



SCIENCE LETTER

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Stem Cell Transplantation for Fanconi Anemia Using Donors Other Than Matched Siblings

New protocols including fludarabine show improved outcomes

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When an HLA-matched sibling is identified, marrow transplantation can cure the hematologic manifestations of FA. The chromosome fragility of FA makes children with FA unable to tolerate high doses of radiation and/or high doses of certain chemotherapy agents. This has limited the ability to perform marrow transplantation using donors other than HLA-matched siblings. For BMT using HLA-matched siblings, cytoreduction with low doses of radiation and chemotherapy has been successful. In the context of alternative donors such as unrelated marrow or cord blood donors, the use of a similar cytoreduction has been associated with high rates of rejection/graft failure and GvHD, resulting in poor overall survival. In addition, other problems have included toxicity and infections (in particular those of fungal origin).

The medical literature reports that approximately 150 patients with FA have received marrow transplants from HLA-matched siblings; the risk of rejection was 8%, the risk of developing acute and chronic GvHD was

45%, and 69% of patients are alive disease-free. Seventy patients have received marrow transplants from closely matched related donors with an increased risk of rejection of 20-37%, a risk of GvHD as high as 50%, and only 1/3 of the patients are alive and disease-free. Very little data exists regarding transplants from related HLA-mismatched donors.

Problems associated with stem cell transplants for FA include: (1) immune complications, namely graft-versus-host disease and graft rejection, (2) infectious complications in particular fungal infections, and (3) organ toxicity and especially severe mucositis. In addition, secondary malignancies are a potential complication for these patients. Therefore, to optimize stem cell transplants for Fanconi anemia using alternative donors, we need (1) a regimen that is immunosuppressive enough to be able to "get a graft in" (allow engraftment), with a low risk of rejection, (2) a regimen of marrow processing (T-cell depletion) that will decrease the risks of GvHD, (3) a regimen that will not give rise to

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Quality of Life after Bone Marrow Transplantation in Patients with Fanconi Anemia

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One of the very important questions asked by patients with Fanconi anemia and their families is how they can expect their life and health will be after a successful bone marrow transplant (BMT). To date, there have been no formal studies conducted to address quality of life in patients with Fanconi anemia after BMT. As a preliminary study, we sent out questionnaires to patients who were at least 1 year after BMT and received 18 responses from 9 males and 9 females. Transplantation occurred between January 1984 and June 1998. Patients' ages ranged from 1.7 to 31.6 years at the time of transplant and 2.8 to 35.4 years at present. It is very important to note that this group of patients was transplanted over a 14 year period and as a result, different preparative therapies, type of donors and supportive care treatments were utilized. In addition, patients were treated at a many different institutions. Therefore these results do not necessarily reflect results of current protocols. Twelve patients received a matched sibling donor BMT, one a matched cord blood transplant and one a matched cousin transplant. There were 4 unrelated transplants.

We first addressed the medical issues of these patients. It is important to keep in mind that patients with Fanconi anemia will have ongoing medical issues regardless of whether a BMT occurred. In fact, many patients and parents noted that life was less complicated after transplantation than before as numerous medical visits were no longer necessary and normal blood counts allowed for a much more normal routine.

There are complications after BMT that are common for all patients as well as unique complications seen in patients with Fanconi anemia. The common complications include lung problems (often asthma-like symptoms), poor heart function, gastrointestinal problems (poor appetite, diarrhea), poor kidney function, cataracts (clouding of the lining of the eye caused by radiation therapy), hypothyroidism (low thyroid function) and poor ovarian function in females requiring hormonal therapy. All BMT patients are more susceptible to dental caries and overcrowding of teeth. In addition, patients with Fanconi anemia have unique complications after BMT, in particular an increased risk for cancer of the head and neck. This risk is highest in patients with chronic graft-versus-host disease, especially if treated with azothioprine. It is very important that patients be followed at least annually by a

physician and dentist and more often if suspicious areas are noted.

Of the 18 patients who responded to our questionnaire, there were 3 who had one or a combination of lung, gastrointestinal, cataracts, hypothyroidism, and tooth decay. Three patients experienced chronic graft-versus-host disease causing dry eyes, dry mouth, poor appetite and skin rashes. There was one case each of heart complications, cancer of the tongue (treated successfully) and the need for estrogen replacement therapy. What is important to note is that most patients had either none or several medical issues after BMT. All of these complications were experienced in 10 patients with 8 patients reporting no chronic medical problems. This is markedly less than in patients with Fanconi anemia who have not undergone BMT and whose medical issues tend to escalate as they age.

The majority of patients report that they lead normal lives and are able to carry out normal activities of daily living with no restrictions. Patients with chronic graft-versus-host disease report somewhat restricted physical ability but rank their quality of life as very good. Some parents reported their children as having difficulty at school, increased self consciousness due to short stature and congenital anomalies, and excessive sibling rivalry. Whether the incidence is different from FA patients who have not undergone BMT is unknown. Nevertheless, most parents report an improvement in overall family life as less medical attention is required.

Most patients report a very good quality of life after bone marrow transplant. It appears that those patients who do well after transplant do very well. Many report that their quality of life has improved as they require less doctor visits and treatments. The quality of life of FA patients with and without transplant needs to be determined. Although it will be difficult to eliminate all complications, improvement can be made. For instance, the role of growth hormone replacement therapy before BMT needs to be addressed. As well, reducing the incidence of chronic graft-versus-host disease may decrease the risk of malignancy after BMT.

Hematopoietic Cell Transplantation for Fanconi Anemia Using Mismatched Related and Unrelated Donors: The University of Minnesota Treatment Protocol

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Background

Hematopoietic cell transplantation (HCT) from an allogeneic donor is the only treatment with curative potential for patients with the hematological complications of Fanconi anemia (FA). However, the majority of FA patients do not have an unaffected (i.e. FA negative) HLA-identical sibling donor. Compared to transplants from HLA-matched related donors, results with HCT from mismatched family donors or unrelated donors have been relatively poor.

For the International Bone Marrow Transplant Registry (IBMTR), Gluckman et al. (1995) analyzed the outcome of 199 patients who received HCT for FA from HLA-identical siblings (n=151), mismatched family member donors (n=29) or unrelated donors (n=19). Myeloid engraftment occurred in 76% of patients who received marrow from alternate donors (i.e., donors other than HLA-identical sibling donors) and grade II-IV acute graft-versus-host disease (GVHD) occurred in 51%. The probability of survival was 29% after alternative donor transplantation in contrast to 66% two year survival after HLA-identical sibling transplantation.

Between June 1993 and July 1998, 29 FA patients were treated on the University of Minnesota protocol of cyclophosphamide 40 mg/kg, total body irradiation (TBI) 450 cGy or 600 cGy and anti-thymocyte globulin followed

by HCT from a mismatched related or unrelated donor. GVHD prophylaxis consisted of cyclosporine A (CSA) for 6 months, short course methylprednisolone between day +5 and +19, and T cell depletion of the marrow. For the entire cohort, the probability of neutrophil recovery was 61%, acute GVHD was 28%, and survival was 34% at 1 year. Survival was best in recipients of TBI 450 cGy (n=21, 43% at 1 year).

Based on all these data, the major obstacle to successful alternative donor HCT for patients with FA is graft failure. Notably, the presence of somatic mosaicism (i.e., the presence of DEB insensitive lymphocytes) was the only risk factor associated with a higher risk of graft rejection. Degree of HLA disparity between the donor and patient, number of red cell and platelet transfusions prior to HCT, and disease status (e.g., aplastic anemia versus myelodysplasia) were not associated with graft failure within this dataset.

As a result of these findings, the protocol was modified in three respects:

- 1) Fludarabine, a potent immunosuppressant, was added to the preparative therapy in an attempt to reduce the risk of graft rejection;
- 2) Screening for fungal colonization/infection and initiation of anti-fungal prophylaxis was instituted in an attempt to reduce the risk of fungal infection after HCT;
- 3) Patients with somatic mosaicism were identified.

Eligibility Criteria

Patients must be <35 years of age with a diagnosis of FA. Patients must have an HLA-A, B, DRB1 identical or 1 antigen mismatched related (non-sibling) or unrelated donor. Patients and donors will be typed for HLA-A and B using serological or molecular techniques and for DRB1 using high resolution molecular typing. Patients with FA must have severe aplastic anemia (SAA), myelodysplastic syndrome (MDS) or acute leukemia with or without chromosomal abnormalities.

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Ralf Dietrich, MD, John Wagner, MD and Margaret MacMillan, MD

Fanconi Anemia 101 - The Basics

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This review of FA was an introduction for the new families. Photographs of several patients were shown, to indicate the heterogeneity of FA patients. The age average at diagnosis of FA is 8-9 years, but at least 10% are not diagnosed until they are over 16 years of age. Physical abnormalities are found in ~75% of cases, and include skin pigmentation such as dark skin or dark (café au lait) or light (hypopigmented) birth marks. Short stature is common, as are abnormal or missing thumbs or kidney malformations. Males may have underdeveloped reproductive organs. Other birth defects are less common and are listed in *Fanconi Anemia: A Handbook for Families and Their Physicians*. The major complications of FA include aplastic anemia, leukemia, and solid tumors.

The inheritance of FA is autosomal recessive (each parent is a silent carrier, and the affected offspring occur in a frequency of 1 in 4). The accepted diagnostic test is chromosome breakage, in which peripheral blood lymphocytes are cultured with a chemical which makes the cells divide, and then with a DNA damaging agent such as diepoxybutane (DEB) or mitomycin C (MMC). The basic genetic material of the cells is in chromosomes, and the FA cells cannot repair the damage from these agents, leading to breaks, gaps, and rearrangements. Flow cytometry, which measures the amount of DNA in the cells, may have a diagnostic role in the future. In some patients, some of the blood cells seem to have fixed their FA defect, leading to a population of blood cells that are "FA," and cells which are not FA, i.e., somatic mosaicism (see Hans Joenje's report).

Blood counts may be subtly abnormal in FA patients, including mild anemia, borderline platelets, and increased red blood cell size (MCV). Patients not known to have FA, but who have slight deviations from normal counts on a blood test, should be evaluated for FA.

FA should be suspected in patients with any of the characteristic physical findings, children or young adults with aplastic anemia or myelodysplastic syndrome (MDS), acute myeloid leukemia (AML), particularly those who are overly sensitive to chemotherapy, children or adults with any of the characteristic types of cancers (see my section on adults), and adults with fertility problems associated with a suspicion of FA.

For known FA patients who are clinically well, blood counts should be done 3-4 times a year, and bone marrows should be examined annually. The bone marrow exam includes an aspirate for cellular morphology (microscope

examination), biopsy to assess the amount of cells in the marrow, cytogenetics to determine whether there is an abnormal clone, as well as special stains and flow cytometry. The latter are done in research laboratories and can be sent to my collaborator at the University of Texas Medical Branch in Galveston, Texas. He can be reached as follows:

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The treatment of blood problems in FA includes male hormones with or without corticosteroids, or bone marrow, peripheral blood stem cell, or cord blood transplant from a related HLA-matched donor. G-CSF may improve the white blood count. Supportive care includes red blood cell and platelet transfusions (do not use family members because they may sensitize and jeopardize a later transplant), and antibiotics as needed. Unrelated transplants have a higher risk of failure (see John Wagner's article on page 3), and gene therapy is a research topic (see Chris Walsh's article on page 8).

Fanconi Anemia and the GI Tract

Sarah Jane Schwarzenberg, MD
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Fanconi anemia (FA) is associated with both anatomic and functional disorders of the gastrointestinal tract. Anatomic abnormalities include esophageal atresia, duodenal atresia, and anal atresia. Functional abnormalities include poor oral intake, nausea, abdominal pain, and diarrhea.

The major goal of the gastrointestinal tract is to provide good nutrition, as exemplified by normal growth, energy to meet demands of daily living, and adequate reserves to face short-term malnourishment during acute illness.

Poor growth in FA can be the result of multiple endocrine abnormalities and/or poor oral intake. Problems resulting in poor oral intake include lack of appetite/interest in food, nausea, and/or pain caused by eating (cramps).

Causes of poor oral intake include gastrointestinal abnormalities, chronic inflammation or infection, medication side effects, and/or neurologic abnormalities or behavioral problems.

Some gastrointestinal problems are related to complications of congenital abnormalities in FA. After esophageal atresia repair, patients frequently have gastroesophageal reflux; 30-50% require anti-reflux surgery. Esophageal replacement is associated with pain eating solids and vomiting.

Complications of duodenal atresia repair include >25% symptomatic with abdominal pain, chronic alkaline reflux, blind loop syndrome, poor duodenal motility above repair, and recurrent obstruction-like episodes. Some of these complications are seen less frequently in patients who have duodenal tapering at the time of their repair.

Complications of anal atresia repair include: 30% have fecal incontinence, 50% have occasional soiling, and some have constipation with or without encopresis.

Evaluation of poor feeding will begin with a good history and physical exam, which may take up to an hour. The child's previous records and a three-day diet history should be available to the physician two weeks before the visit. Other tests which might be ordered include:

- Barium study of gastrointestinal tract
- Gastric emptying study
- Blood for CRP, ESR, Helicobacter pylori antibody, zinc level
- Stool for ova and parasites, cryptosporidium;
- Urine culture
- Endocrine studies
- Endoscopy with biopsy

Some clinical situations suggest certain problems. Abdominal pain + Nausea suggest:

- Mechanical obstruction
- Abnormal gastrointestinal motility
- Small bowel overgrowth
- Gallbladder disease

Nausea alone suggests:

- Infection
- Urinary tract infection
- Sinusitis
- Behavioral problem
- Medication side-effect
- Gastric emptying delay

If a diagnosis cannot be made, a trial of therapy to improve symptoms is warranted. Treatment options include:

- Trial of acid suppression: ranitidine, famotidine, omeprazole
- Trial of motility-promoting agents: cisapride, metoclopramide, erythromycin

Note that cisapride (Propulsid) must be used with caution (if at all) in FA, as many FA children have cardiac abnormalities.

- Trial of anti-nausea agents: ondansetron
- Trial of small bowel overgrowth treatment: metronidazole
- Supplemental nutrition

Supplemental nutrition can be administered by two methods: supplemental enteral feeds or supplemental parenteral feeds. Supplemental parenteral feeds require placement of a central line, are associated with increased risk of infection and metabolic disorders. Their use is limited to patients unable to meet needs enterally. Supplemental enteral feeds are used when a child persistently is less than 85% expected weight for height OR fails to gain weight over 3-6 month period. Lasting benefits may require long-term therapy.

Enteral feeds are supplemented at night, over 8-10 hours, to allow for appetite during the day. After the child reaches the "goal" weight, flexibility is possible with "nights off" to teenagers, for example. Problems include development of heartburn, decrease in daytime appetite,

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Issues for Adults and Teens with FA

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FA males have underdeveloped male organs and may have decreased production of sperm and decreased ability to father children. FA females have delay in onset of menstrual periods, irregular periods, and some decrease in the ability to have children. In a study of the literature, at least 20 FA women had 30 pregnancies, with 8 spontaneous abortions in 4 patients, 22 babies delivered, and 21 living. FA was diagnosed late, at ages 12 to 44, and prior to pregnancy in only 9. Pregnancies were at ages 18 to 34. Five Cesarean sections were required. There was worsening of anemia in 10 cases, and decreased platelets in 8. These blood problems were managed with transfusions, and counts improved after the deliveries. Eight later developed cancer, and 7 died from their cancer.

Malignancies have been reported in many FA patients: 10% leukemia, 7% myelodysplasia, 5% solid tumors, 4% liver tumors. These are minimal figures. Sixty cancers were reported in 55 patients, with more in females than males. The cancers include head and neck (tongue, mouth, throat), gastrointestinal (esophagus, stomach, colon), gynecologic (anus, vulva, cervix, breast), brain, and other areas. Several tumors occurred in FA patients who had received bone marrow transplants. The tumors were tongue or mouth, 2 to 12 years after transplant, and were mainly in the context of chronic graft versus host disease (which may include oral lesions), and also irradiation.

Liver cancers are primarily in patients who have taken androgens and can be monitored with tests of liver enzymes and alpha-fetoprotein every 2-3 months, and ultrasound examinations at 6 to 12 month intervals. They often reverse when androgens are stopped. Although serious, these tumors are usually not the cause of death, although they may be found with other tumors, leukemia, or severe aplastic anemia.

Cancer surveillance includes 6-12 month gynecologic exams for women, good general physical exams at 4-6 month intervals, careful oral exams by hematologists and dentists, and more invasive testing as soon as symptoms occur. It is very important that the patient inform his or her doctor about any mouth sores, difficulty swallowing, hoarseness, vomiting, loss of appetite, unusual bleeding, etc., so that problems can be identified early.

Leukemia is not uncommon in FA and is usually myeloid (hard to treat), rather than the more common childhood leukemia which is lymphoid. My colleague at the University of Texas Medical Branch in Galveston, Dr.

Tarek Elghetany, and I are searching for early markers of preleukemia. Although patients with leukemia may have abnormal bone marrow clones, our preliminary data do not support the suggestion that the finding of a clone means that leukemia is inevitable. Clones are identified by examination of 20 dividing bone marrow cells, and thus statistically clones may be missed, or may appear to fluctuate because of the small number of cells examined. More sophisticated methods, such as FISH (fluorescence in situ hybridization) may be more useful, and research is currently being done with these methods. Some patients have had clones for up to 12 years without leukemic transformation. Microscopic evidence for myelodysplastic syn-

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Fanconi Anemia and the GI Tract

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vomiting, or tubes may become dislodged.

Routes for enteral feeds include nasogastric tube, nasojejunal tube, or gastrostomy tube. Nasogastric tubes are soft feeding tubes, passed through the nose into the stomach. They may be removed daily or left in place. They may become dislodged at night (this is a higher risk in infants). They are unattractive and may cause sinusitis, but are good for short term (<3 months) of supplementation or to determine if a gastrostomy tube feeding would be successful.

Nasojejunal tubes are soft feeding tubes, passed through the nose into the small intestine by a radiologist. They cannot be removed daily, but they reduce the risk of reflux.

A gastrostomy tube is a flexible tube placed into the stomach through the abdominal wall. Placement requires a minor surgical procedure. Complications are generally limited to local irritation and/or infection. Rarely, disruption of the tube can cause a more serious infection.

The choice of enteral methods must be made by the family and child together after they are educated in options. Before gastrostomy tube placement, it is important to do a trial of NG feeding to show that feeds will work. The choice should have minimal impact on the child's social situation and the family's lifestyle when possible.

Matched Related Donor Transplantation for Fanconi Anemia

Richard E. Harris, MD
Children's Hospital, Cincinnati

Children with Fanconi anemia who undergo successful matched related donor stem cell transplant for Fanconi anemia have an excellent chance for cure of the aplastic anemia and reduction in the risk of later developing leukemia or myelodysplastic syndrome. Published data from 151 matched sibling donor transplants reported to the International Bone Marrow Transplant Registry (Blood 86: 2856, 1995) showed a 2-year survival of 66%. Graft failure was seen in 9%, grade II-IV GVHD in 42%, and chronic GVHD in 44%. Factors associated with a worse outcome were age over 10 years, a low platelet count, no ATG in the preparative therapy, a cytoxan dose over 100 mg/kg, and the use of methotrexate for GVHD prophylaxis. An update in 1998 of IBMTR data in 285 matched sibling donor transplants showed a 5-year survival of 61%, but more importantly, much better survival in more recent years. Since 1994, 2-year survival has been over 80%.

The most successful transplant preparative therapy regimens to date have been the regimens utilizing low dose cytoxan (about 20 mg/kg) combined with thoraco-abdominal radiation 400-500 cGy. Dr. Eliane Gluckman in Paris reported a survival of 76% in 45 patients receiving this approach. A similar regimen but with the addition of ATG to the preparative therapy at the Children's Hospital Medical Center in Cincinnati has resulted in a 2-year survival of 86% among 26 patients and a very low risk of acute or chronic GVHD. The Seattle and Brazil groups are jointly reporting improved success with their cytoxan only regimen. Their studies have looked at de-escalating the dose of cytoxan from 200 mg/kg to much lower doses and are achieving survival also in the range of 80%.

The Cincinnati protocol is available to any transplant center which agrees to provide patient data and research samples through the FARF or directly to Dr. Richard Harris (richard.harris@chmcc.org). The Seattle/Brazil protocol is available through Dr. Mary Flowers (mflowers@fhcrc.org).

The Cincinnati protocol utilizes a preparative therapy of cytoxan 5 mg/kg/day for four days and 400 cGy of thoraco-abdominal irradiation. Also, ATG is given both before and after transplant, and patients receive cyclosporine for GVHD prophylaxis. The marrow is not manipulated to remove any lymphocytes. Either marrow or cord blood may be used. If the patient has a cytogenetic clone, myelodysplasia, or leukemia, the patient receives the radiation as total body irradiation. Entry criteria include a

clear-cut diagnosis of FA by DEB, an available matched related donor, and low blood counts (at least one of the following: platelet count < 50,000, hemoglobin < 8.0, or neutrophil count < 1000). Patients need not have previously received either cytokines or androgens prior to going to transplant. Pre-transplant studies include: 1) DEB testing, 2) skin biopsy for skin fibroblasts for research purposes, 3) peripheral blood sample for developing a cell line of immortalized B-cells for research purposes, 4) registration with IFAR or Eufar, 5) central review of the bone marrow slides for myelodysplasia or leukemia, 6) bone marrow cytogenetics and FISH for monosomy 7, and 7) an organ function evaluation. It is expected that from 10-20 patients per year will undergo transplant on this protocol.

Following presentation of the above protocol, Dr. Harris presented his recommendations for alternatives if no matched sibling donor is available, including when to initiate androgens or cytokines such as G-CSF, erythropoietin, or interleukin-11, and when to receive an unrelated donor transplant. He also discussed the new Cincinnati regimen for unrelated donor transplant in FA which utilizes cytoxan 5 mg/kg/day for 4 days, fludarabine 10 mg/m²/day for 6 days, and total body irradiation 450 cGy along with ATG, cyclosporine, and T-cell depletion of the donor marrow. So far, one patient has received this new regimen with rapid full engraftment and no evidence of GVHD. She is now out about 5 months from transplant with normal blood counts and no significant complications.

Issues for Adults and Teens with FA

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drome (MDS) may be more serious than a clone. Our data suggest that a high risk unrelated transplant might not be wise in the context of an abnormal clone by itself, but that clinical status including microscopic MDS might be a more compelling indication.

I have prepared a questionnaire to identify FA patients who have/had any type of cancer, as a first step in research into the reason for specific types of cancer in FA, and early identification of causes and treatment. This questionnaire can be obtained from the FA Research Fund.

Genetic Correction of Fanconi Anemia

Christopher Walsh, MD, PhD

University of North Carolina, Chapel Hill, NC

The generation of Fanconi anemia group C knockout mice now allows scientists to determine if genetic correction of bone marrow failure in FA is feasible. Knockout is a term used by geneticists to describe mice which are created without a functioning gene. The FANCC knockout mice (FANCC KO) used in our experiments in collaboration with Dr. Markus Grompe produce no FANCC protein. The FANCC KO mice exhibit some but not all of the features commonly found in FA patients and can best be described as having a mild form of the disease. The mice maintain normal blood counts throughout their lives. However, upon treatment with drugs such as mitomycin C or irradiation, they develop bone marrow failure. Bone marrow failure is dose-dependent; if sufficient drug or x-ray is provided, the bone marrow failure leads to death, whereas lesser doses will lead to temporary impairment of marrow failure. KO mice do not develop leukemia or tumors. The lack of cancer development is an important difference between KO mice and FA patients.

Will gene therapy for FA patients work? To test this we used a retrovirus modified to carry a normal or correct version of the human FANCC gene and infected bone marrow isolated from the FANCC KO mice. The bone marrow stem/progenitor cells were infected with the virus and carried the new correct copy of the FANCC gene in their chromosomes. They produced daughter cells with the new gene. If this occurs, the bone marrow and the peripheral blood cells will carry this new gene. To test this, we then challenged the mice with either drug or x-rays. Doses of mitomycin C known to cause irreversible bone marrow failure were given to the mice. The mice all survived and all maintained normal blood counts!!! To be certain that these results were not in error, we performed the same experiment using a virus carrying the FANCA gene which does not correct the FANCC cells. All those mice died after receiving MMC.

In order to demonstrate that we are able to transfer the correct FANCC gene into stem cells, we must fulfill certain requirements. Do all the cell types in the peripheral blood carry the gene? Yes! Can you take the bone marrow from the experimental animals

who received the gene and put it into new mice, and will those mice be resistant to MMC? Yes. Therefore, in this animal model system we have corrected the hematologic defect in FA.

How do we use this information for treatment of FA patients? The results described above are quite encouraging. The results demonstrate for the first time that gene therapy for FA will succeed. However, the transfer of genes into human hematopoietic cells is much more difficult. Why? The vectors currently used to transfer genes into cells were modified from viruses known to infect mouse cells. These older vectors have particular characteristics allowing them entry into mouse cells. This is not to say that they will not work in patients. New methods for producing viruses and infecting cells have dramatically improved gene transfer. However, vectors based on viruses known to infect human cells, such as lentiviruses, may be more suited to infecting human stem cells. One particular lentivirus is derived from the virus that causes AIDS. Thus, identifying virus vectors which are both safe and effective for infection of human bone marrow stem cells is currently an open question.

An alternate strategy shifts the focus from viruses to the target cells. Can other cells be used as a source of hematopoietic stem cells that are more easily infected by viruses? It is now clear that cells capable of regenerating the bone marrow of mice originate in skeletal muscle,

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Christopher Walsh, MD, PhD gives lecture at FA Gene Therapy symposium.

Stem Cell Transplantation for Fanconi Anemia Using Donors Other Than Matched Siblings

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excessive toxicity in children with FA, and (4) aggressive strategies for diagnosis, prevention and treatment of infectious complications.

T-cell depletion can be an effective way to prevent graft-versus-host disease. Several methods are available including (1) negative selection (depletion of T-cells and using the leftover cells including the stem cells). Examples include antibodies to T-cells, elutriation (as pioneered by Dr. John Wagner) or soy-bean agglutination and E-rosetting (as pioneered by Memorial Sloan-Kettering) and (2) positive selection (selection of the stem cells by an antibody which binds stem cells; CD34 antibody). One could use a combination of the two types of selection. T-cell depletion can prevent GvHD very effectively.

There is another potential advantage of T-cell depletion. Chronic GvHD puts non-FA patients at higher risk of squamous cell carcinomas of the oral mucosa and the skin. Although correlation of chronic GvHD and secondary squamous cell carcinoma is difficult in children with FA because they already are at risk for this complication, C-GvHD may increase this risk in these children. Adequate T-cell depletion can decrease the risk of chronic GvHD significantly, thereby decreasing the chances of squamous cell carcinoma post transplant.

Graft rejection can be prevented by two mechanisms: (1) by increasing the number of stem cells from the donor and/or (2) by increasing the immunosuppression of the patient (host). Stem cells can be obtained directly from the blood especially after stimulation with a growth factor such as G-CSF (1-2 subcutaneous injections/day x 5 days); they can be harvested thereafter by leukapheresis. In autologous transplants, the use of PBSC instead of BM was associated with faster engraftment and faster patient recovery. These findings can be seen in the context of allogeneic transplants as well. The product of the leukapheresis can contain a 2-5 fold increase number of stem cells as compared to bone marrow.

In 1992, fludarabine was found to be an effective agent against indolent (low grade) leukemias and lymphomas. In 1993, fludarabine was also used effectively for the treatment of acute leukemia (AML). In 1995-1998, it was found to have some effect against auto-immune disorders. Only one major side-effect was found with this agent: potent immunosuppression. In 1996, Martelli's group in Italy used fludarabine successfully as part of its cytoreduction in the context of related mismatched transplants. In 1997-1998, Slavin's group in Israel used fludarabine successfully for BMT and cord blood transplant from HLA-

matched siblings in 2 patients with Fanconi anemia.

Therefore, the addition of fludarabine to the cytoreduction (TBI/Cy + ATG/steroids) will enhance immunosuppression, thereby potentially decreasing the risk of rejection without additional toxicity. The use of G-CSF mobilized PBSC will increase the number of stem cells, potentially decreasing the risk of rejection. The use of T-cell depletion of the stem cells will decrease the number of immune (T) cells in the graft, potentially decreasing the risk of acute and chronic GvHD (and potentially of secondary malignancies). In the context of mismatched transplant, additions made to the regimen post-transplant include G-CSF for more rapid engraftment and FK506, an agent similar to cyclosporin to decrease the risk of GvHD.

Our experience at Sloan-Kettering included two patients we were able to transplant successfully using 2 antigen mismatched (or 4/6 matched) family donors. The first patient, Jack, is a 5-year-old boy with FA who developed aplastic anemia refractory to standard treatment with steroids, androgens and hematopoietic growth factors. He had been transfused multiple times. He received the standard approach of TBI (500 cGy), cyclophosphamide and anti-thymocyte globulin (ATG), steroids, cyclosporin A and G-CSF followed by a 4/6 matched cord blood transplant. He developed graft failure. We gave him a secondary cytoreduction with fludarabine, cyclophosphamide, ATG/steroids and FK506 and G-CSF post-transplant. He received G-CSF mobilized, T-cell depleted (CD34 positive, E-rosette negative) peripheral blood stem cells from his 4/6 matched (B-DR MM) father, day +46 after the first transplant. He engrafted by day 11 post transplant, with minimal toxicity and no GvHD. He is now 15 months post transplant with no chronic GvHD. He developed a complete immune recovery by 8 months post transplant. He is back to school.

The second patient, Justin, is a 10-year-old boy with FA who developed aplastic anemia and then myelodysplastic syndrome (RAEB). He also had been transfused multiple times. He had no closely matched donors (family or unrelated). He received cytoreduction with TBI (450 cGy), ATG/steroids and fludarabine and cyclophosphamide and received FK506 and G-CSF post transplant. He received G-CSF mobilized T-cell depleted (CD34+E-) peripheral blood stem cells from his HLA 4/6 matched sister. Toxicity included minor mucositis, minor hemorrhagic cystitis, and steroid-dependent diabetes. These have all resolved. Justin engrafted day +10 post transplant. He is now 5 months post-transplant. He is on a taper of his FK506. He is off steroids, off G-CSF, and is transfusion independent.

Although this is early data on only two patients, it is

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Hematopoietic Cell Transplantation for Fanconi Anemia Using Mismatched Related and Unrelated Donors

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Preparative Therapy

Preparative therapy will be identical for all patients. It will include total body irradiation (450 cGy); cytoxan (10mg/kg/day for 4 days); fludarabine (35 mg/m²/day for 4 days); methylprednisolone and ATG.

Results

At this time, 6 patients have been enrolled into this trial using fludarabine in combination with cyclophosphamide and total body irradiation. Patient age has ranged from 6 years to 26 years. Five received unrelated donor bone marrow (matched or mismatched at one locus) and one received a two antigen mismatched umbilical cord blood graft. All tolerated the therapy. All patients engrafted; none had graft-versus-host disease; and, all are alive. The follow-up, however, is short (maximum 7 months). While encouraging, more patients and longer follow-up are needed before any conclusions can be made.

Thus far, prescreening for fungal infection has demonstrated colonization of yeast in one and aspergillus in another. Pretreatment with itraconazole has been well tolerated. While the value of prescreening and itraconazole prophylaxis remains to be determined, these early results already indicate that asymptomatic FA patients may be colonized with fungus.

New Directions

We hope that the use of fludarabine will improve engraftment and survival. We are also developing new strategies for reducing further the risk of chemotherapy/radiation therapy side effects, graft rejection, graft-versus-host disease and infection. For example, we are investigating non-transplant approaches for improving bone marrow function, studying the biological significance of stem cell somatic mosaicism, and evaluating methods for predicting cyclophosphamide and irradiation sensitivity.

In collaboration with Dr. Arleen Auerbach, we are investigating endocrine (hormone) function after transplantation. In collaboration with Ear, Nose and Throat (ENT) specialists at the University of Minnesota, we are studying methods of ameliorating the effects of androgens on the vocal cords. Furthermore, we have just developed a group of investigators interested in treating squamous cell carcinoma of the head and neck (more details to follow).

Genetic Correction of Fanconi Anemia

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liver, brain and non-adult tissue such as embryonic stem cells. These surprising findings lead us to think of stem cells as omnipotent (capable of becoming several tissues) in different tissues throughout the body. Given the right experimental conditions, these very rare cells can be coaxed to become hematopoietic cells. FA patients have decreased blood counts which reflects diminished functioning stem cells. For patients with severe disease, the number of stem cells in the blood and bone marrow may be either too few or too damaged for gene transfer to be effective. Perhaps we should consider the use of omnipotent stem cells from other organs as alternate sources of stem cells to repopulate the bone marrow.

So what is available now for FA patients? The gene therapy trial for patients in FA-A at UNC uses many of these innovations described above. This trial is approved and funded for 10 patients. We expect to begin the trial in late December 1999 or early 2000. Inquiries can be made by patients and their physicians to Dr Chris Walsh, UNC Gene Therapy Center, (919)966-9116 or by e-mail: cwalsh@med.unc.

Stem Cell Transplantation for Fanconi Anemia Using Donors Other Than Matched Siblings

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exciting because: (1) for one patient this represented a second transplant, which is usually very hard to perform successfully, (2) both patients had had multiple transfusions, which also makes the likelihood of engraftment more difficult, (3) none of the patients developed GvHD, and (4) the patient's immune reconstitution was very rapid, possibly due to the use of peripheral blood stem cells.

Based on this very exciting data, we are developing a new protocol of stem cell transplantation for children with FA who lack an HLA-matched sibling donor. This will be a joint protocol between Memorial Sloan-Kettering Cancer Center in New York and Hackensack Medical Center in New Jersey. This protocol will include low dose TBI, fludarabine, cyclophosphamide and ATG/steroids, with FK-506 and G-CSF post-transplant. The choice of donors will be based upon availability of unrelated donor or cord blood. The choice will be: (1) 5/6 related (2) 6/6 or 5/6 unrelated donor, (3) 6/6, 5/6 or 4/6 unrelated cord blood with a very good cell dose (> 100 x 10⁶ nucleated cells/kg), (4) 4/6 or 3/6 related donor. The graft will be G-CSF mobilized T-cell depleted (CD34+E-) peripheral blood stem cells for related or unrelated donors.



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