age range of 14–62 (median 46) years, treated with a modified fludarabine-based NST including busulfan and cyclophosphamide (Table 7) (personal communication). All patients engrafted (one displaying mixed chimerism). The protocol was well tolerated and carried out in an outpatient clinic. No patient developed acute GVHD, and only one developed chronic GVHD.

Bornhauser et al from Dresden, Germany used the Jerusalem protocol but eliminated ATG for patients with matched siblings (Table 7) (personal communication).
Out of a total of 11 patients with AML treated with PBSC from matched siblings, six are in CR (age range 38–65) and seven patients are alive and well (range 90–546 days). Only three patients developed GVHD < grade II (Table 8). The same group has also used the Jerusalem protocol, with ATG (Merieux) 2.5 mg/kg x 4 for MUD recipients (Tables 7,8). A total of 21 patients, 16 with AML and five with MDS, with age range of 16–63 (median 50) years received MUD allografts. Mixed chimerism was observed in five cases whereas the others converted to 100% donor type cells. Fourteen patients showed no or no more than grade I GVHD and their outcome is shown in Tables 7 and 8.

Seven patients with AML who received MUD allografts were reported by Niederweiser from Leipzig, on behalf of the cooperative group, including Seattle, Stanford and Leipzig. Patients were conditioned with a slight modification of the Seattle protocol (Table 7). All patients engrafted and full donor type chimerism was confirmed in all seven patients (median 100; range 95–100%) (Table 7). At the time of this report, one patient has relapsed and six patients are alive and disease free with a median follow up 228 days (range 156–367 days) following NST.

Craddock and colleagues from the Hammersmith Hospital in London pioneered the use of NST using a fludarabine/busulfan-based protocol with T cell depletion by positive selection of CD34+ stem cells (Table 7) (personal communication). In a group of CML and AML patients, all recipients of T cell-depleted allografts engrafted. No GVHD ≥ grade II was seen in any of the nine recipients of T cell-depleted allografts, suggesting that following NST, at least for CML, T-cell depletion may be considered for induction of host versus graft tolerance, followed by DLI. However, only three of the patients had AML so there is not yet enough information to evaluate if such a protocol is safe and effective for patients with AML and MDS.

Pre-Grafting Combined with Increased Post-Grafting Immunosuppression

The conditioning regimen developed in Seattle included TBI 200 cGy pre-transplant on day 0, and post-grafting immunosuppression with CSA and MMF (Table 7). Eligibility for the study required a contraindication to the use of conventional allografting because of age, prior high-dose therapy, or organ dysfunction. Forty-six patients with a median age of 56 years (range 31–72) were recently reported with a variety of hematologic malignancies. Transplants were very well tolerated with mild myelosuppression, no development of mucositis and no alopecia. The only significant regimen-induced toxicity included reversible hepatotoxicity in three patients. Of the 46 patients treated, non-fatal graft rejection occurred in nine (16%) patients. (Four patients were not evaluable because of death.) Spontaneous acute GVHD requiring treatment occurred in 36% of patients. Transplant-related deaths occurred in three patients (6.5%). With median follow-up of 160 (range 30–450) days, significant disease responses have been observed in the majority of patients with sustained engraftment after transplant. Responses have frequently been gradual in onset, occurring over a periods of 4–12 months. This is an important difference from conventional allografting in which detection of disease post transplant is usually considered failure of the treatment approach. Residual disease following NST served as an indication for DLI. An update of the results of the cooperative study group (Seattle, Leipzig & Stanford) focusing on a cohort of 14 patients at a median age of 60 (36–71) years with AML (nine in first CR; three with induction chemoradiotherapy failure; one in second and one in third CR) transplanted with cells from a matched sibling, with a median follow-up of survivors of 14 (range 1–25) months found that the protocol was extremely well tolerated. Nine of 14 are alive, seven in complete remission (one is a mixed chimera) (Table 7). No transplant-related mortality was observed, but seven of 14 relapsed and five died of relapse. One patient rejected the graft and another is a mixed chimera (P. McSweeney, personal communication). Of 32 patients receiving MUD allografts reported on behalf of the same cooperative group, seven patients had AML. Patients were conditioned with the Seattle protocol plus additional fludarabine. All patients engrafted and full donor type chimerism was confirmed in all seven patients (range 95–100%) (Table 7). No graft rejection was observed. One patient relapsed. At the time of reporting 6 patients were alive and disease free with a median follow up 228 (range 156–367) days (D. Niederweiser, personal communication).

Tumor Debulking with High Dose Chemotherapy and Autologous Stem Cell Support Followed by NST for Induction of GVL Effects

The concept of using allogeneic cell therapy, analogous to DLI, for eradication of minimal residual disease following high dose chemoradiotherapy in conjunction with autologous BMT was pioneered in 1992. This concept was improved by combining autologous BMT for maximal tumor debulking, and NST for induction of durable GVL effects following induction of host-versus-graft tolerance for patients with advanced malignancies. The NST regimen developed in Genoa was based on the use of the combination of fludarabine and cyclophosphamide protocol developed at the M.D. Anderson Cancer Center. A total of 25 patients, mostly with lymphoma but also including five MDS and one AML-M4 patients, are
Table 9. Potential advantages of non-myeloablative stem cell transplantation (NST).

- Minimal procedure-related toxicity and mortality.
- Minimal risks of late effects in young individuals (e.g. growth retardation; endocrine adenopathies; sterility, etc).
- For elderly individuals with AML and MDS.
- For patients with poor performance status.
- For continuous GVL effects by DLI in mixed chimeras.
- Transient mixed chimerism may allow better control of GVHD (yet to be proven).

Conclusions and Future Developments
Non-myeloablative conditioning associated with a sufficient degree of immunosuppression can be very well tolerated and can offer the possibility of engraftment of donor stem cells, which results in induction of transplantation tolerance to donor alloantigens. Engraftment of donor T cells present in the graft, or DLI administered following NST, can effectively eradicate otherwise incurable hematologic malignancies as well as additional life-threatening non-malignant diseases correctable by replacement of host with donor stem cells. NST is well tolerated in patients of all age groups, including those with poor performance status. Although larger numbers of patients need to be investigated for longer observation periods to fully assess the benefits of various NST regimens for AML and MDS, it appears that one of the major advantages of NST is its better immediate outcome. NST is associated with shorter periods of pancytopenia, reduced incidence of side effects, low incidence of mucositis, lower consumption of blood products and reduced mortality. It is therefore anticipated that NST may eventually provide a safer therapeutic option that may help accomplish the goal of improved disease-free survival and better quality of life for recipients with matched siblings donors or MUD. For the young age group, NST may offer all the benefits of BMT with a lower incidence of long-term procedure-related hazards, possibly even avoiding sterility. Furthermore, NST makes it possible to offer safer outpatient BMT procedures. NST may open the field of stem cell transplantation to a larger number of patients for a wider spectrum of clinical indications for both malignant and non-malignant diseases. Following NST, mixed chimerism or recurrent disease may be effectively reversed with DLI. With respect to AML, if the benefits of NST are confirmed in larger series, NST will need to be compared prospectively with conventional BMT.

In the future, specific attention must be given to better control of GVHD. Once accomplished, NST may provide the proper setting for more intensive cell-mediated immunotherapy by adoptive transfer of specifically immune rather than naïve lymphocytes. Future developments should include either pre- or post-grafting immune regulation to control GVHD, or clinical application of innovative protocols for induction of graft-versus-host unresponsiveness in vitro with subsequent immunotherapy/tumor vaccination (see Section III).

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IV. Treatment of Myeloid Leukemias with (NST)


