Aplastic Anemia & MDS International Foundation (AA&MDSIF) created this report of the presentations given at the first annual International Bone Marrow Failure Scientific Symposium in Washington, D.C. from October 17-19, 2005. This document offers a three part summation of each presentation: a very brief summary written in easy-to-understand language; a more detailed description written by a medical reporter; and the speaker’s abstract as they appeared in the Program Guide distributed to symposium attendees. Presentations are listed in the order they were given, under the topic of their plenary session.
Dear Colleagues,

We are excited to greet colleagues from all over the world who are visiting Washington, D.C in order to participate in a conference dedicated to bone marrow failure syndromes. Organized by the Aplastic Anemia and MDS International Foundation, sponsored by the National Institutes of Health, and funded by NIH institutes as well as generous contributions from private industry, the conference is an excellent example of tripartite collaboration among government, academia, and drug manufacturers with a special interest in these diseases and their amelioration. The major objects of the conference are to compare and contrast the pathophysiology and treatments of historically diverse syndromes now increasingly recognized to share both common mechanisms of disease and responsiveness to therapy. For, aplastic anemia, paroxysmal nocturnal hemoglobinuria, and myelodysplasia, the relationship between the clinical manifestations of disease and basic biological processes can often be easily discerned, making them ideal subjects for laboratory study. It is important that we have been able to apply knowledge in the laboratory to the benefit of patients, and conversely that study of these diseases has yielded fundamental biological information. In this conference, we will have the opportunity to hear the experts from Europe, the United States, and Asia. We hope for animated discussions, enthusiasm and criticism, and inspiration for the younger members of the audience!

Sincerely,

Jaroslaw Maciejewski, M.D., Ph.D.  
Co-Chair

Neal S. Young, M.D.  
Co-Chair
September 20, 2005

Dear Colleagues:

It is with great pleasure that the Aplastic Anemia and MDS International Foundation (AA&MDSIF) is able to present the Bone Marrow Failure Scientific Symposium which you are now attending. The Symposium represents an unparalleled opportunity to hear from virtually all of the world’s experts on the biology and treatment of Aplastic Anemia, Paroxysmal Nocturnal Hemoglobinuria, Myelodysplastic Syndromes and related disorders. This is a unique opportunity to be able to focus on these issues, consider what is known, and be stimulated to think collectively about development of new ideas and directions.

The AA&MDSIF is committed to the rapid translation of scientific discoveries toward patient benefit. Improvement in the quality of life and overall survival of patients with aplastic anemia, PNH, and myelodysplastic syndromes has been the primary goal of the foundation since its inception. Research grants, patient education, and patient centered meetings have been the mainstay of the organization. This symposium affords the opportunity to enhance research in our focus area.

The symposium contents are a stimulating and relevant combination of a review of basic research concerning the immunological and other mechanisms of primary bone marrow failure as well as an up-to-the-minute review of current treatment approaches. These approaches range from novel agents like lenalidomide in the myelodysplastic syndromes, anti-cytokine therapy in PNH, immunomodulation in aplastic anemia and stem cell transplantation for each of these diseases. The internationally respected speaker panel in this meeting represents a most impressive array of accomplished contributors. The basic biology of the stem cell and the bone marrow microenvironment is covered in depth as is the epidemiology of myelodysplastic syndrome, a topic to which much current energy is being focused. In summary, I am extremely proud that the AA&MDSIF under the leadership of Marilyn Baker and under the scientific direction of Drs. Maciejewski and Young offers this stellar symposium.

Sincerely,

Richard M. Stone, M.D.
Chairperson
AA&MDSIF Medical Advisory Board
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Hôpital Saint Louis, France

Andre Tichelli, M.D.
Universitätsspital Basel, University Clinics, Switzerland
AGENDA

STEM CELL TRANSPLANTATION
Chair: Theo De Witte, Ph.D.

Severe aplastic anemia: conventional and alternative donor transplantation
David Margolis, M.D., Children’s Hospital of Wisconsin, Wisconsin

The role of graft engineering in transplantation
Rupert Handgretinger, M.D., St. Jude Children’s Research Hospital, Tennessee

Long-term outcomes after transplantation for aplastic anemia
Gerard Socie, M.D., Ph.D., Hôpital Saint Louis, France

Hematopoietic stem cell strategies in patients with myelodysplastic syndromes and secondary acute myeloid leukemia: the role of reduced intensity conditioning regimens
Theo de Witte, M.D., Ph.D., University Hospital Nijmegen, Netherlands

IMMUNOSUPPRESSIVE TREATMENT OF BONE MARROW FAILURE SYNDROMES
Chair: Neal S. Young, M.D.

Treatment of aplastic anemia in children
Seiji Kojima, M.D., Ph.D., Nagoya University Graduate School of Medicine, Japan

Short and long-term outcomes after ATG therapy
Neal S. Young, M.D., National Heart, Lung, and Blood Institute, Maryland

Advances in current immunosuppressive therapies in AA: European experience
Andre Tichelli, M.D., Universitatsspital Basel, University Clinics, Switzerland

Immunosuppressive treatment for myelodysplastic syndromes
A. John Barrett, M.D., PRCP, FRCPath, National Heart, Lung and Blood Institute, Maryland

NOVEL THERAPIES
Chair: Alan List, M.D.

New treatment strategies in MDS: non-cytokine hematopoietic promoters
Alan List, M.D., H. Lee Moffitt Cancer Center & Research Institute, Florida

Novel therapeutic approaches in paroxysmal nocturnal hemoglobinuria
Peter Hillmen, M.D., FRCP, FRCPath, Ph.D., Leeds Teaching Hospitals NHS Trust, United Kingdom

Cytoxic vs. non-cytoxic therapy for high-risk MDS
Elihu Estey, M.D., University of Texas MD Anderson Cancer Center, Texas
STEM CELLS AND BONE MARROW FAILURE DISORDERS
Chair: Judith CW Marsh, M.D.

Qualitative and quantitative defects in the stem cell function in aplastic anemia
Judith C.W. Marsh, M.D., St. Georges Hospital Medical School, United Kingdom

Paroxysmal nocturnal hemoglobinuria as a stem cell disease
Lucio Luzzatto, M.D., FRCP, FRCPath, Instituto Toscan Tumori, Italy and University of Genova, Italy

Mechanisms of clonal evolution in bone marrow failure
Elaine M. Sloand, M.D., National Heart, Lung, and Blood Institute, Maryland

Application of high resolution genomic scan in bone marrow failure syndromes
Jaroslaw P. Maciejewski, M.D., Ph.D., Taussig Cancer Center, Cleveland Clinic Foundation, Ohio

EPIDEMIOLOGY OF THE BONE MARROW FAILURE SYNDROMES
Chair: David W. Kaufman, Sc.D.

Epidemiology of the bone marrow failure syndromes: aplastic anemia and myelodysplastic syndromes
David W. Kaufman, Sc.D., Boston University School of Public Health, Massachusetts

Aplastic anemia in the Orient
Surapol Issaragrisil, M.D., FRCP, FACP, FRCPath, Siriraj Hospital, Mahidol University, Thailand

Genetic risk factors for bone marrow failure
Neal S. Young, M.D., National Heart, Lung, and Blood Institute, Maryland

IMMUNE PATHOPHYSIOLOGY OF BONE MARROW FAILURE SYNDROMES
Chair: Jaroslaw P. Maciejewski, M.D., Ph.D.

Autoimmunity in MDS: friend & foe
Jeffrey J. Molldrem, M.D., University of Texas MD Anderson Cancer Center, Texas

Autoantigens of marrow failure syndromes
Shinji Nakao, M.D., Ph.D., Kanazawa University Graduate School of Medical Science, Japan
Severe Aplastic Anemia: Conventional and Alternative Donor Transplantation

David Margolis, M.D., Medical College of Wisconsin, Milwaukee, Wisconsin

**Capsule Summary:** Many patients with severe AA receive transplants from a matched sibling donor. Advances in drug therapy have achieved excellent results in these patients without radiation. Patients with unrelated or unmatched family donors face greater challenges. However, new drug therapies (or lower doses of old ones) promise improved outcomes and reduce late side-effects.

**Detailed Summary:** For patients with severe aplastic anemia (SAA), the conventional donor transplant is bone marrow from a matched sibling donor. Such transplants are a well-established approach to treating SAA, although outcomes vary. Challenges include survival, acute or chronic graft vs. host disease (GvHD), rejection, late cancers, and fertility problems. Long term studies may provide some answers.

In a study beginning in 1988, 81 SAA patients received cyclophosphamide (CY) and anti-thymocyte globulin (ATG), plus methotrexate (MTX) and cyclosporine (CSA) to prevent GvHD. One-year rejection rates were below 5%, and 87% of patients survived 16 years or more. The risk of developing chronic GvHD was greater in patients over 38 years old and those who received a higher dose of nucleated marrow cells – a lower cell dose may actually be better. A 12-year follow-up found that more late cancers had developed in patients with cGvHD. On the positive side, 17 successful pregnancies (patients or partners of patients) showed that the CY regimen preserves fertility.

Another long term study compared the effects of CY-plus-radiation to CY-plus-ATG. Survival was higher among patients who did not receive radiation. Both acute and chronic GvHD were less frequent in non-radiation patients, but long term data show that chronic GvHD is a problem requiring attention. Newer treatments such as Campath (alemtuzumab, a monoclonal antibody) or graft engineering (to enrich the population of marrow-repopulating cells) are decreasing the risk of cGvHD. In general, CY/ATG provides excellent results for patients with an HLA-matched sibling donor.

**Alternative donor transplants** are from an unrelated donor or mismatched family member. Sources include marrow, peripheral blood cells (natural or depleted/enriched), or cord blood. Historically, the major barriers to survival have been rejection and GvHD. A therapeutic approach developed in Milwaukee tries to balance the use of total body irradiation (TBI; to prevent graft rejection) against T-cell depletion (to prevent GvHD). Over 18 years, about 55% with 45% of patients showed event-free survival. The discrepancy is secondary to two cases of therapy related cancer. Survivors reported problems such as short stature, short-term memory loss, fertility, bone, and gynecological issues, and dental problems - in general related to TBI. We need an alternative donor transplant regimen that prevents rejection, prevents GvHD, prevents late effects, and has excellent long term survival.

A five year study gave CY/ATG to prevent rejection, MTX/CSA for GvHD prevention, and "de-escalated" total body irradiation. After two years, 58% survived, with higher survival rates in younger patients and those with shorter disease duration. This study is the basis for a current
North American trial using de-escalating doses of CY. The European Group for Bone Marrow Transplantation has also published recently showing even better survival with an even reduced intensity regimen. Future decisions include the use of fludarabine-based therapy (with or without Campath, ATG, CY, or TBI). Marrow transplants may include T-cells or be T-cell depleted, or cells may come from peripheral blood stem cells or cord blood. Alternative donor transplants may come from unrelated or haplo-identical donors.

The outcomes for alternative donor transplants are improving, as scientists develop regimens leading to fewer late effects.

**Presenter's Abstract:** Allogeneic Hematopoietic Progenitor Cell (HPC) transplant is an established curative approach to severe aplastic anemia (SAA). By definition, a conventional donor transplant is a transplant utilizing an HLA matched sibling while an alternative donor transplant uses HPC from anyone else. The risks and benefits to transplant are often based on the medical condition of the patient as well as the histocompatibility between the patient and the donor. Historically, the major barriers to survival after a HPC transplant have been rejection and graft versus host disease. Because allogeneic transplants have been utilized for over 25 years for SAA and because newer transplant packages have emerged with exciting data, this talk will focus on the following questions:
1. What have we learned from the long-term follow-up data from transplants done in the past?
2. What are we learning from the short-term data from transplants done recently?
3. What do we need to learn/research regarding the natural history and pathophysiology of SAA in order to maximize the role of transplant as a curative approach for this disease?

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**The Role of Graft Engineering in Transplantation**

*Rupert Handgretinger, Peter Lang, Xiaohua Chen, Raymond Barfield, Wing Leung, Mario Otto, Stanley Chaleff, Paul Woodard, Gregory Hale. St. Jude Children's Research Hospital, Memphis, USA and Children's University Hospital, Tübingen, Germany.*

**Capsule Summary:** In clinical studies scientists can now choose cells from bone marrow or peripheral blood that are best suited to the patients that receive them. Transplanting the cells that suit each patient's needs will improve graft success, and decrease problems like graft vs. host disease, rejection, and relapse.

**Detailed Summary:** Whether bone marrow or peripheral blood is used as a source of stem cell transplants, graft versus host disease (GvHD) has remained a problem. The problem is especially serious for patients who have trouble finding a well-matched donor. But scientists are beginning to “engineer” cell grafts and tailor them to the needs of the patient and their disease. These tailor-made cell grafts, already being used at St. Jude's and various centers across Europe, frequently reduce the risk of GvHD and other complications.

CD34+ lymphocytes are very effective for repopulating the bone marrow, whereas T-cells are programmed to attack foreign substances. Scientists can now “sort” donor cells in a magnetic field, selecting for CD34+ cells and rejecting T-cells. In fact, so few T-cells are retained in the graft that toxic pre-transplant conditioning can be greatly reduced. Three patients with sickle cell disease received CD34+ enriched stem cell transplants from partially matched family donors after reduced intensity conditioning (RIC). In spite of the partial match and the reduced conditioning regimen, two of the three grafts have been successful.

Natural killer (NK) cells benefit some patients by killing mutant and virus-infected cells. Patients with acute lymphoblastic leukemia (ALL) are also less likely to relapse after receiving donor NK cells that are not inhibited by the patient’s HLA system (a so-called “perfect mismatch”). Graft engineering techniques have been used to increase the concentration of stem cells and enrich NK cells in the transplant. In this protocol, anti-thymocyte globulin (ATG) is excluded from the therapy because of its anti-NK cell activity.
These are only two examples of the exciting opportunities offered by graft engineering. The techniques can be applied to peripheral blood stem cells and tailored to select the cell populations most beneficial to each patient. Because graft engineering usually decreases the intensity of the rejection response, it broadens the applications of RIC and increases the donor pool for many patients.

**Presenter’s Abstract:** Allogeneic Hematopoietic Stem Cell Transplantation (HSCT) is for a variety of malignant and nonmalignant disorders the only known curative approach. While in the beginning, bone marrow has been the major donor source, mobilized peripheral stem cells are increasingly used in both malignant and nonmalignant diseases. Despite considerable progress over the years, Graft-versus-Host Disease (GvHD), infections and relapse of the underlying disease are still major problems in allogeneic HSCT. In addition, HLA-mismatched haploidentical donors are increasingly selected for patients with otherwise no HLA-suitable donor. Recent advances in stem cell manipulation technologies, which allow the large scale clinical processing of bone marrow and PBSC allow the preparation of defined stem cell or other immune effector cell products adjusted to the patients’ underlying disease.

We have developed positive selection strategies of CD34+ or CD133+ stem cells from PBSC for the transplantation of patients with malignant and nonmalignant diseases from matched unrelated and haploidentical donors and the different strategies will be discussed in the context of specific diseases. For patients who might benefit from the infusion of alloreactive Natural Killer (NK) cells, we have developed negative depletion strategies of CD3+ T-cells. Such T-cell depleted PBSC grafts are highly enriched in NK and dendritic cells. We have used this T-cell depletion strategies in myeloablative haploidentical transplantation. In addition, we have shown that CD3 depleted PBSC from haploidentical donors allow the use of Reduced Intensity Conditioning (RIC) in three-loci mismatched transplantation. In our preliminary clinical experience, haploidentical transplantation using RIC is associated with a very rapid immune reconstitution and low transplant-related toxicity and is currently used in patients with hemoglobinopathies and aplastic anemia. In addition to stem cell manipulation strategies, techniques are now available for the clinical large scale purification of other important effector cell populations, such as NK cells or regulatory T-cells. These technologies present a platform which allows graft engineering strategies adapted to the patients’ disease and to the type of donor.

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**Long-term Outcome after Transplantation for Aplastic Anemia**

*Gerard Socie, M.D., Ph.D., Hôpital Saint Louis, France*

**Capsule Summary:** Successful stem cell transplants depend on factors such as the use of radiation, patient’s age, graft vs. host disease, and previous immunosuppressive therapy. Radiation can cause other cancers, so its use in stem cell transplants (SCT) is decreasing. For patients under 40, the effects of immuno-suppression are being questioned – future research will show which AA therapies are best.

**Detailed Summary:** When patients with aplastic anemia (AA) receive a transplant from an HLA-identical sibling, they are usually prepared with anti-thymocyte globulin (ATG) and cyclophosphamide (CY). For patients under 20 years old, the five-year survival rate after transplant is 84%. For patients 20-40, and over 40 years old, the survival rates are 67% and 68% respectively. Graft versus host disease (GvHD) is a major problem for transplant patients. GvHD develops most often during the first year after transplantation, although some cases appear five to 10 years later.

Radiation therapy reduces graft rejection, but is often associated with chronic GvHD and secondary tumors. If the patient’s conditioning includes total body irradiation (TBI), they may develop problems with fertility, growth and development, thyroid and dental problems. TBI, chronic GvHD, and corticosteroid therapy can all cause cataracts, “dry eyes,” osteoporosis, and lung complications. Secondary tumors may develop, or the quality of life can decrease: this includes emotional and social functions, body pain, vitality, and general health.

Studies of marrow transplantation in severe AA involved 212 patients at the Fred Hutchinson Cancer Research Center and 133 patients at Hôpital St. Louis. Some patients were
conditioned with CY and ATG, while others received CY and thoraco-abdominal radiation (TAI). Patients who received CY/ATG achieved nearly 99% survival. Factors with negative effects on survival were: age over 14 years, GvHD, and CY/TAI. Of eleven solid tumors diagnosed in the study, nine were in patients who received TAI.

The next question is: “Why do patients develop chronic GvHD?” The contributing factors are previous acute GvHD and the use of methotrexate alone (without cyclosporine) for GvHD prevention. CY/ATG conditioning is usually associated with less acute GvHD, and reduces the occurrence of chronic GvHD more effectively than CY/TAI conditioning does.

A study of 24,011 patients with stem cell transplants included 58 patients with squamous cell carcinoma (SCC; a cancer affecting cells of body surfaces) and 125 patients with non-SCC. Chronic GvHD and its treatment increased the occurrence of SCCs but not the occurrence of non-SCCs. More SCCs occurred with longer GvHD therapy, use of azathioprine, and severe chronic GvHD. Because these factors often overlapped, their individual effects are not known.

Another study compared Fanconi’s anemia patients who received stem cell transplants with those who did not. The risk of SCC was 4.4 times higher in transplant patients than patients without transplants. SCCs occurred in younger patients in the transplant group. Both acute and chronic GvHD increased the risk of developing SCC.

Researchers agree that CY/ATG produces better results than radiation conditioning. Both TAI and GvHD reduce survival, and radiation may cause secondary tumors. Although chronic GvHD is less frequent with CY/ATG, it is still the leading factor in transplant-related mortality.

**Presenter’s Abstract:** Allogeneic bone marrow transplantation (BMT) from an HLA-identical sibling is a curative form of therapy for patients with acquired severe aplastic anemia (SAA). Survival has significantly improved over the past three decades, mainly with the introduction of cyclosporine A (CsA) in the early eighties, which resulted in reduced graft rejection and deaths due to infections: the actuarial risk of rejection has been reduced from 24% in the seventies to the current 7%. While irradiation pre transplant also reduces the incidence of graft rejection, it is associated with early and late sequelae such as chronic graft versus host disease (GvHD) and secondary tumors. Improved results with survival in excess of 90% has been reported in sensitized patients conditioned with the association of cyclophosphamide and anti-thymocyte globulin by the Seattle group and by other investigators. Even today, nearly a third of BMT currently performed for SAA are done with irradiation-based conditioning both in Europe and in the United States (European Group for Blood and Marrow Transplantation and International Bone Marrow Transplant Registry).

Immunosuppressive therapy (IST) based on antithymocyte globulin (ATG) and CsA has been given with increasing success to patients without an HLA identical sibling, the results being correlated with the severity of neutropenia at the time of treatment. The combined use of ATG and CsA together with hematopoietic growth factors has produced promising results also in patients with very low neutrophil counts. Thus it turned out that some authors advocate the use of IST upfront, even in patients with severe disease and with a sibling donor, and use BMT only in case of IST failure or relapse.

Factors previously related to lower survival after BMT include, older age, interval between diagnosis and transplant, number of transfusions, absence of CsA for GvHD prophylaxis. However, most studies were performed from retrospective analyses of registries or included few patients, and only two studies reported detailed analysis on long-term outcome following BMT for SAA.

In 1991 we reported our experience using the combination of cyclophosphamide (CY) and thoraco-abdominal irradiation (TAI) as conditioning regimen before BMT for SAA. In this previous analysis we showed that although leading to survival rate in the range of 70%, Cy-TAI was associated with increased risk of development of secondary malignancies. We thus decided to definitively stop the use of irradiation based conditioning for SAA. Following the report by Storb et al in 1994 showing excellent survival and few rejections with the association of CY and ATG, we uniformly used the Seattle conditioning regimen. With a 13.6 years follow-up, the main findings of our study included decreased survival associated with the use of Cy-TAI, and a detrimental effect on the long term of any IST before transplantation for aplastic anemia.
Hematopoietic Stem Cell Strategies in Patients with Myelodysplastic Syndromes and Secondary Acute Myeloid Leukemia: The Role of Reduced Intensity Conditioning Regimens

Theo de Witte M.D., Ph.D., Department of Hematology, Radboud University Medical Centre Nijmegen, The Netherlands

**Capsule Summary:** The success of stem cell transplants is growing, due to better donor-patient matches, earlier transplants, and fewer infections. Transplants from matched unrelated donors are nearly as successful as those from sibling donors. And for patients over 60, reduced intensity conditioning, introduced in 1997, has increased the use of allogeneic stem cell transplants.

**Detailed Summary:** The results of stem cell transplants (SCT) are improving, due largely to better cell typing and the ability to select more closely matched donors. Closer matches decrease the occurrence of graft vs. host disease (GvHD) and infections related to GvHD and immunosuppression. Rates of transplant-related mortality have fallen from 50% to around 30%, and survival has increased from 35% to 50% among those transplanted in recent years. A study compared four year survival rates for several types of allogeneic SCTs (transplants from someone other than the patient). Whether the cells came from the bone marrow of a matched unrelated donor, the bone marrow of an HLA-identical sibling, or the peripheral blood of an HLA-identical sibling, four year survival rates were essentially the same. Early transplants and anti-fungal and anti-viral agents contributed to this improvement.

Another improvement in allogeneic SCTs is the introduction of reduced intensity conditioning (RIC). RIC was introduced in 1997, and its use is increasing. There is confusion about RIC because so many different regimens are used. Some advocate moderate cytoreduction (reduction of cell number) as opposed to myeloablative conditioning in which all marrow stem cells are destroyed. Other RIC regimens increase immunosuppression, or combine cytoreduction with immunosuppression. Since the introduction of RIC, more allogeneic SCTs have been done in patients over 60. RIC is now used more frequently than myeloablative conditioning in older patients. Comparing standard (myeloablative) conditioning with RIC: failures after standard conditioning were about equally divided between relapse and treatment-related mortality (TRM) – but in older patients TRM was more frequent. RIC had the same overall failure rate, but fewer failures were due to TRM. A five-year study is underway to compare the outcomes of RIC and standard conditioning.

Finally, the choice of a treatment regimen and its success are influenced by a combination of factors. These include patient age, percent of blasts (immature cells) in the bone marrow, and donor availability. Younger patients generally do better than older patients. Patients with fewer than 10% blasts (immature cells) in their marrow generally have better results than patients with more than 10% blasts. Because the outcome of SCT is superior for patients with fewer blasts in their marrow, chemotherapy is advised before SCT in patients with more than 10% blasts. For younger patients with fewer than 10% blasts but no sibling donor, we recommend allogeneic SCT with an alternate donor. For patients over 50 or 60, chemotherapy is advised.

**Presenter’s Abstract:** Allogeneic stem cell transplantation (alloSCT) is the treatment of choice in the majority of young patients with advanced stages MDS or sAML if they have a suitable donor. Long-term disease-free survival may be attained if the transplant is performed in an early stage of the disease. Since outcome of transplantation is superior for patients with a low blast percentage, this supports the use of chemotherapy prior to transplantation in patients with high blast marrow infiltration. The allogeneic transplant procedure continues to carry a high treatment-related risk, but results have improved progressively over the years. The results of transplantation using phenotypically matched voluntary unrelated donors have improved recently, mainly due to significantly reduced transplantation-related mortality rate (largely due to molecular typing and the ability to...
select more closely matched donors). The upper age limit for transplantation has moved to 65-70 years after the introduction of reduced intensity conditioning regimens (RIC). The place of RIC remains to be determined also in older patients since the transplant results utilizing conventional regimens have also improved in recent years. For patients lacking a suitable donor the choice is ambiguous. Although the number of reports on autologous stem cell transplantation is still limited, the outcome seems similar to allogeneic SCT with donors other than HLA-identical siblings. The presence of sufficient numbers of residual polyclonal stem cells and achieving a complete remission after chemotherapy form a prerequisite for a successful autologous transplantation. AutoSCT is a valid option for patients who fulfill these criteria. For patients who fail to enter complete remission alloSCT with unrelated donors may be a treatment option in young patients. Further development of accurate prognostic classification systems will allow a risk-adapted strategy for an individual patient.
IMMUNOSUPPRESSIVE TREATMENT OF BONE MARROW FAILURE SYNDROMES
Chair: Neal S. Young, M.D.

Treatment of Aplastic Anemia in Children
Seiji Kojima M.D., Ph.D., Nagoya University Graduate School of Medicine, Japan

Capsule Summary: Patients with AA must often decide between several treatments. Scientists in Japan found that treatments with similar survival rates may have very different rates of “failure-free survival.” They also stress the importance of good donor/recipient matches for bone marrow transplants, and offer advice to patients who have trouble finding a fully-matched donor.

Detailed Summary: Deciding between alternative treatments is often difficult for patients and their physicians. Researchers in Japan tackled this problem, focusing on AA therapies in children. Many patients under 18 years old don’t respond to immunosuppressive therapy (IST). Their second-line treatment options are stem cell transplantation (SCT) if a suitable donor is available, or a trying a second round of IST. In this study, “failure-free survival” means survival of the patient with response to therapy. “Failure” means no response after six months, progression of AA requiring other treatments, relapse, or death. The difference between “survival” and “failure-free survival” is very important. The patients started either SCT or IST as a second-line therapy. After five years, survival rates for both groups were above 90%. However, IST was much less effective at normalizing blood counts – the failure-free survival rate was nearly 84% for SCT but less than 10% for IST. SCT offered a much better chance for failure-free survival.

The next question was: What is the best therapy for non-severe AA in children – antithymocyte globulin (ATG) alone, or ATG and cyclosporine (CSA)? Survival rates in both treatment groups were well above 90%. However, failure-free survival occurred in 55% of patients receiving ATG and CSA, but in only 27% of patients getting ATG alone. The combination therapy was superior in terms of failure-free survival.

The following project was not limited to younger patients: How important is the match of human leukocyte antigens (HLA) between a bone marrow recipient and an unrelated donor? Matching the donor/recipient HLA-A and -B antigens is the most crucial survival factor in an unrelated bone marrow transplant. But HLAs come in many varieties, and the importance of HLA-C, HLA-DRB1 and HLA-DQB1 required further study. The researchers analyzed 79 recipient/donor pairs in the Japan Marrow Donor Program. Some of these pairs matched for all antigens, some had one mismatch, or two, or even three. Apparently, a single mismatch doesn’t hurt survival rates. However, if HLA-A or -B don’t match, mismatches of other antigens significantly reduce survival. These HLA antigens should also be considered when matching donors with recipients.

The researchers then compared the effectiveness of tacrolimus and short-term methotrexate vs. cyclosporine and short-term methotrexate for preventing graft vs. host disease (GvHD) in patients who receive bone marrow transplants (BMT) from an unrelated donor. Approximately 30% of patients in both groups developed acute GvHD. Chronic GvHD occurred less frequently in the tacrolimus group. In addition, 84% of tacrolimus patients survived at least five years, compared with 56% of patients receiving cyclosporine. The advantages of the tacrolimus/methotrexate therapy are obvious.

Finally: “What treatment is best for patients who don’t respond to IST but cannot find matched donors for SCT?” The research above provides an answer: “Bone marrow
transplantation from an unrelated donor with one HLA antigen mismatch." For patients who fail to respond to intensive IST and do not find a fully-matched donor, this may be the treatment of choice.

**Presenter’s Abstract:** A significant proportion of children with aplastic anemia (AA) are refractory to immunosuppressive therapy (IST). Although the efficacy of repeated IST and stem cell transplantation (SCT) from an alternative donor has been confirmed, a direct comparison between these two procedures has not been yet conducted. We prospectively compared the efficacy of these two treatments. A total of 120 very severe AA and 86 severe AA children received antithymocyte globulin (ATG) and cyclosporine (CyA). Sixty patients were non-responders to first-line IST and were eligible for 2nd-line therapies. Of these, thirty-one patients received SCT from an alternative donor. Twenty-one patients lacking suitable donors received 2nd IST. The overall probability of survival at 5yrs from the start of 2nd-line treatment was 90.3% in SCT group and 95.2% in the IST group (p=0.49). However, trilineage response to a 2nd course of IST was seen in only 2 of 18 evaluable patients. The estimated FFS at 5yrs was significantly better (p<0.0001) in the SCT group (83.9%) than in the IST group (9.5%). Our previous study revealed that mismatching of HLA-A and -B antigens, and not of HLA-DRB1 antigen was the most crucial risk factor for survival after unrelated BMT. Because only the data of HLA-A, -B and DRB1 were available, the role of HLA-C and DQB1 mismatching was not clarified in the previous study. We aimed to evaluate the effects of HLA-C and DQB1 mismatching. We selected 79 recipient-donor pairs from database of JMDP in which molecular analysis of HLA-A, -B, -C, -DRB1, and DQB1 were performed. Of the 79 pairs, 26(33%) were found to be matched at HLA-A, -B, -C, -DRB1, and DQB1; 18(23%) were mismatched at a single HLA antigen (6 HLA-A or -B, 7 HLA-C, 5 HLADRB1 or HLA-DQB1); 31(39%) were mismatched at two HLA antigens (5 HLA-DRB1 and HLA-DQB1, 18 HLA-A or –B and HLA-C or -DRB1 or -DQB1, 8 HLA-C and HLA-DRB1 or -DQB1); and 9(11%) were mismatched at 3 HLA antigens. Of 79 patients, 44 survived. The 5-year survival rate did not differ between recipients transplanted from a full matched donor (68.5%) and those from any single HLA mismatched donors (60.7% to 80.0%). In addition, the 5-year survival rate was not worse (62.5%) in patients transplanted from HLA-C and HLA-DRB1 or -DQB1 mismatched donors. However, the 5-year survival rate was significantly worse (38.9%) in recipients who transplanted from HLA-A or -B mismatched and –C or -DRB1 or -DQB1 mismatched donors and it was 0% in patients transplanted who were mismatched at 3 antigens. In multivariate analysis, the relative risk for HLA-A or -B and -C or -DRB1 or -DQB1 mismatching was 4.24 (95% CI, 1.57-11.5) and for 3 antigen mismatching, it was 20.0 (95% CI, 5.57-71.6). The current JMDP study showed that mismatching of a single HLA antigen did not result in increased mortality. However, in patients with HLA-A, or -B mismatching, additional mismatching at HLA-C, -DRB1, or -DQB1 had a significant adverse effect on survival. Matching of HLA-C and -DQB1 should be incorporated into algorithms for unrelated donor selection.

**Short and Long-Term Outcomes after ATG Therapy**
Neal S. Young, M.D., Elaine Sloand, and Philip Scheinberg, M.D., Hematology Branch, NHLBI, Bethesda MD

**Capsule Summary:** AA therapy has gone through many changes in the past 40 years. Today, ATG and CSA are commonly used to suppress immune responses in AA patients. Some patients respond well to immune therapy, but others benefit from bone marrow transplants. Scientists have found that a patient’s age and blood counts are the best predictors of their response to therapy.

**Detailed Summary:** In the 1960s and 70s, aplastic anemia (AA) was treated with transfusions, antibiotics, and corticosteroids. Even with these therapies, most patients with severe AA died in one or two years. Then anti-thymocyte globulin (ATG) came into use for people whose transplants failed. ATG increased their cell counts. In combination with cyclosporine (CSA), ATG could suppress immune function and block the activity of T-cells (white blood cells programmed to attack foreign substances). Nearly two-thirds of patients responded to this therapy. Yet, survival is not always the best definition of a good response to therapy. The best indication of response to AA therapy is a blood count. When counts no longer qualify as AA, the outcome is good. Patients who respond well, with platelet counts above 50,000, have a survival rate greater than 90%.
Relapse is a problem, and probably means that the immunosuppressive therapy (IST) isn’t adequate. But relapse does not appear to decrease survival. Patients can go back on CSA, with monitored dose and reactions, until counts improve again. Another problem in long term survivors is monosomy 7 or trisomy 8 – in each case, a group of mutant cells in the bone marrow gains a survival advantage over other cells. The risk of this occurring in 10 years is 20%. Trisomy 8 has 100% survival, but the outlook for monosomy 7 is less encouraging.

Although IST is often considered the ideal therapy for older patients, most of those over 60 will die of the disease. However, studies in Japan and in Germany showed that children with severe AA respond very well to IST. From 1981 to 1991, IST and BMT survival improved greatly due to CSA use. Weighing the advantages of bone marrow transplant (BMT) vs. IST, major factors seem to be a patient’s age and neutrophil counts. BMT is preferable with low counts and in young patients; IST is recommended with higher counts and in older patients.

The action of a drug called mycophenolate mofetil (MMF) is synergistic (enhances the effect) with CSA. When used together, CSA dosage can be reduced, decreasing kidney damage. MMF blocks an increase in the number of lymphocytes. However, when MMF was tested in 104 patients, the outcomes were nearly the same as those with ATG/CSA. Another drug, sirolimus (Rapamune), acts on T-cells and is complementary to CSA but less toxic. Some studies found improved survival when sirolimus was given with CSA, but statistical analysis detected a trend toward lower response with sirolimus. Patients also developed more bleeding complications, so the use of sirolimus is not being pursued. Instead, National Heart Lung and Blood Institute (NHLBI) is currently looking at rabbit ATG, alemtuzumab, and tapering CSA after the initial response to ATG/CSA. Additional approaches to IST involve Campath and Daclizumab, antibodies that bind to lymphocytes and other white blood cells.

IST for severe AA has progressed from corticosteroids (response rate about 10%), to ATG (40-50%), to ATG plus CSA (60-70%). The response limit seems to be about 70%. Factors that limit success are a severe stem cell deficit, relapse, and the formation of groups of resistant cells (as in monosomy 7 and trisomy 8). Failure to respond to IST seems to correlate with lower numbers of stem cells. It would be helpful to have some indication of a patient’s response before treating them with IST. A patient’s age and blood counts – particularly counts of immature red blood cells – are probably the best predictors of their response to IST.

**Presenter’s Abstract:** Antithymocyte globulin (ATG)-based immunosuppressive regimens have markedly improved the prognosis of patients with severe aplastic anemia who are unable to undertake allogeneic stem cell transplantation. Retrospective analyses of collected European data have indicated no statistical differences in survival between patients who have been transplanted compared to those treated with ATG, although specific subgroups may do better with transplant (younger or more neutropenic cases) or ATG (older or less neutropenic patients). In contrast to transplant using a sibling donor, immunosuppressive treatment has more long-term complications related to the underlying hematologic disease, especially relapse and evolution to late clonal disease such as myelodysplasia and acute leukemia. Transplant from unrelated donor sources, which historically have yielded inferior results to standard protocols utilizing matched siblings, have shown improved outcomes with more immunosuppressive conditioning regimens. Nevertheless, most Aplastic anemia is currently initially treated with ATG, usually in combination with cyclosporine. Efforts to improve immunosuppression have focused on increasing early hematologic response rates and decreasing late complications. Accurate numbers for response to ATG alone vary widely from center to center, ranging from 20-80%, probably depending on criteria for response as well as differences in patient selection. Cyclosporine represented a significant advance in immunosuppressive protocol design, improving response rates and survival. At NIH, the addition of cyclosporine to ATG raised increased hematologic response rates measured at 3 or 6 months from about 40% to >60%, using our strict criteria of no longer meeting the Camitta requirements for severe pancytopenia. Early robust responses (platelet or absolute reticulocyte levels = 50,000/μL) correlate with long-term survival. However, efforts to improve outcomes by addition to the ATG plus cyclosporine regimen of agents that act synergistically (mycophenolate, a tolerizing drug through its inhibition of the purine salvage pathway of lymphocytes) or complement cyclosporine's mechanism of action (rapamycin, an inhibitor of transcription that does not affect calcineurin) have not yielded the expected improvement in response rates. In the few randomized trials conducted, ATG has usually proven superior to cyclosporine alone. These data suggest that further improvements to immunosuppressive therapy should focus on cytotoxicity for lymphocytes rather than inhibition of their activity. In our experience, high dose cyclophosphamide's myelosuppression resulted in unacceptable toxicity due to prolonged neutropenia. Small uncontrolled study of fludarabine has
suggested that this agent may be effective and equivalently simple to administer. Repeated courses of ATG, usually rabbit following horse, have produced responses in refractory aplastic anemia patients. One current NIH protocol compares rabbit ATG to Campath 1H, a monoclonal antibody to CD52, in refractory disease: good hematologic responses have been seen with both treatments. More limited monoclonal antibody therapy with Daclizumab (anti-CD25), which can be administered in the clinic and has little toxicity, produces hematologic remission in about 50% of patients with moderate aplastic anemia and pure red cell aplasia. Our new protocol for severe aplastic anemia at presentation will randomize to three arms: 1) horse ATG with a long-course of cyclosporine at low dose; 2) rabbit ATG with cyclosporine for 6 months; and 3) Campath without cyclosporine. Ultimately, immunosuppressive approaches to aplastic anemia may be limited by the residual stem cell number, perhaps predictable by presentation blood counts. Earlier application of transplant from unrelated donors and experimental approaches to increase stem cell number through ex vivo manipulation may better serve patients with markedly deficient hematopoietic reserves.

**Advances in Current Immunosuppressive Therapies in AA: European Experience**

**André Tichelli, M.D., Universitatsspital Basel, University Clinics, Switzerland**

**Capsule Summary:** Five-year survival rates for AA have increased to 87% in patients receiving immune therapy, and new drugs are being tested for patients who don’t respond to immune therapy. A drug that decreases infections in AA patients is being studied in eight European countries.

**Detailed Summary:** Survival is no longer an unusual outcome in aplastic anemia. Five-year survival rates increased from 54% before 1980 to 73% in the 90s, and recent data for AA treated with immunosuppression show 87% survival. The most potent immunosuppressive treatment combines antithymocyte globulin (ATG) and cyclosporine (CSA). Higher rates of remission and failure-free survival result from this combination than from ATG alone, and patients treated with ATG/CSA recover faster. However, many remissions are unstable, and event-free survival (without relapse or complications) has decreased. Although 35-40% of patients have relapsed after ten years and about 25% depend on CSA to maintain remission, repeated courses of immunosuppression are usually effective.

A more serious problem confronts patients who don’t respond to immunosuppression. Some scientists believe that AA in these patients originates with a stem cell defect rather than their immune system. Tests of mycophenolate mofetil (MMF), an immunosuppressive drug, were unsuccessful in patients who had not responded to standard immunosuppression. This suggests a non-immune cause for AA in these patients. It also alerts researchers to the shortcomings of testing new drugs only in resistant patients. By contrast, Campath-1H (an experimental drug used in multiple sclerosis) produced rapid responses in patients with autoimmune neutropenia (AIN) who had failed to respond to other therapies.

Survival rates are typically worse for patients over 60. This is unrelated to response or relapse rates, but reflects a higher risk of death early in treatment from bleeding or infections. To prevent infections, granulocyte-colony stimulating factor (G-CSF) has been used to increase the number of neutrophils (a type of white blood cell that combats infection). When three months of G-CSF treatment was combined with ATG/CSA therapy, survival and response rates were unchanged, but patients developed fewer infections. Surprisingly, when two different G-CSF doses were tested, the lower dose was better in terms of remissions, percent survival, and event-free survival. G-CSF also improves survival rates in children, although no effect was seen in adults. Scientists in eight European countries are collaborating on an extensive study of the effects of G-CSF on failure-free survival, mortality, and rate of hematologic (blood related) response in severe AA patients treated with ATG/CSA. This project, which will eventually enroll 340 patients, focuses on the relationships between patient age, severity of disease, and G-CSF, as well as on the occurrence of early mortality in older patients.

**Presenter’s Abstract:** Immunosuppression is the treatment of choice for patients with aplastic anemia not eligible for bone marrow transplantation. So far, the combination of ATG and Cyclosporine represents the gold
standard to which new protocols have to be compared. The combination provides the best results in respect of response, event free survival, and recovery time. However, remissions are unstable and the patients have a relapse rate at 10 years of between 35-40%. Furthermore, about 25% of the patients treated with a Cyclosporine containing regimen are dependent on the drug to maintain remission, some of them during more than 10 years. Relapse does not affect outcome of the aplastic anemia patients, since most relapses are repeatedly re-treatable with immunosuppression. In contrast, patients refractory to previous immunosuppression have a worse prognosis, and are unlikely to respond to a further course. It could be hypothesized that the mechanism of aplastic anemia in refractory patients may be different and, rather than an autoimmune etiology, there may be an intrinsic stem cell defect. This could be one of the reasons why none of 14 patients with acquired aplastic anemia failing with standard immunosuppressive treatment responded to mycophenolate mofetil. This should be considered when designing studies for the evaluation of new immunosuppressive drugs in aplastic anemia.

In contrast to hemopoietic stem cell transplantation, immunosuppressive treatment has no upper age limit. Patients older than 70 years have been successfully treated with ATG and Cyclosporine. However, the overall survival of elderly patients is inferior when compared to younger adults, because of the higher risk for early death due to bleeding and infections. Response rates and the risk of relapses were similar to younger patients. Older patients had a lower standardized mortality ratio because of the inherent higher risk for death in elderly healthy persons. Therefore, to some extent at least, the decrease in survival among older patients is due to the deaths that can be expected with increasing age in a general population. The improvement of outcome in elderly patients could be obtained by shortening the period of low neutrophil count to lower the risk of infections. Early death due to infection and bleeding is still of concern during the first months after treatment, before immunosuppression starts to work. A prospective randomised European study comparing ATG and Cyclosporine with or without three months of G-CSF showed no difference in survival or response rates but did show a reduction in the incidence of serious infections. The lack of difference in survival and event free survival could be due to the small number of patients. Indeed in order to find a difference of 10% in survival and/or disease free survival in between the two arms for an expected overall survival at 5 years of 60-80% and a disease free survival of 30-50%, it is anticipated that a minimum of 170 subjects have to be enrolled on each arm. Therefore, to evaluate the effect of G-CSF on mortality and failure free survival in patients treated with ATG and Cyclosporine, a further and larger prospective randomized study is in progress. In September 2005, 162 of the projected 340 aplastic anemia patients were included.

Immunosuppressive Treatment for Myelodysplastic Syndromes

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Capsule Summary: Nearly a third of MDS patients respond to standard immune therapy – but which ones are they? Studies show that younger patients with low platelet counts and short term dependence on red cell transfusions are most likely to benefit. Successful therapy lengthens their survival and does not increase their chance of developing leukemia.

Detailed Summary: An autoimmune process may cause low cell counts in some patients with myelodysplastic syndromes (MDS). Immunosuppressive therapy (IST) with antithymocyte globulin (ATG) – alone or combined with cyclosporine (CSA) – increases blood cell production in about 30% of MDS patients with cytopenia (low cell counts). Centers throughout the world have obtained response rates of 30-45% in MDS patients treated with ATG. It would be useful to predict which MDS patients will benefit from IST, and what the long term outcomes will be.

MDS biology involves chromosome abnormalities, disordered blood cell formation, and an expanded population of T-lymphocytes which damage the blood-forming system. When the T-cell population expands, their cytotoxicity (toxic effect on cells) kills cells and lowers cell counts. IST stops this process in its tracks so normal blood cell formation can occur.

One study treated 61 MDS patients with ATG. In the 34% that responded, initial neutrophil counts were low but more than doubled after ATG therapy. The response was rapid and lasted five years or more in 76% of the patients. Among non-responders, initial neutrophil counts were higher before treatment.
A statistical analysis of MDS patients treated with ATG and CSA revealed that hematological (blood-related) response to IST depends on a patient’s age, platelet levels, length of dependence on red cell transfusions, and HLA-DR15 (a leukocyte antigen more common in people with MDS or AA). Response to IST is highest in younger patients with HLA-DR15+, short transfusion dependence, and severely low platelet levels. A follow-up study confirmed that these are good predictors: IST response occurred in two-thirds of the patients predicted to respond, but in none of those predicted to fail.

Records of survival, response, and leukemic progression in IST patients were compared with those of untreated controls. It is known that in non IST-treated MDS, age (below 60 is better) and IPSS stage (IPSS is a scoring system that estimates length of survival based on bone marrow make-up, cytogenetics, and cytopenias.) are the major factors that affect survival. Average survival for patients receiving IST was about 16 years, compared to 5 years for untreated patients. Progression to leukemia was less frequent in IST recipients whether or not they responded; and IST responders had the best long term leukemia-free survival. In patients over 60 years old, IST had no significant effect on survival. To summarize: the response of MDS patients to IST is predictable, durable, and responders have prolonged survival. IST patients fare better than untreated controls, and those under 60 have longer leukemia-free survival.

**Presenter’s Abstract:** There is both clinical and laboratory-based evidence that the cytopenias in myelodysplastic syndromes (MDS) have an autoimmune component and that immunosuppressive therapy (IST) can improve cytopenias in some patients. Using antithymocyte globulin (ATG) with or without cyclosporine, about 30% of unselected cytopenic MDS patients can be expected to respond to treatment with clinically significant improvement in hematopoiesis as measured by durable independence from platelet or red cell transfusions and correction of profound neutropenia. Here we present further studies to determine factors predictive for response, the fate of patients given IST, and the optimum way to give IST. A multivariate analysis of patients treated with ATG and cyclosporine at NIH formed the basis for a predictive score for hematological response to IST based on HLA DR15 type, age and duration of red cell transfusion-dependence, where HLA-DR15+, younger age and short transfusion dependence periods were favorable. Patients designated as high probability responders (HP) had a 90% chance of hematological response while patients with intermediate or low probability of response (LP) had less than 20% chance of response. To evaluate long-term outcome after IST, we studied 129 patients with MDS (94 int-1, 13 int-2, 16 low and 6 high International Prognostic Scoring System [IPSS]) given IST on investigational protocols between 1998-2004. Seventy-four received ATG 40mg/kg x 4 days; 42 received a combination of ATG and cyclosporine (CsA); and 13 received CsA alone for up to 6 months. Median follow-up was 43 months (range 3 weeks to 10 years). Overall, 29% IST patients responded. The prognostic score was highly predictive for hematological response to IST: 38/55 (69%) responses in HP patients vs. 0% in 77 LP patients, p<0.0001. ATG+CsA was more likely to induce response than ATG alone (p=0.015). Only two responders relapsed and required re-treatment with ATG; both responded to the second treatment. Seven of eight (87%) int-1 patients< 60 with trisomy 8 as the sole abnormality and 16/34 with normal cytogenetics (47%) responded to one course of ATG; all 11 int-1 patients with 5q were > 60 years of age and none responded to IST. Marrow cellularity did not influence response to IST. We then compared survival, response, and leukemic progression in IST patients with historical non-IST controls submitted to the International MDS Risk Analysis Workshop (MRAW) database. Age and IPSS stage were the major factors affecting survival in IST treated and untreated patients. The survival of int-1 patients age = 60 was >16.4 years for the 44 IST patients and 5.2 years for the untreated 87 IMRAW patients (p=0.0016). Progression to leukemia (time for 25% patients to develop AML) in int-1 patients < 60 years was also less frequent in the IST recipients: >16.4 vs 6.9 years for IMRAW patients, p<0.001. There was no significant difference in survival between IST and IMRAW int-1 patients > 60 years of age. These results identify a younger MDS population with a high probability of durable hematological responses to IST. Results suggest that more intensive IST regimens have the best chance of inducing response.
NOVEL THERAPIES
Chair: Alan List, M.D.

New Treatment Strategies in MDS: Non-Cytokine Hematopoietic Promoters
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Capsule Summary: New approaches are being developed for MDS therapy. Some focus on modulating the activity of the immune system. Others modify chemical pathways that control the formation and death of blood cells. Current clinical trials are adding to our understanding of MDS, and will contribute to more effective treatments in the future.

Detailed Summary: Drug companies are currently developing new myelodysplastic syndromes (MDS) therapeutics to target the ineffective blood cell formation fundamental to MDS. These categories of drugs have distinct modes of action. Among the novel therapeutics are anti-angiogenic agents – which include a subset of immunomodulatory drugs (IMiDs) – and survival signal modifiers.

Anti-angiogenic agents inhibit tumor growth by preventing or limiting the development of new blood vessels. IMiDs include thalidomide and its less famous relative, lenalidomide. Lenalidomide (also known as CC-5013, or Revlimid™) is not toxic to the nervous system and does not cause birth defects. It increases immune responses such as interferon production and the activity of natural killer cells. Most importantly, it increases the signaling of erythropoietin receptors – signals that stimulate red blood cell (RBC) formation. Lenalidomide itself has some effect on RBC formation and the effect of erythropoietin is even greater. But when erythropoietin and lenalidomide act together, the formation of RBCs increases dramatically. In 36 MDS patients treated with lenalidomide, 21 experienced a “major erythroid response.” Responses were durable: 14 of the 21 were still responding two years later. Lenalidomide was effective in patients who had failed prior treatment with erythropoietin or were unlikely to benefit from it. Because marrow suppression is a common side effect, causing low neutrophil and platelet counts, close observation and the use of growth factors are required.

An interesting aspect of lenalidomide is its toxic effect on acute myelogenous leukemia (AML) cells with a deletion in chromosome 5 (del 5q). Lenalidomide selectively induces apoptosis (programmed cell death) in del 5q cells. Thus, the effect of lenalidomide in MDS is two-pronged: it promotes RBC formation in non-del 5q cells, but is toxic to del 5q cells. Clinical development of lenalidomide involved groups of patients with and without the 5q deletion. About 67% of patients with del 5q achieve transfusion independence with lenalidomide therapy, and this rate of success is unaffected karyotype complexity. However, suppression of neutrophil and platelet counts is more severe in del 5q patients. Lenalidomide is currently in Phase III trials in the U.S.

Novel therapeutics which modify survival signals:
- TLK199 (Telintra™) promotes blood cell formation by enhancing signals that stimulate marrow formation. A Phase I/II study has been completed in MDS patients. Toxic effects were nausea/vomiting and hypotension with the liposomal formulation.
- P38α/MAPK modulates the inhibitory signals that lead to programmed cell death (apoptosis).
- SCIO469 inhibits p38α/MAPK and promotes the survival of progenitor cells (cells that generate more cells) including CD34+ cells, and CD71+ cells which lead to RBC formation. SCIO469 suppresses del 5q cells and enhances the survival of cells with normal chromosome numbers. So far, no drug-related toxicity has been reported.
• Additional new drugs (such as Fibrogen, FB-2216) promote red cell formation and increase iron utilization. Other therapeutics focus on increasing the number of platelets and megakaryocytes (large marrow cells that form platelets). These agents include Src family kinase (SFK) inhibitors, and AMG 531 and SB 497115-GR which begin trials next year. As our understanding of MDS increases, therapeutic advances will enable doctors to tailor therapy to the biology of the disease.

Presenter’s Abstract: The development of non-cytokine therapeutics with specificity for critical targets implicated in the ineffective hematopoiesis phenotype represents the primary goal for lower-risk MDS. Mechanistically, such agents can be broadly categorized as either anti-angiogenic or survival signal modifiers. Investigations of anti-angiogenic agents in MDS have shown that the predominant responsive lineage is erythroid. Thalidomide and its analogue, lenalidomide, are the most active members of the immunomodulatory drug (IMiD) class. Lenalidomide is a 4-amino glutarimide derivative that lacks the neurologic toxicity and teratogenicity of thalidomide, and is a more potent modulator of ligand-induced responses. The activity of lenalidomide in MDS was first studied in an open-label, single-center phase I/II efficacy and safety trial in which all patients had either failed prior treatment with EPO or had low probability of benefit. Twenty of 36 evaluable patients (67%) experienced an erythroid response according to the International Working Group criteria, with 21 patients achieving sustained transfusion independence. Response rate was highest among patients with a chromosome 5q31.1 deletion (91%), a subset that also had a high frequency of cytogenetic response, a finding consistent with preclinical studies that lenalidomide is directly cytotoxic to deletion 5q clones. A 148 patient multi-center trial (MDS-003) confirmed the activity of lenalidomide in transfusion-dependent patients with Low or Int-1 risk MDS with a chromosome 5q31 deletion. In an intent-to-treat analysis after 24 weeks of treatment, 111 patients (75%) experienced an erythroid response, with 99 patients (67%) achieving transfusion independence, reaching a median hemoglobin of 13.5 mg/dL. Responses are durable, with 75% of the patients remaining transfusion free at a median follow-up of 58 weeks. Cytogenetic responses were observed in 70%, with complete cytogenetic response in 44%. Moreover, cytologic dysplasia resolved in 36% of patients evaluable for central pathology review. Neutropenia and thrombocytopenia were the most common adverse effects necessitating dose adjustment. This highly active agent was recently reviewed by the Oncology Drug Advisory Committee of the FDA, which recommended full approval for patients with del 5q.

Survival Signal Modifiers. The modulation of survival signals by selective inhibition of non-receptor signal intermediates is a strategy that has been exploited initially in higher-risk patients with MDS in an effort to alter the natural history of the disease. Two classes of survival signal modifiers are under investigation, i.e., those agents that target hematopoietic inhibitory signals, including farnesyl transferase, p38a mitogen-activated protein kinase (MAPK), and glutathione S-transferase (GST) P1-1, and those that disrupt anti-apoptotic signals such as the Ras farnesyl transferase inhibitors. TLK199 is a liposomal glutathione derivative that promotes granulopoiesis in vitro and in animal models. The compound selectively inhibits GST P1-1, an enzyme that attenuates Jun kinase and MAPK signaling. In a preliminary dose-finding study, 25 patients received escalating doses of TLK199 for 5 days every 2 weeks with multi-lineage in the dose-ranging phase. Hematologic improvement was reported in 6 patients (30%). No dose-limiting toxicities were observed with the liposomal formulation; however, an orally bioavailable analog of TLK199 that offers more convenient dosing will soon enter clinical studies.

Preclinical investigations of the role of p38a MAPK, a pivotal effector of external inhibitory stimuli initiated by death receptors or inflammatory cytokines, have shown that the enzyme is activated in MDS precursors and that pharmacologic inhibition enhances survival of marrow progenitor and erythroid fractions. SCIO-469, a selective oral inhibitor of p38a MAPK, is currently in phase I/I clinical investigation in patients with lower risk MDS. Characterization of cellular targets integral to the disease process is yielding promising new selective therapeutics for MDS. Given the biologic complexity of MDS, such agents should complement existing therapies and permit tailoring of treatment to the biology of disease.

Novel Therapeutic Approaches in Paroxysmal Nocturnal Hemoglobinuria
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Capsule Summary: PNH blood cells lack a surface protein that protects them from attacks by a part of the immune system called complement. Complement-mediated destruction of blood cells causes anemia and hemoglobin in the urine, and leads to intestinal cramps. A drug called Eculizumab blocks complement activation, reduces the need for transfusions, the frequency of cramps and improves the quality of life for PNH patients.

Aplastic Anemia & MDS International Foundation, Inc.
**Detailed Summary:** Three clinical signs characterize paroxysmal nocturnal hemoglobinuria (PNH): destruction of circulating blood cells, formation of clots in the veins, and associated bone marrow failure. Patients with many PNH cells have massive hemolysis (destruction of blood cells) and a very high risk of clot formation. Those with fewer PNH cells show little hemolysis but appear to have aplastic anemia (AA). Overall, venous clots occur in about 40% of PNH patients. Although warfarin can prevent clot formation, it increases the risk of internal hemorrhage.

Cells with the PNH mutation are missing an “anchor” molecule that attaches proteins to cell membranes. One of these proteins – CD59, known as protectin – protects cells from destruction by the immune system. When the immune system is “turned-on,” a set of chemicals called the “complement cascade” springs into action. Some of them bind to cell membranes, allowing water and ions to rush into cells until they burst. If CD59 were attached to the cell membranes, they would be protected. But when CD59 is missing, cells are destroyed. This is why hemolysis occurs in PNH.

Two approaches seem to hold promise. First, a synthetic linking molecule could attach CD59 to PNH cell membranes. In test tube experiments, a synthetic CD59 complex coated PNH red blood cells for more than three days, and inhibited hemolysis by the complement cascade. In mice, red blood cells were coated by the CD59 complex and protected from human complement for up to 48 hours. Unfortunately, it’s difficult to isolate and produce enough linked-CD59. This technology is not currently in clinical trials.

A second approach blocks the series of reactions that destroy PNH cells. Eculizumab is an antibody that binds and inactivates C5, a critical molecule of the complement cascade. It has been used in patients with various conditions, with no major safety concerns. In theory there could be a possible increase in meningitis during eculizumab therapy but this complication has not been seen since a policy of meningococcal vaccination two weeks before starting eculizumab therapy has been instituted. In a pilot study of 11 transfusion-dependent patients with PNH, eculizumab was given intravenously – initially every week, then once every two weeks. The six patients in this study with normal platelet counts (presumed to indicate good bone marrow activity) have experienced a dramatic improvement in symptoms and have either become transfusion independent or have only very occasional transfusions. Among five of these patients with low platelet counts, one has had no more transfusions, three have received fewer transfusions but have become transfusion independent with the addition of erythropoietin therapy to improve red cell production, and one patient with a very low platelet count showed no change in transfusion frequency. Two of the eleven patients had dark urine immediately prior to a dose of eculizumab (on day 13 or 14) and required a slight increase in the frequency of eculizumab infusions to every 12 or 13 days. Neither patient has had any further clinical evidence of hemolysis since then.

The paroxysms experienced by some PNH patients involve abdominal pain, difficulty swallowing and severe lethargy. These symptoms are probably due to low levels of nitric oxide – a chemical that is taken up by hemoglobin released from ruptured red blood cells. With eculizumab treatment, the average frequency of paroxysms in the 11 patients decreased from three days each month to approximately one day every five months.

Eculizumab should in theory have positive effects on platelet activation in PNH as this seems to be complement-mediated. In addition intravascular hemolysis probably directly leads to clot formation. It is therefore likely that eculizumab will protect PNH patients from the development of clots but this has yet to be proved in the eculizumab trials. The decreased transfusion frequency and improved quality of life with eculizumab has been maintained for more than three years in patients from the initial Pilot study. Development of eculizumab is being pursued in the SHEPHERD study (closed to recruitment September 2005) and the TRIUMPH study whose results are expected in 2006. Further investigation of the role of anticoagulation (warfarin), replacement of CD59 on PNH cells, and complement blockade by eculizumab promises new therapeutic options for patients with PNH.
Presenter's Abstract: Paroxysmal nocturnal hemoglobinuria (PNH) is characterized by the classic triad of intravascular hemolysis, venous thrombosis and an association with bone marrow failure. The intravascular hemolysis and, most likely, the propensity to venous thrombosis are due to the unopposed action of complement resulting from a deficiency of the terminal complement regulator, CD59, from the surface of PNH red cells and platelets, respectively. The characteristic symptoms of PNH, such as marked lethargy, severe dysphagia, abdominal pain and erectile failure are postulated to result from the consumption of nitric oxide by the action of free "soluble" hemoglobin with resulting dysfunction of smooth muscle. In addition, the venous thrombosis, the most feared complication of PNH, appears most likely to be due to a combination of platelet activation by complement as well as the presence of intravascular hemolysis. Therefore the protection of PNH red cells and platelets from the action of complement would be expected to abrogate the symptoms and major complications of PNH. Two possible approaches are either to replace the CD59 on the surface of PNH cells or to block the activity of terminal complement. There are theoretical and logistic problems that make the correction of the genetic lesion in PNH currently impossible and undesirable. The transfer of GPI-anchored CD59 to PNH cells is precluded by the technical difficulty of isolating or producing sufficient GPI-linked CD59. Prodaptin-CD59 is CD59 attached to an artificial GPI-like glycolipid which behaves in a similar manner to GPI-linked CD59 in that it rapidly coats PNH red cells in vitro protecting them from complement mediated lysis and coats murine red cells in vivo protecting them from human complement. This technology is, however, not yet in clinical trials in PNH. The inhibition of terminal complement may appear to be potentially detrimental; however the only apparent problem experienced by individuals with inherited deficiencies of terminal complement is an increased propensity for attenuated infections with Neisseria species. Eculizumab is a humanized monoclonal antibody specific for the complement C5 molecule which binds tightly to C5 preventing its proteolysis and subsequent activation. Eculizumab has been used in a large number of individuals with a variety of conditions with no major safety concerns. Eculizumab given intravenously on a schedule of 600mg weekly for the first 4 weeks followed by 900mg every other week maintains trough plasma levels of greater than 35mcg/ml which is adequate to totally block terminal complement activity. The initial Pilot study in which 11 transfusion-dependent patients with PNH were treated with eculizumab demonstrated a marked reduction in intravascular hemolysis and transfusion requirements with an associated significant dramatic improvement in objective measures of Quality of Life. Ten of these 11 patients remain on eculizumab with continuing benefit. Two trials of eculizumab in PNH – a randomized placebo-controlled efficacy trial and a non-randomized safety trial – have now completed the enrollment of approximately 200 patients with PNH and the results of these trials are awaited with keen interest. The use of eculizumab promises to alleviate many of the symptoms and complications of PNH.

Cytotoxic vs. Non-cytotoxic Therapy High-Risk MDS
Elihu Estey, M.D. Anderson Cancer Center, Houston

Capsule Summary: Therapy for patients with high-risk MDS ranges from very toxic to non-toxic. Less toxic treatments cause fewer treatment-related deaths, but greater toxicity often provides more complete remissions. Scientists have found that the partial response to less toxic treatments may prolong survival even though MDS is not cured.

Detailed Summary: In the past, patients with high-risk myelodysplastic syndromes (MDS) were often treated with the same cytotoxic therapies (toxic to cells) used in patients with acute myeloid leukemia (AML). The diseases have several things in common, such as increased numbers of blasts (immature cells) and similar histories. Unfortunately, this approach to MDS was unsuccessful, especially in patients over 60. Recently, MDS has been treated with non-cytotoxic therapies such as tipifarnib, PKC412, or decitabine. Two year survival rates improved but the change in therapy had little influence on five-year survival. Non-cytotoxic therapies may not be able to reverse the bone marrow failure which usually causes death in high-risk MDS.

A clinical trial in patients over 65 with untreated AML compared Zarnestra (tipifarnib; one of the non-cytotoxic therapies) with IA (idarubicin plus cytoarabine; a cytotoxic therapy). Complete remission (CR) was much more frequent with IA (44% vs. 16%), and partial response including improvement in the blood profile was slightly more frequent with IA (15% vs. 11%). Overall, 73% of the patients who received non-cytotoxic Zarnestra failed to respond. The results favor IA by 24 to 1. Lower intensity therapy may cause less treatment-related mortality, but it won’t necessarily lead to longer survival.
Further trials investigated the importance of complete remission (CR) vs. CRp (complete remission with incomplete platelet recovery). In CRp, immature cells (blasts) are not found in the blood and make up less than 5% of the marrow; the number of neutrophils (a type of white blood cell) is recovering; and the patient is transfusion-independent. CRp shows that a treatment has some activity, and may affect survival. Undoubtedly, patients with CR live longer than patients without remission, and long term survival reflects the time spent in CR. CRp takes longer to develop than CR – for instance, three-fourths of patients who go into CR do so within 38 days. By contrast, three-fourths of patients who will achieve CRp do so within 60 days. The four-year survival of patients who were alive on day 60 is very similar in CR and CRp patients. However, the percent survival in CRp patients was very stable, while survival in CR patients continued to decline. CRp will not result in a “cure,” but it may prolong survival.

**Presenter’s Abstract:** For many years M.D. Anderson patients with untreated high-risk MDS (IPSS int-2 or high) received the same cytotoxic therapies given to patients with AML. In general this approach was unsuccessful, particularly in patients age 60 and above. Recent years have seen use of non-cytotoxic therapy, e.g. tipifarnib, PKC412, or decitabine in such patients; this trend is likely to continue. As a result of its presumed specificity for MDS blasts, non-cytotoxic therapy, which also may include combinations of low-dose ara-C with drugs such as tipifarnib or PKC412, may produce less treatment-related mortality than cytotoxic therapies.

Although the appeal of such non-cytotoxic therapies is obvious, they may have insufficient anti-MDS effect, with such effect (e.g. CR) necessary to reverse bone marrow failure, which is the usual cause of death in high-risk MDS. An example of this phenomenon is provided by a comparison we undertook in patients age 65 and above with untreated AML between tipifarnib (140 patients) and the cytotoxic combination of idarubicin + ara-C (IA, 124 patients). IA was given at MDA and tipifarnib at one of 5 centers; data on the tipifarnib patients was provided by Johnson and Johnson, the sponsor of the tipifarnib trial. All patients had a performance status (PS) < Zubrod, 30-35% had abnormalities of chromosomes 5 and/or 7; the median age of the tipifarnib patients was 74 vs. 70 for the IA patients (p < .0001). CR rates were 44% with IA and 16% for tipifarnib; CRp or hematologic improvement was seen in 15% of the IA patients and 11% of the tipifarnib patients. After accounting for the covariates noted above, we compared survival in patients treated at each tipifarnib center with survival in IA patients. The Bayesian posterior probabilities provided in the following table suggest that, with the exception of center 2, survival was highly likely to be shorter in patients given tipifarnib.
Quantitative and Qualitative Defects in Stem Cell Function in Aplastic Anaemia
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Capsule Summary: In AA, bone marrow cells function abnormally. A series of studies looked at cells in bone marrow to see which types were impaired. Supporting cells (stroma) functioned normally, but didn’t protect blood-forming cells as much as usual by suppressing T-cell function. Blood-forming cells were generally less able to form colonies and more likely to die.

Detailed Summary: Although defective stem cells are typical of aplastic anemia (AA), we know that some normal stem cells (able to replicate themselves and develop into various cell types) must survive because cell counts can return to normal when patients recover. Several studies looked at deficits in cell quality and quantity that persist even after recovery.

Megakaryocytes are marrow cells that eventually form platelets. In marrow from patients with aplastic anemia, the number of “colony forming units” (CFUs) is lower than normal, and megakaryocytes from AA marrow don’t proliferate or differentiate as well.

Long term bone marrow cultures (LTBMC) from AA patients post-treatment have reduced ability to generate new cells. In one study, the only new cells were from an untreated patient who had spontaneously recovered. Are the blood-forming cells impaired, or is the problem in their marrow environment? Scientists cultured normal stem cells on stromas (supporting cells) from AA marrow, and cultured AA stem cells on stromas from normal marrow. The normal stem cells grew well, but the AA cells grew poorly despite their normal environment. Apparently the problem is in the blood-forming stem cells and not their environment.

Mesenchymal stem cells differentiate into fibroblasts and other stromal components of the marrow. Fibroblast colonies develop in AA marrow much as they do in normal marrow. Mesenchymal stem cells in AA cell cultures seem to have normal ability to support cell growth and differentiation. In normal bone marrow, mesenchymal stem cells suppress T-cell function and provide a “protected” environment where blood-forming cells develop. However, T-cell proliferation – and production of interferon-γ, a chemical produced by some T-cells – is not suppressed as well in AA patients.

The percentage of CD34+ cells is reduced in AA. This cell population includes cells which have the ability to repopulate the bone marrow, and CD34+ counts predict recovery of platelet and neutrophil counts. When AA cells are cultured with various combinations of growth factors, many of the cultures fail. However, if G-CSF (granulocyte-colony stimulating factor) is added, their growth improves. This is one of the rationales for considering treating patients with G-CSF after they have received immunosuppressive therapy.

The numbers of long term colony initiating cells (LTC-IC), CD34+ cell counts, and colony forming cells (CFC) are very low in AA patients compared with normals. Even patients with physiological recovery have counts far below normal. LTC-ICs (e.g. in CD34+ cells) remain low for years after physiological recovery. Granulocyte-macrophage colony forming units (CFU-GMs) recover more quickly after bone marrow transplant than after immunosuppressive therapy; but LTC-ICs remain low after both types of therapy. AA blood-forming cells may not proliferate normally, and apoptosis (programmed cell death) of CD34+ cells increases in AA. After ATG therapy, the rate of apoptosis returns to normal.
Telomeres are structures that “seal” the ends of chromosomes and shorten slightly with each cell division. In AA, telomeres are shorter than in age-matched normals. Telomere length decreases with time after diagnosis with active AA; but when patients have recovered normal blood counts, this correlation is not seen. When adjusted for patients’ age, there is no correlation between telomere loss and the duration of AA.

The most “primitive” stem cells are quiescent and normally resistant to 5-fluouracil (5-FU), a substance that impairs DNA synthesis and is most harmful to actively-dividing cells. A study compared normal and AA cell cultures treated with 5-FU in terms of their production of CFUs. The normal cultures increased cell production for 6 weeks before dropping to zero in week 7. The production of cells in AA cultures fell off after the second week of culture, and dropped to zero by week 5. Were there just fewer CFUs in the original AA cultures? To investigate the role of cell concentrations, researchers reduced the concentration of CD34+ cells in normal cultures to replicate the levels in AA cultures. Despite the low initial cell counts, the generation of CFUs followed the same pattern as before. Conversely, when AA CD34+ cultures were started with higher (normal) cell concentrations, their cell production fell off as rapidly as before. The failure of AA cell cultures was not due to low concentrations of CD34+ cells. Instead, their susceptibility to 5-FU suggests that very active cell division was occurring in these normally quiescent cells.

General conclusions:

- AA bone marrow functions abnormally in trying to generate CFUs in long term culture. A defect in the CD34+ stem cells is likely. This deficit is not just due to the reduced number of CD34+ cells.
- Since 5-FU eliminated many AA cells early in the culture, cells must have been actively dividing at that time. Increased cell divisions in AA bone marrow may be an attempt to maintain blood-formation.

**Presenter’s Abstract:** Aplastic anaemia (AA) is characterised by defects in the stem cell compartment, but pluripotent haemopoietic stem cells must persist as lymphocyte counts may be normal, sustained and complete haematological recovery can occur after immunosuppressive therapy (IST) and stromal function in vitro is normal. Short term clonogenic cultures of bone marrow reveal absent or markedly reduced multipotent, bipotent and lineage specific haemopoietic progenitors. Megakaryocyte colonies also show reduced differentiation. Long term bone marrow cultures (LTBMC) show a profound defect in haemopoiesis which, on cross-over experiments, is due to a defect in the stem cell compartment and not the stroma, with reduced marrow repopulating ability. Both immature and mature CD34+ cells are reduced. Long term culture initiating cells (LTC-IC) when scored in limiting dilution assays using various endpoints are markedly reduced and also abnormal qualitatively as shown by reduced clonogenic capacity. The reduction in LTC-IC is not simply due to a reduction in CD34+ cells but the proportion of LTC-IC within the CD34+ population is reduced. LTC-IC remain very low many years after successful BMT and IST. LTC-IC assays assume stem cells proliferate and differentiate at uniform rate, but AA stem cells do not proliferate normally so LTC-IC assay may be unreliable for assessment of early AA progenitors.

AA marrow CD34+ cells are dysfunctional with reduced clonogenic potential but this can be corrected by G-CSF in vitro. Furthermore, more primitive haemopoietic stem cells are also dysfunctional. 5-fluouracil (5-FU)-resistant (quiescent) AA haemopoietic cells show reduced ability to generate colony forming units (CFU) on stromal layers (despite correcting for reduced numbers). This indicates that 5-FU has a greater effect on CFU production from AA CD34+ cells compared with normal controls, implying that in AA the reduction in CD34+ cells leads to an increase in cell cycling in an attempt to maintain steady state haematological activity. Reduced survival of the AA CD34+ marrow population has been demonstrated by increased apoptosis and expression of Fas-antigen. This is associated with shorter telomere lengths of peripheral blood leucocytes in AA than in age-matched controls. On haematological recovery after IST, telomere lengths in AA granulocytes show no difference to normal controls. This is despite the persistent reduction in LTC-IC that occurs in AA. This might indicate that the damage to the stem cell compartment may not be great enough to require increased compensatory cell divisions in the remaining cells under steady state conditions. But, an alternative and possibly more likely hypothesis is that this is consistent with an initial immunological attack not at the level of the pluripotent haemopoietic stem cell compartment but at a more mature progenitor cell level, and the extra number of cell divisions required in the pluripotent stem cells may be too small to detect when measuring telomere length in peripheral blood cells at time of haematological recovery.
Paroxysmal Nocturnal Hemoglobinuria as a Stem Cell Disease
Lucio Luzzatto, Università di Genova and Istituto Toscano Tumori, Firenze, ITALY

**Capsule Summary:** A stem cell can make copies of itself and develop into different types of cells. Cells that descend from the same stem cell are called a clone. PNH may occur when a stem cell mutation combines with an immune reaction, killing normal cells while mutant cells survive and multiply. Understanding these processes better will shed light on PNH and on AA as well.

**Detailed Summary:** Stem cells are undifferentiated, primitive cells that can divide and yield at least one new stem cell and differentiate into specialized cells of several types. Some primitive cells can replicate themselves, others can differentiate, but true stem cells can do both. In hematopoiesis (blood cell formation) a bone marrow stem cell forms blood cells with many different forms and functions.

Paroxysmal nocturnal hemoglobinuria (PNH) was identified in 1969 as a clonal disorder – that is, a disorder of cells descended from a common “parent” cell. Part of the evidence for its clonality is its long lasting effects on blood formation: the same mutation may be found in blood cells for decades. Also, the mutation (which causes protein abnormalities in PNH cells) affects the blood cells. Almost by definition, PNH must originate with a stem cell mutation.

If you examine the bone marrow from one patient with PNH and another with aplastic anemia (AA), they look very different. The PNH marrow is crammed with cells, while the AA marrow is practically empty. However, 95% of the cells in PNH marrow may be abnormal. Remove them, and the marrow would look like AA. Marrow from PNH patients contains both PNH cells and normal cells, but the normal (non-PNH) cells are very reduced in number.

Telomeres are structures that “seal the ends” of chromosomes and gradually shorten with each cell division. In PNH cells, the telomeres are shorter than usual – probably because the cells have gone through more divisions than normal. In fact, the larger the PNH cell population, the shorter the telomeres. Oddly enough, short telomeres are also found in non-PNH cells of PNH patients. There is evidence that hematopoiesis in non-PNH cells is far below normal levels, sometimes as low as that found in AA.

A possible explanation for PNH goes like this: When a stem cell develops the PNH mutation, its clonal descendents lack a molecule that helps anchor proteins to their cell membranes. Normal people have these PNH cells, about 10-50 cells in every million. However, sometimes killer T-cells arise that can differentiate between PNH cells and normal cells. In people who develop PNH, these T-cells attack normal stem cells but leave PNH stem cells unharmed. Studies have found that these T-cell clones make up more than 50% of the T-cell clones in PNH patients but fewer than 5% in healthy individuals. The struggle may go on for years, but the result is a kind of natural selection in which cells descended from the PNH stem cell make up more and more of the hematopoietic population.

By itself, the PNH stem cell mutation has no clinical consequences. By itself, the attack of killer T-cells would result in aplastic anemia. But when the stem cell mutation combines with the autoimmune attack, the result is PNH. Understanding these mechanisms more fully will help scientists to develop new approaches to PNH, and perhaps to AA as well.

**Presenter’s Abstract:** The evidence that paroxysmal nocturnal hemoglobinuria (PNH) is a clonal disorder dates back to over thirty years ago; and the fact that blood cells of all lineages are affected by the PNH abnormality provides compelling proof that the PNH clonal population originates in a hematopoietic stem cell (HSC). At the same time, a unique characteristic of PNH is that normal and abnormal blood cells co-exist in the bone marrow and in the peripheral blood. Therefore, in this condition we must consider both (i) PNH HSCs and (ii) non-PNH HSCs: both of them are abnormal.
PNH HSCs are, of course, abnormal because they are severely or totally deficient in glycosylphosphatidylinositol (GPI)-anchored proteins. Interestingly, there is evidence that somatic mutations of the PIG-A gene, producing the GPI- phenotype, are relatively common amongst both hematopoietic progenitor cells and HSCs. The GPI- qualitative abnormality must be regarded as severe (indeed, it is lethal when it affects the whole body); yet, it does not impede active and effective hematopoietic activity. In some cases we have direct proof that one and the same HSC (identified by a specific PIG-A mutation) supports hematopoiesis in a PNH patient for years. However, we must expect that the hematopoietic potential of a single stem cell will be eventually exhausted: consistent with this, the telomeres of PNH granulocytes are short when compared to appropriate controls. As long as hematopoiesis is largely supported by one or few GPI- HSC, the clinical picture will be dominated by the consequences of abnormalities in cells of all lineages present within the progeny of those HSCs.

Non-PNH HSCs in patients with PNH are, by definition, GPI+, but that does not mean that they are normal. Indeed, there is abundant evidence that GPI+ hematopoiesis is quantitatively reduced: in some cases, as severely as in severe AA. From the qualitative point of view, non-PNH HSCs have also short telomeres in PNH patients, again similar to what applies in AA.

Thus, PNH is eminently a stem cell disease. Probably the most crucial outstanding question in the pathogenesis of PNH is what determines the rate of proliferation and the fate of both the GPI+ (non-PNH) and the GPI- (PNH) stem cells. At this time, evidence is accumulating that an immune process may be important in this respect, and may be a primary agent in producing clinical PNH. There is also evidence that GPI+ cells may be more vulnerable than GPI- cells to certain types of cytotoxic attacks. PNH is a prototype clonal non-neoplastic stem cells disorder, in which we see Darwinian selection of somatic cell populations in action. Understanding more fully the mechanisms involved may help to develop new approaches to controlling PNH, and perhaps also AA.

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Mechanisms of Clonal Evolution in Bone Marrow Failure

_Elaine Sloand, M.D., John Barrett, M.D., and Neal Young, M.D. Hematology Branch, National Heart, Lung, and Blood Institute_

**Capsule Summary:** Groups of abnormal cells may develop after AA is diagnosed or treated. Some have extra chromosomes, some have too few, but they grow and survive. Scientists are studying the factors that increase the numbers of these cells and decrease their death.

**Detailed Summary:** After aplastic anemia (AA) is diagnosed, or even after its successful treatment, an abnormal clone (group of genetically-identical cells descended from the same parent cell) may start to grow and make up an increasing part of the cell population. These cells must have some advantages that allow their numbers to increase. Either they multiply faster or survive longer than normal bone marrow cells – or perhaps they do both. Starting with this assumption, researchers studied cell growth and survival in three of these clonal disorders:

- trisomy 8 - three copies of chromosome 8 are found in each cell;
- monosomy 7 - only one copy of chromosome 7 is found in each cell; and
- paroxysmal nocturnal hemoglobinuria (PNH).

There is evidence for immune involvement in trisomy 8. A certain type of T-cell (Vβ) is more common in trisomy 8 patients. After patients receive immunotherapy, trisomy 8 colonies increase in number and Vβ T-cells decrease. A gene called c-myc is more active in trisomy 8 cells, possibly a direct result of having three copies of chromosome 8. c-myc stimulates cell division and gene activity, and energizes other genes (including cyclin D1 and survivin) that advance the cell cycle and inhibit processes leading to cell death. Increased division and decreased death give trisomy 8 cells a survival advantage over normal cells.

Monosomy 7 typically arises in patients who have responded poorly to immunotherapy. Cells with monosomy 7 have an unusually high number of granulocyte-colony stimulating factor receptors (G-CSFR). Strangely, this makes the cells less responsive to normal levels of G-CSF; but when G-CSF levels increase in bone marrow failure or during therapy, monosomy 7 cells...
multiply. Attempts to cause monosomy 7 in cell culture by giving high doses of G-CSF were unsuccessful. However, in myelodysplastic syndromes (MDS) where some monosomy 7 cells are already present, G-CSF increases this population. In a normal G-CSF receptor, one region controls proliferation and another regulates maturation. In many monosomy 7 cells, the receptor region for maturation is missing. These cells can multiply but not mature, and so they rarely die. Increased proliferation and decreased death produce a larger cell population.

PNH clones often increase during immune-mediated marrow failure such as AA. In normal cells, a protein anchors the urokinase-plasminogen activator receptor (u-PAR) to the cell membranes. In PNH cells, this protein is missing. Instead of binding u-PAR to their cell membranes, PNH cells secrete soluble u-PAR – which stimulates their own growth and decreases their death. Because soluble u-PAR has just the opposite effect on normal cells – decreasing their number and increasing mortality – PNH cells have a survival advantage.

**Presenter’s Abstract:** Myelodysplasia, leukemia and paroxysmal nocturnal hemoglobinuria are clonal disorders which may become evident following a diagnosis of aplastic anemia. The risk of developing these disorders is substantial (5-19%), increases with age (risk increases by 40% for each decade of life) and may follow successful treatment of aplastic anemia. Monosomy 7 and trisomy 8 are the predominant cytogenetic abnormalities. Monosomy 7 typically occurs in the setting of poor response to immunotherapy or clinical relapse and frequently progresses to leukemia. In contrast, trisomy 8 is associated with a high rate of response to immunotherapy and does not appear to be associated with an increased risk of leukemic progression. Although paroxysmal nocturnal hemoglobinuria (PNH) was once regarded as a late complication of AA, more sensitive flow cytometric measurements generally detect PNH cells at time of diagnosis. We examined the pathophysiology underlying the development of these clonal abnormalities in bone marrow failure syndromes, assuming that expansion of each of these clones requires a proliferative or survival advantage over native cells. We found that c-myc a gene present on chromosome 8 was upregulated in trisomy 8 cells; this finding may be a consequence of a gene-dosage effect and a direct result of the trisomy. Upregulation of cyclin D1 mRNA and protein in these cells likely results from increases in c-myc. Cyclin D1 increases cell proliferation and inhibits apoptosis via upregulation anti-apoptotic proteins. WT1 mRNA is also upregulated in trisomy 8 and may be instrumental in producing an immune response directed against trisomy 8 cells. Monosomy 7 cells express increased amounts of the GCSFR isform IV which accounts for the cells inferior responses to physiologic levels of granulocyte colony stimulating factor. However, monosomy 7 cells proliferate at the higher concentrations of this cytokine as occur in bone marrow failure or with administration of GCSF in pharmacologic doses. PNH clones also expand in the setting of immune-mediated marrow failure, and functional and molecular studies suggest an escape mechanism. In the setting of aplastic anemia, normal CD34 cells are apoptotic while PNH CD34 cells are not. Preliminary studies suggest that urokinase plasminogen activator receptor, a protein normally linked by the glycosylphosphatidylinositol (GPI) anchor to the cell membrane but secreted by PNH cells, plays an exocrine function, stimulating growth of PNH cells and inhibiting their apoptosis, while having the opposite effect on GPI-positive cells.

**Application of High Resolution Genomic Scan in Bone Marrow Failure Syndromes**
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**Capsule Summary:** About half of all MDS patients have chromosomes that are abnormal in size, shape, or number, and many others have smaller genetic defects. People with unstable chromosomes may be more likely to develop bone marrow failure. New technologies can now detect very small genetic variations and identify people at higher risk for bone marrow disease.

**Detailed Summary:** Myelodysplastic syndromes (MDS) are a collection clinically diverse conditions. Between 40% and 60% of MDS patients have an abnormal karyotype (the chromosomal complement of an individual, including the number of chromosomes and any abnormalities). In MDS patients with normal karyotypes, the genetic variations may simply be too small to show up at this level of analysis. The factor common to all these patients is probably an increased susceptibility to genetic variation. This susceptibility leads to unstable
chromosomes and genetic defects. At some point in the lives of these patients, a group of defective cells expanded enough to overtake other cells in the bone marrow. The result was MDS.

Scientists are asking whether chromosomal defects can be defined better with new techniques. Array-based genomic hybridization (A-CGH) has better resolution than karyotype analysis and can scan a patient’s entire genome. When A-CGH was used to analyze the marrow from a person with a “normal” karyotype, it uncovered several small genetic defects. In marrow from 39 MDS patients whose karyotypes appeared normal, A-CGH detected abnormalities in 26. Regions on chromosomes 7, 8, 10, and 22 were among those frequently affected.

A technique with even higher resolution is SNP array-based genotyping (SNP-A). A “SNP” is a single nucleotide polymorphism – a variation in one of the units that string together to make up a strand of DNA. SNP analysis can screen for 100,000 polymorphisms at once. A recent study enrolled 39 patients with MDS, and 20 with aplastic anemia (AA) and paroxysmal nocturnal hemoglobinuria (PNH). Computer analysis revealed several interesting genetic variations: one was present in 25% of MDS patients but only 2% of the general population; eight polymorphisms were found in 20% to 30% of patients but fewer than 5% of the general population; and five polymorphisms occurred in more than 60% of AA and PNH patients but in less than 10% of the general population.

The use of these new technologies permits more precise and sensitive detection of chromosomal defects in MDS. Chromosome sites which are frequently affected in bone marrow failure can be identified. SNP technology will help us recognize patients showing high numbers of random defects, and identify the genetic patterns that contribute to their chromosomal instability.

**Presenter’s Abstract:** Chromosomal damage is a hallmark of myelodysplastic syndromes (MDS) and can also be present in other forms of bone marrow failure. The inability to detect an abnormal karyotype in a portion of MDS and perhaps some aplastic anemia (AA) patients is consistent with the theory that large lesions may represent an extreme of a possible DNA damage spectrum present in these diseases. New gene array technologies facilitate detailed genomic analysis. These techniques can be used to investigate genomic structure in MDS and include BAC array-based comparative genomic hybridization (A-CGH) and high-density SNP microarrays. In conjunction with expression arrays, these techniques allow for the comprehensive evaluation of molecular lesions in bone marrow failure states. We have applied A-CGH for high resolution analysis of chromosomal defects in bone marrow failure patients. Our results showed a superior precision of this technology over metaphase cytogenetics, demonstrating the presence of discrete lesions in patients with normal or non-informative karyotypes. Even more precision can be achieved with SNP arrays. In addition to the determination of the gene copy number, this technique allows for the identification of tag SNP, which, based on the principle of linkage disequilibrium, marks functional genetic elements that coincide with the disease. To date, studies dealing with the complex pathogenesis of bone marrow failure have been based on empirical approaches, allowing for very limited insights into the possibly complex genetic traits and DNA changes during evolution of the disease. SNP arrays may overcome these limitations of traditional studies.

Our results demonstrate the potential value of high-density SNP arrays in the precise analysis of clonal genomic lesions and loss of heterozygosity, as well as complex genotypic profiles that may potentially contribute to an inherited susceptibility.
Epidemiology of the Bone Marrow Failure Syndromes: Aplastic Anemia and Myelodysplastic Syndromes

David W. Kaufman, Sc.D., Slone Epidemiology Center at Boston University, Boston University School of Public Health

Capsule Summary: Aplastic anemia (AA) and myelodysplastic syndromes (MDS) are the two major types of bone marrow failure. AA is rare and affects all ages. MDS is more common and mainly affects the elderly. In most cases, the cause of these diseases is unknown. To learn more about them, the Slone Epidemiology Center is starting a registry for patients with MDS or multiple myeloma (MM). Patient enrollment will begin in February 2006.

Detailed Summary: Aplastic anemia (AA) is a type of bone marrow failure affecting at least two cell lines. The disease develops over several weeks or months, with initial symptoms of weakness, fatigue, infections, and sometimes bruising or bleeding. This gradual onset makes it difficult to establish a relationship between AA and its causes. This is even more challenging because the disease is rare: only 2 to 7 cases per million. Among various studies, data from three – the International Agranulocytosis and Aplastic Anemia Study (IAAAS), the U.S. Blood Dyscrasia Study, and Thai studies of Agranulocytosis and Aplastic Anemia – have shed some light on the disease. AA affects both young and old, although the incidence in women increases with age in the IAAAS study, and peaks in men and women 15-24 and over 60 in the Thai study. As late as the 1980s, the fatality rate was over 50%, but it is considerably lower with currently available treatment. Primary causes of AA include drugs and chemicals (the relative risk for chloramphenicol is no greater than for many other medications), with indications of viral and genetic involvement. The cause of most AA cases is still unknown.

Myelodysplastic syndromes (MDS) is a group of conditions characterized by ineffective and abnormal formation of blood cells. MDS is much more common than AA (3 to 12 cases per 100,000), especially in older people. In children, there are fewer than 5 cases per million per year. MDS affects more elderly people than acute myeloid leukemia (AML), and often progresses to AML. Because most patients are older and early symptoms are hard to recognize, MDS is probably under-diagnosed. Chemotherapy is a well-established risk factor, and possibly chemical exposures, smoking, and genetic factors. Genetic abnormalities including “5q-syndrome,” trisomy 8, and monosomy 7, are present in more than 50% of MDS patients. As with AA, the cause of most MDS cases is still unknown.

To remedy some of the knowledge gap for MDS, the Slone Epidemiology Center at Boston University is working to establish a patient registry for MDS (together with a parallel effort for multiple myeloma). Enrollment is planned to begin in 2006. Objectives of the registry are to:

- obtain information on the outcomes of different treatments, including clinical events, economic outcomes, and quality of life measures
- measure time trends in the use of different treatments
- obtain information on adherence to therapy and disease treatment patterns
- evaluate other issues among patients, such as their reasons for participating in research, and their knowledge/behavior and attitudes toward different types of therapy.
**Presenter’s Abstract:** Aplastic anemia (AA) and myelodysplastic syndromes (MDS) are the two major bone marrow failure syndromes. AA is rare, with the incidence in different parts of the world ranging from about 2-8 per million per year. It is generally more common in Asia than in Western countries. There are not striking differences in incidence between males and females; in some series, a bimodal pattern has been observed according to age. AA is a life threatening condition and until the advent of modern therapy more than 50% of cases were fatal. Well documented risk factors include some medications, benzene, and pesticides. However, these and other factors such as post-hepatitis/aplasia syndrome explain less than half of the etiology, and many cases remain "idiopathic." Recent data from Thailand are consistent with an infectious etiology for some cases. Genetic factors are clearly relevant because the vast majority of individuals exposed to documented risk factors do not develop AA.

By contrast, MDS, which comprises a heterogeneous group of conditions characterized by ineffective hematopoiesis, is at least an order of magnitude more common than AA, with the reported overall incidence ranging from about 5-15 per 100,000 per year, a higher frequency of occurrence than acute myeloid leukemia (AML). It is largely a disease of the elderly: mean and median ages in most series are 70 or greater and incidence rates of up to 50 per 100,000 have been reported in those over 70. Myelodysplasia is also more common in men. Many cases progress to leukemia, particularly AML, and the eventual fatality rate is quite high; cytogenetic abnormalities are common and relevant to the clinical course. MDS occurs among children but is rare, with incidence of the order of 5 per million per year. Familial MDS has been reported, but again infrequently. Due to definitive issues, predominance in the elderly, and the relatively mild symptoms of early disease, it is likely that MDS is underdiagnosed, with the incidence underestimated, perhaps substantially. For these reasons it is also difficult to study epidemiologically. The etiology is not well understood and few risk factors are established; these include most prominently, chemotherapeutic agents. Other reported factors include benzene, chemical exposures, radiation, and various occupational groups. There is a need for large scale epidemiological studies to better determine the incidence of MDS and its etiologic factors.

**Aplastic Anemia in the Orient**

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**Capsule Summary:** Aplastic anemia is 2-3 times more common in Asian countries. A recent study in Thailand found that contaminated drinking water, exposure to ducks and geese, and the use of animal fertilizers all increase the risk of developing AA. This suggests that viruses or bacteria may be involved in AA, and provides a new clue to the causes of the disease.

**Detailed Summary:** Aplastic anemia (AA) occurs more often in Asian countries. The causes were assumed to be wide use of chloramphenicol, exposure to pesticides, and the hepatitis virus. However, a large case-control study supported by the National Heart, Lung and Blood Institute (NHLBI) of NIH recently explored the causes of AA in Thailand. The study focused on Bangkok region, with a population over 8 million, and Khonkaen region, with 7.6 million people in northeast Thailand. AA was diagnosed more often in patients 15-24 years old and in those over 60, which suggests the influence of environmental factors. Although AA was previously considered a disease of poverty, the current study showed no difference in occurrence based on household income.

In exploring the effects of medical drug use, sulfonamides, mebendazole and thiazide increased the risk, but chloramphenicol turned out to be insignificant. Non-medical needle exposure contributed to the occurrence of AA, but post-hepatitis cases of AA were rare. Among solvent exposures, benzene stood out as a risk factor compared with fuels, glues and other solvents.
The most interesting effects uncovered by this study involved rural or agricultural factors. For example, agricultural pesticide exposure contributes to AA, while household pesticides do not. The most harmful agricultural pesticides were DDT and carbamates. Animal fertilizers also increase the occurrence of AA, but chemical fertilizers do not. Associating with cattle, water buffalo or chickens poses no significance, pigs have a marginal effect, but exposure to ducks or geese (on the farm, not the table) carries an increased risk of developing AA. Finally, the source of drinking water was of no association in Bangkok, but in rural Khonkaen, any water other than bottled or distilled carried an increased risk. The possibility that infectious agents in the water, the animals, or the animal products contribute to AA requires further investigation.

**Presenter's Abstract:** It has long been believed that aplastic anemia is more frequent in the Orient than in the West. The high prevalence was thought to be related to exposure to toxic chemicals, administration of chloramphenicol, and viral hepatitis. However, our recent systemic epidemiological study of aplastic anemia in Thailand indicated that the annual incidence rate was 3.9 per million in Bangkok metropolitan and 5 per million in Khonkaen. The incidence rate was 2-3 fold higher in Thailand than in the West; it was not as high as we thought because 60% of cases in large hospitals were from outside catchment areas. Based on the results of a case-control study which enrolled 541 patients and 2,261 controls, we observed significantly elevated relative risk estimates for benzene (3.5) and for sulfonamides (5.6), thiazides (3.8), and mebendazole (3.0).

Chloramphenicol use was infrequent and no significant association was observed. Agricultural pesticides were implicated in Khonkaen: there were significant associations with organophosphates (2.1), DDT (6.7), and carbamates (7.4). We found significant risks for farmers exposed to ducks and geese (3.7). There was a significant association with drinking other than bottled or distilled water (2.8) in Khonkaen. Non-medical needle exposure was positive associated (3.8). There was no significant association with history of hepatitis and jaundice. Only two patients were posthepatitis aplastic anemia.

The most striking finding was the large etiologic fraction in the rural region accounted for animal exposures and drinking of water from sources such as wells, taps and rain water, consistent with an infectious etiology for a large proportion of aplastic anemia in rural Thailand.

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**Genetic Risk Factors for Bone Marrow Failure**

**Neal S. Young, M.D. and Rodrigo Calado, M.D., Ph.D., Hematology Branch, National Heart, Lung, and Blood Institute**

**Capsule Summary:** Mutations in genes that affect the immune system or the blood-forming system may increase the chances of developing AA. Some of these genes act at the ends of chromosomes, making them shorter each time a cell divides. Extra-short chromosomes can be detected in the cells of certain AA patients and their families, and help explain their tendency to develop AA.

**Detailed Summary:** For most of its history, aplastic anemia (AA) has had no recognized cause. Its onset was attributed to clinical events such as drug or chemical exposure, pregnancy or hepatitis. Scientists are now seeing links between acquired and constitutional (congenital or hereditary) forms of marrow failure. AA may even have something in common with multiple sclerosis and type I diabetes, diseases mediated by T-cell activity. In AA, T-cells may wrongly target hematopoietic stem and progenitor cells (blood-forming cells) in bone marrow by increasing production of inflammatory cytokines (proteins that regulate cell function) such as TNFα, IFNγ or IL-6. Scientists are asking whether these T-cells might be genetically abnormal cells which fail to shut down when a proper target is unavailable.

On the “target” side of this genetic equation, a disease called dyskeratosis congenita affects skin, nails, and tongue. People with this disease have short telomeres – structures that cap the ends of chromosomes and gradually shorten with each cell division. Most cells divide many times as a person ages. Eventually telomeres become so short that a cell can no longer divide – one of the hallmarks of aging cells. Dyskeratosis congenita can be caused by mutation
of an X-linked gene (DKC1) that codes for a telomere protein. Another genetic cause is not sex-linked, but involves mutation of the TERC gene that codes for telomere RNA.

Oddly enough, short telomeres are often found in the cells of AA patients. An abnormal TERC gene was recently traced in the family of a man with "acquired" AA who received a stem cell transplant from his sister. The sister had been slightly anemic all her life, and the transplant team was unable to obtain enough cells from her marrow for a successful graft. Scientists at NIH became interested in the family and found individuals over several generations with a single TERC mutation. Those affected generally showed mild macrocytic anemia (a type of anemia in which red blood cells are abnormally large) and some thrombocytopenia (low platelet counts), but no severe problems or frank blood disease. Sometime later, another patient presented with “acquired” AA expecting to receive a transplant from her brother. Although the brother’s health seemed normal, his marrow was very deficient. The same pattern was later discovered in four generations of the family. Another mutant TERC gene was the underlying cause.

Another gene called TERT encodes for the important enzyme in telomere repair; mutations in TERC have been identified in aplastic anemia patients who have short telomeres and reduced blood-forming activity. Five different mutations of the TERT gene were found among seven patients, and in each case their telomeres were much shorter than normal. When mutations occur in genes that control telomere function and repair, there is often increased susceptibility to AA. This suggests a common underlying mechanism based on a diminished marrow compartment and a decreased capacity for repair.

To summarize, genetic risk factors for AA involve mutations in genes that:

- control immune functions such as cytokine production and target recognition; or
- influence stem cell number, repair, and function.

Further support for a genetic tendency toward AA is found in the high incidence of the disease in 0-14 year old Asian children in British Columbia (approx. 7 cases /million per year compared with 2.4 /million per year overall). With increasing evidence that genetic factors are involved in acquired AA, it is even more important for patients to tell their doctors about family disease patterns in order to arrive at an accurate diagnosis.

**Presenter's Abstract:** Genetic risk factors for acquired aplastic anemia have been identified that affect both the immune response and hematopoietic cell number and function. The class II histocompatibility antigen HLA-DR2 (*1501) is more prevalent in both Asian and Western patients and may correlate with responsiveness to immunosuppressive therapies. Polymorphisms in type I cytokine genes, especially in the genes for interferon- and tumor necrosis-a, are also more prevalent; these genetic alterations correspond to hyper-responsiveness of cytokine production in vitro. Alterations in other cytokine genes, such as the gene for interleukin-6, may also be more prevalent among marrow failure patients. For hematopoiesis, mutations have been identified in genes of the telomere repair complex as causative of the constitutional marrow failure syndrome dyskeratosis congenita: DKC1 is abnormal in the severe, X-linked form and TERC in the autosomal dominant form. Both gene products are related to telomere repair; TERC encodes the crucial RNA template. Telomeres are short in patients with DKC and also in a large proportion of patients with acquired Aplastic anemia. We have described patients with apparently acquired aplastic anemia, presenting as adults and without either physical anomalies or a family history of aplastic anemia, with mutations in TERC. In other patients with acquired aplastic anemia and short telomeres, TERT, the gene encoding the reverse transcriptase, is mutated. Telomeres were short in vivo, and in vitro studies established that the mutated genes produced an inactive RNA in telomere assays; haploinsufficiency is the mechanism for telomere shortening in these patients. Other genes relating to telomere repair are also abnormal in acquired aplastic anemia, suggesting a common mechanism of susceptibility based on a congenitally diminished marrow compartment and/or defective repair capacity. Androgens augment telomerase activity in vitro, acting via the estradiol receptor, and this mechanism likely explains their benefit in dyskeratosis congenita and other marrow failure syndromes.
**IMMUNE PATHOPHYSIOLOGY OF BONE MARROW FAILURE SYNDROMES**
Chair: Jaroslaw P. Maciejewski, M.D., Ph.D.

Autoantigens of Marrow Failure Syndromes
Shinji Nakao, MD, PhD, Cellular Transplantation Biology, Kanazawa University Graduate School of Medical Science

**Capsule Summary:** Marrow failure can be caused by an immune attack on the patient’s bone marrow, an attack that may come from the patient’s own cells. Two non-foreign substances have been found that stimulate antibody formation in AA patients. If AA patients are PNH+ and have both these antibodies, a good response to immune therapy is highly likely.

**Detailed Summary:**
Autoantigens are cell proteins that stimulate a reaction by a person’s own immune system. This anti-self reaction causes diseases like insulin-dependent diabetes and types of arthritis. It may also cause some cases of bone marrow failure, but the autoantigens in bone marrow failure are hard to identify: Aplastic anemia (AA) is not associated with bacteria or viruses, and there is no good assay to identify the antigens that stimulate the immune attack by CD4+ T-cells (a type of lymphocyte that reacts against foreign substances). The number of blood-forming cells (hematopoietic progenitor cells, or HPCs) targeted by T-cell attacks is too few to study.

Recently, scientists looked for autoantigens in AA patients who have high numbers of PNH+ cells. PNH+ cells are numerous in paroxysmal nocturnal hemoglobinuria, and a high percentage of blood cells in AA may be PNH-positive. The response rate to anti-thymocyte globulin and cyclosporine (ATG/CSA) therapy is higher when AA patients are also PNH+. Complex immunological studies identified antibodies against a substance called DRS-1 (diazepam binding inhibitor related protein) in the serum of AA patients who are PNH+, but not in those who are PNH-. DRS-1 is also found in some lines of leukemia cells as well as in normal CD34+ cells (cells with a high ability to repopulate bone marrow). One experiment showed that certain leukemia and lymphoblastic cell lines are attacked by DRS-1-specific T-cells. Immune responses to DRS-1 are now recognized frequently in PNH+ AA patients. Detecting their anti-DRS-1 antibodies may be useful in diagnosing and understanding the immune pathology in AA. However, it is still not known whether the autoimmune response directed against DRS-1 contributes to the bone marrow failure in AA.

Another candidate autoantigen is a protein called moesin. This protein, and antibodies against it, is found in serum of AA patients but not in the serum of normal individuals. Moesin normally functions inside cells to link the cell membrane to internal structures. However, it is secreted by some types of blood cells. Specific antibodies vs. moesin were present in 53% of PNH+ AA patients, 27% of PNH- AA patients, but only 9% of healthy individuals. Levels of anti-moesin antibodies were higher in PNH+ than in PNH- individuals. Because it is secreted, moesin can be detected in the liquid culture medium of various leukemia cell lines. It’s possible that moesin secreted by blood-forming cells could induce antibody formation as well as an attack by cytotoxic T-cells specific for moesin.

The individual factors discussed here – anti-DRS-1 antibodies, anti-moesin antibodies, and PNH+ cells – do not have a significant effect on a patient’s response to immunosuppressive therapy (IST) when considered separately. However, a small pilot study found that patients positive for all three factors (both antibodies and PNH+) responded to IST better than patients positive for one or two factors did. Clinical trials are currently being planned.
**Presenter’s Abstract:** Identification of antigens which elicit development of bone marrow failure has been a major challenge for hematologists. Aplastic anemia (AA) patients showing an increase in the proportion of CD55-CD59- (PNH-type) cells with HLADRB1*1501 are the best subject when trying to identify such autoantigens of AA because antigen-specific T cells are likely to contribute to destruction of hematopoietic progenitor cells in this subset of AA patients. In these patients with immune-mediated AA, B-cell response to hematopoietic stem cell antigens which are recognized by autoreactive T cells may also take place. When we examined serum of the subset of AA patients for the presence of antibodies to a megakaryocytic leukemia cell line, UT-7, using immunofluorescence analysis, the cytoplasm of the cell line was brightly stained by the sera of some patients. Immunoscreening of cDNA library derived from UT-7 cells identified diazepam binding inhibitor-related protein (DRS-1), a peroximal protein which mediates ? oxidation of unsaturated fatty acid. Specific antibodies to DRS-1 was detectable in 38% of AA patients showing an increase in the proportion of PNH-type cells. DRS-1 gene was abundantly expressed by several myeloid leukemia cell lines and CD34+ cells from normal individuals. The frequency of CD4+ T cells specific to a DRS-1 peptide which shows high affinity to HLA-DR15 is increased in patients carrying HLADRB1*1501 as well as anti-DRS-1 antibodies, and DRS-1-specific T cells showed cytotoxicity against autologous LCL cells transfected with DRS-1 gene. These findings suggest that DRS-1 specific T cells may be involved in the pathogenesis of AA in patients carrying HLA-DR15.

On the other hand, immunoblotting using UT-7 lysate and patient's serum coupled with peptide mass fingerprinting identified another putative autoantigen, moesin. Moesin is a membrane-cytoskeleton linker protein essential to apical membrane traffic and regulation. When sera of AA patients were screened for the presence of antibodies specific to recombinant moesin protein using Western blotting and ELISA, significantly high titer of the antibodies was detected in 32% of AA patients. The prevalence of anti-moesin antibodies in AA patients showing increased PNH-type cells (41%) was significantly higher than in those without PNH-type cells (12%). Although moesin is an intracellular protein which is localized adjacent to the cell membrane, LCL cells and some cancer cells reportedly secrete moesin as a form of exosome. When supernatants of cells incubated for 1 hour were examined for the presence of moesin, the membrane linker protein was detectable in the culture supernatant of some myeloid leukemia cell lines such as UT-7, K562 and OUN-1. These findings indicate that B-cell response to moesin may be involved in immune pathophysiology of AA and that moesin secreted from hematopoietic progenitor cells may be efficiently processed by antigen-presenting cells leading to induction of antibodies and possibly T cells specific to moesin in immune-mediated AA. Roles of antibodies specific to DRS-1 and moesin and PNH-type cells in predicting response to immunosuppressive therapy are discussed.

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**Autoimmunity in MDS: Friend & Foe**

Jeffrey J. Molldrem, M.D., Transplantation Immunology Section, University of Texas M.D. Anderson Cancer Center, Houston

**Capsule Summary:** One of the causes of MDS is T-cells that attack both normal and abnormal blood-forming cells. Now scientists are learning to use T-cells to benefit patients. When T-cells can focus their attacks on harmful cells, they become a patient’s friend instead of a foe.

**Detailed Summary:** Patients with myelodysplastic syndromes (MDS) have abnormally low blood cell counts, as both red blood cells and platelets are attacked by the patient’s immune system. Among the immune system culprits are T-cells (a kind of white blood cell). MDS patients have increased numbers of certain CD8+ T-cells, usually a clone of genetically identical cells descended from the same parent cell. All are programmed to attack the same target. T-cell attacks contribute to the low blood cell counts in MDS by chemically targeting abnormal blood-forming cells. Unfortunately, the attack is often non-specific and destroys healthy cells as well. How do T-cells recognize which blood-forming cells to target?

Anti-thymocyte globulin (ATG) is a common therapy for MDS. In one clinical trial, ATG increased cell counts in 34% of the patients, while 66% did not respond. Scientists suspected that CD8+ T-cells had been suppressing blood cell formation in MDS patients who responded to ATG. To test this hypothesis, they removed CD8+ cells from patients’ marrow preparations, and noted that blood cell formation increased. If they added CD8+ cells to the patients’ marrow preparations, blood cell formation decreased. In both cases, ATG treatment returned blood cell formation to normal within six months and reduced the destructive effects of CD8+ cells. MDS
involves a large population of an abnormally dominant CD8+ clone, but ATG reduces the dominance of a single clone of CD8+ cells and restores a more natural distribution.

Up to this point, the role of CD8+ T-cells in MDS sounds pretty negative. However, scientists began to wonder whether CD8+ cells could be “taught” to recognize certain abnormal (dysplastic) cells among the blood-forming population. A small protein known as “PR1 leukemia-associated antigen” is present on myeloid leukemia cells. T-cells that “target” PR1 are called PR1-CTLs (i.e. PR1 cytotoxic T-lymphocytes). Other studies had shown that PR1-CTLs kill abnormal cells in MDS and in acute and chronic myelogenous leukemia (AML and CML), and lead to remission in these diseases. Perhaps this activity could be strengthened.

PR1 vaccine was given to 45 patients with AML, CML, or MDS in a series of three vaccinations, and was very well tolerated. Among the patients, 25 showed immune responses and 15 did not. Among 16 patients who had both immune and clinical responses, none have died. Among 12 patients who had neither immune nor clinical responses, 6 have died. Event-free survival and overall survival were also higher in AML/MDS patients who had an immune response to the PR1 vaccine.

After the vaccinations, the T-cells with PR1 targets showed a higher specificity, and often a higher affinity for their PR1 target. Vaccination with PR1 selectively boosted the CD8+ immune response to abnormal blood-forming cells. The action of CD8+ T-cells, which causes many of the problems in MDS, can be harnessed to attack abnormal cells in the blood-forming population. Advantages of PR1 vaccine-induced immunity are:

- high T-cell receptor avidity
- targets more dysplastic (abnormal) blood-forming cells than normal blood-forming cells
- can be induced in both low-risk and high-risk MDS patients
- does not induce low blood cell counts
- vaccination with PR1 restores blood counts, induces remission, and is not toxic.

[No Presenter's Abstract was available]
For information on the symposium contact:

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