



SCIENCE LETTER

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Decision-Making Guidelines for Treatment and Therapy of FA

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There are many potential therapies for FA. Which ones you should consider will depend upon your child's situation and your personal approach to risk. How willing are you to take a big risk for a possible cure of your child's bone marrow failure? You, as a parent, should become familiar with your child's specific situation and with the advantages and disadvantages of the potential therapies.

First, you should find out certain information about your child's situation, such as: Is the diagnosis certain? Is it known what complementation group your child belongs to or has the exact gene defect been determined? What are the blood counts? What is the cellularity of the bone marrow? Is there any evidence in the marrow of a clone, myelodysplastic syndrome (MDS) or leukemia? How well do your child's kidneys, heart, and gastrointestinal system work? Might these problems prevent a safe transplant? Has HLA typing been done on the family members? If there is no sibling match, has an unrelated donor search been initiated? How knowledgeable is your child's physician about FA? Do you have time to consider gene therapy or pre-implantation genetic diagnosis/*in vitro* fertilization?

Second, learn about the options. It is well known that a matched sibling donor

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Cancer: Risks, Screening, and Prevention

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As patients with FA get older, the risk of cancer increases dramatically. At the cellular level, cancer may develop due to a series of mutations in: oncogenes, which lead to increased growth of cells (accelerators), tumor suppressor genes, which remove the ability to restrain growth (brakes), telomerase genes (which are involved in the integrity of DNA), or DNA repair genes, which fail to repair carcinogenic mutations. Cancer pathways reflect an accumulation of various types of mutations, which initiate changes in cell growth, promote malignant transformation, progress to tumor cells, and ultimately become cancers which can invade and metastasize.

The major cancers reported in the medical literature in FA are leukemia, liver tumors, and tumors of the oral cavity and pharynx, esophagus, vulva, and cervix. A small number of tumors were also reported in the skin, brain, breast, kidney, lung, lymphoid system, stomach, colon, bone and eye. Several tongue cancers have been reported following bone marrow transplant, but current data are insufficient to determine whether BMT (and its associated preparative regimens) causes a further increase in cancer risk.

Cumulative risks of development of leukemia, MDS, and solid tumors were calculated from the literature reports.

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Transplants in FA: Improvements and Prospects

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There are still many controversies about the desirability and timing of matched sibling or alternative donor transplants in FA. The same is true for the type of preparative regimen for such transplants. The aim of the German Fanconi Anemia Study (GEFA-Study) is to define prognostic factors in FA, and develop transplant protocols with decreased early and late side effects by avoiding radiation and critical cross-linking agents in conditioning regimens. Therefore, two fludarabine based protocols were initiated. The GEFA I protocol consisted of fludarabine in combination with *in vivo* T cell antibodies, for patients with matched sibling donors and without clonal disease. The GEFA II protocol consisted of fludarabine, low-dose busulfan in combination with *in*

vivo T cell antibodies, for alternative donor transplants as well as all patients with clonal disease. Other pediatric transplant centers also took part in the transplant protocol (Munich-Schwabing/Germany n=1, Münster/Germany n=1, Madrid/Spain n=1).

Altogether, 17 patients have been transplanted thus far, with a mean follow-up of 18 months. The patients came from various countries (Germany n=11, Spain n=1, Greece n=1, Italy n=3, USA n=1). There were 9 males and 8 females with a median age of 9.3 years (range 1 – 16 years). Disease status, transplant variables, survival with functional grafts (surv) and probability of survival according to the Kaplan-Meier analysis (pSurv) are summarized in the following table:

	N	Surv	pSurv
Total FA-patients	17	13	0.72 ± 0.12
SAA before transplant	8	8	1.00 ± 0.00
MDS before transplant	6	4	0.67 ± 0.19
AML before transplant	3	1	n.d.
MRD transplants	6	6	1.00 ± 0.00
MMRD transplants	2	1	n.d.
MUD transplants	9	6	0.62 ± 0.18
MUD transplants **	8	6	0.70 ± 0.18

** according to the current GEFA II protocol w/o T-cell depletion

There were 4 deaths, one leukemia-related and three infection-related. Toxicity was very limited. Engraftment was the main problem. Thus, 5 treatment failures occurred (29%). It was possible to engraft all of these patients with a second transplant (n=4) or a third transplant (n=1). With the current GEFA II protocol, including fludarabine 180 mg/m², busulfan 2 mg/kg, ATG before transplant, OKT3 post transplant, and no *ex vivo* T-cell depletion, there was no graft failure using well matched unrelated donors and rather high stem cell

doses. The engraftment failures occurred in *ex vivo* T-cell depleted MUD- or MMRD transplants (n=3) or in 2/6 MRD transplants in the attempt to use only fludarabine and antibodies. For such transplants it might be necessary to add also the low-dose busulfan or another alkylating agent, especially if the sibling donor size allows only a limited harvest of stem cells. Otherwise, the data are encouraging, particularly with unrelated donors. The final proof of the concept will be the late results and the incidence of secondary tumors.

The FANCD2 Protein Test for FA

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Dr. Alan D'Andrea's laboratory has recently reported a new test for FA. Six of the FA genes have been identified and their protein products appear to work together in a common pathway. Activation of the FA pathway in response to DNA damage leads to a change in the FANCD2 protein. The resulting larger modified FANCD2 protein (FANCD2-L) is absent in FA cells. The test for the FANCD2-L protein offers a simple alternative to the standard test for FA, the DEB test for chromosomal instability.

We have explored the clinical applications of this new simple, rapid test for Fanconi anemia. The FANCD2 test was compared to the traditional DEB test for FA. FANCD2-L was absent in fresh blood samples from 13 FA patients tested with increased chromosomal breakage by DEB testing. In contrast, FANCD2-L was present in 26 control blood samples drawn either from normal volunteers or from patients in whom FA was ruled out by DEB testing. Absence of FANCD2-L appears to be specific for FA since it is not affected in the other bone marrow failure syndromes or other chromosomal breakage syndromes tested. Introduction of the appropriate functioning FA gene restores FANCD2-L in FA cells. The FANCD2-L test provides a useful rapid way to perform FA subtyping analysis. The FANCD2 test also provides a way to monitor the effects of gene therapy. We are currently exploring whether the FANCD2 test offers a useful way to assess the efficacy of clinical treatments for FA.

To develop these new tests for FA, we need fresh samples of blood or bone marrow. To avoid subjecting patients to additional procedures, these samples can be obtained at a time when they are being drawn for clinical care. If you are interested in contributing samples for our studies, please contact Dr. Shimamura

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FA and the Gastrointestinal Tract

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FA is associated with both anatomic and functional disorders of the gastrointestinal tract. Anatomic abnormalities include esophageal atresia, duodenal atresia, and anal atresia. These abnormalities affect only a small number of patients with FA. Functional abnormalities include poor oral intake, nausea, abdominal pain, and diarrhea. The number of patients with these complaints is not known.

Patients with FA also have short stature, compared to the general population. Some patients have endocrine abnormalities causing their short stature. For others, the cause is not yet determined, but is felt to be related to the genetic cause of FA.

The major function of the gastrointestinal tract is to provide good nutrition, as exemplified by normal growth (for genetic potential), 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, the underlying genetic abnormality, and/or poor oral intake.

There is increasing evidence that patients with FA may have endocrine abnormalities. In one study of 56 patients with FA, 81% had some endocrine abnormality, although not all required medical therapy. The patients in this study had hypothyroidism (36%), growth hormone deficiency (44%), and glucose dysregulation and increased risk of diabetes (25%). Any of these abnormalities may contribute to poor growth. Some studies have demonstrated hypogonadism in some patients with FA; many go through puberty late.

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 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 fecal incontinence (30%), occasional soiling (50%), and constipation with or without encopresis (soiling due to leakage around a chronic impaction).

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 2 weeks before the visit. Other tests which might be ordered include:

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

Some clinical situations suggest certain problems.

Abdominal pain and Nausea suggest:

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

Nausea alone suggests:

- Infection
- Urinary tract
- 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: metoclopramide, erythromycin
- Trial of anti-nausea agents: ondansetron
- Trial of small bowel overgrowth treatment: metronidazole
- Supplemental nutrition

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, vomiting, 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. There 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.

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Androgens: a View from the Past

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Androgens are responsible for the symptoms of puberty as well as the increased hemoglobin seen in post-pubertal males. They stimulate hematopoietic stem cells to proliferate and differentiate, and may also increase levels of erythropoietin, the hormone which is critical for red blood cell production. In the late 50s and early 60s, androgens were used to treat patients with acquired and also inherited aplastic anemia. Despite what appeared to be responses in both types of disorders, it is now clear that patients with inherited aplastic anemia, particularly those with FA, are the primary beneficiaries of this medication. In fact, there are patients who have been treated with androgens for up to 40 years, as well as patients who took androgens for 5 to 10 years, after which they became androgen-independent, perhaps due to development of somatic mosaicism.

The indications for treatment of FA patients were agreed upon at the previous Consensus meeting (held in May 1998): one or more of Hb <8 g/dl or symptoms from anemia, platelets <30,000/mm³, and neutrophils <500/mm³. Patients with consistent low blood counts were recommended for transplant if they had an HLA-matched sibling donor. Alternative donor transplants were considered high risk, and thus reserved for those with refractory aplastic anemia, myelodysplastic syndrome (MDS), or leukemia. Although this latter recommendation may change as transplant technology improves, it is not clear that there is sufficient evidence to make this policy change at this time. For the patients for whom transplant is not possible, not indicated, or not chosen, androgens remain the mainstay of therapy.

The side effects of androgens are potentially extensive, although many are reversible when the treatment is discontinued. The masculinizing components include excess facial and pubic hair, male-pattern hair loss, deepening of the voice, enlargement of the genitalia, acne, flushing, fluid retention, increased appetite, weight gain, increased muscularity, accel-

erated growth and skeletal maturation (but with early fusion of growth plates and thus possible short stature from the treatment), heart disease, and pubertal mood swings. More clinically serious but yet also reversible are increased liver enzyme levels, decreased liver function, cholestatic jaundice, and hepatomegaly (enlarged liver). Of most concern are peliosis hepatis (blood lakes, where hemorrhage may occur), and adenomas and hepatomas (hepatocellular carcinomas). Adenomas are benign tumors, while hepatomas are malignant; these distinctions depend on the appearance of the cells under the microscope, and whether they are invasive and metastatic. However, FA liver tumors have never been subjected to centralized pathologic review, and none of the 34 reported cases appeared to be truly "malignant." These tumors often regress after androgens are stopped.

The Consensus recommendation was to use 2 to 5 mg/kg of oxymetholone (Anadrol), orally, taken once a day. Hb usually improves in >50% of patients within 1 to 3 months, platelets in a smaller proportion by 4 to 6 months, and neutrophils may respond, less commonly and more slowly. The desired response need not be to normal blood counts, but should be sufficient so that the patient does not need transfusions, and to sustain a good quality of life. Each patient is unique in the response that is achieved, and the drug dose required to maintain the response without too many side effects. Injectable androgens such as nandrolone decanoate (Decadurabolin) have much less risk of liver toxicity and tumors, but do require weekly intramuscular injections, which may be painful, and may lead to a local bruise or even an infection.

Danocrine (Danazol) is an attenuated androgen, in which the masculinizing side effects have been weakened, although liver adenomas have been reported in non-FA patients. There is some theoretical concern that the bone marrow stimulation effect may also be

attenuated. We are aware of a few FA patients in whom there has been some response to this agent, but data are clearly insufficient at this time to determine the role of this agent in the management of FA patients. This may be an area for a randomized clinical trial, but parental or patient biases may prevent such a trial from occurring.

Monitoring of patients on androgens includes following liver enzyme levels every 2 to 3 months, and liver ultrasounds every 6 to 12 months. The dose should be decreased or even stopped if the liver enzymes reach 3-fold normal, or if any abnormalities appear on the ultrasound.

There is some concern that patients who have received androgens for "a long time" may be poor transplant candidates. It is important to consider the issue of "causality" here, however. Androgens may not always be the cause of transplant problems, but may be suffering from "guilt by association" in patients whose cytopenias lead to high doses of androgens with inadequate monitoring. For example, androgens are not causal of infections, but may be in use in patients with severe neutropenia. We cannot deny the importance of liver function in transplant, but suggest that appropriate use of androgens has a definite role in the longevity of many patients.

The FANCD2 Protein Test for FA

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(akiko_shimamura@dfci.harvard.edu) or Lisa Moreau (lisa_moreau@dfci.harvard.edu). We are also very interested in using the FANCD-L test to assess tumors that arise in patients with FA. For further information regarding these studies or for instructions on sending the samples, please visit our web site at <http://research.dfci.harvard.edu/dandrealab>. Interested readers are also referred to our web site for a description of the clinical and consultative services available through our center.

Use of Androgens in the Treatment of Bone Marrow Failure in Patients with FA

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It has long been established that androgen therapy with or without prednisone may ameliorate the cytopenias (i.e., deficiencies in red cells, neutrophils and/or platelets) characteristic of FA. Prior to the early 1980s, it was the only therapy with known beneficial effect. As expected, however, its place in the treatment plan of patients with FA has changed remarkably over time. By the late 1980s and early 1990s, androgen therapy was virtually eliminated as a treatment option in patients with an HLA-matched sibling donor. This practice reflected 1) the superior survival results observed in recipients of HLA-matched sibling donor BMT, 2) high incidence of untoward side effects associated with androgen therapy, and 3) the modest effect of androgens on all hematopoietic lineages and long-term survival.

For patients without an HLA-matched donor, androgens are often used at the onset of a cytopenia. Androgen therapy, however, is associated with numerous side effects. They include:

Common side effects

- Acne/oily skin
- Behavioral changes
- Enlarged penis/clitoris+
- Breast enlargement/soreness+/-
- Hoarseness/deepening of voice+
- Hot flashes
- Hair growth/unusual hair loss
- Decreased size of testicles+/-
- Erections
- Amenorrhea+/-

Less common side effects

- Darkening of skin
- Stool/urine discoloration
- Depression
- Thrombophlebitis

- Unusual tiredness
- Fluid retention
- Bone pain
- Hemorrhagic cystitis
- Sore tongue
- Nausea/vomiting/anorexia

Rare side effects

- Intra abdominal hemorrhage+
- Stroke+
- Pancreatitis
- Decreased platelet counts
- High BP (secondary to fluid retention)

Some side effects are irreversible + or only partially reversible +/-, while others disappear with androgen discontinuation. While some side effects would necessitate withdrawal of androgen therapy, the most common reason for discontinuation of such therapy is 1) loss of response to therapy with recurrence of cytopenias and 2) toxicity, such as liver abnormalities: most notably, marked elevation in liver function tests (i.e., increased ALT, AST, or bilirubin), or development of hepatic tumors (adenoma). Occasionally the development of severe behavioral disturbances or liver cancer necessitates discontinuation, but these are rare complications. For these reasons, close monitoring is required—1) liver function tests every 2 months, 2) alpha fetoprotein level every year, 3) liver ultrasound/scans every year.

This screening strategy is meant as a guideline (minimum) but may be required more frequently if there are dose changes or other drugs are added that may interfere with liver function and/or metabolism of drugs in general. Drugs to be concerned about include the following:

- Acetaminophen (Tylenol)
- Antiepileptics (Tegretol, Dilantin, Depakote)

- Chemotherapy
- Phenothiazines (Compazine)
- Immunosuppressants (CsA, FK506)
- Itraconazole
- Oral contraceptives (estrogens)

According to the drug manufacturers, the use of androgen therapy would be contraindicated (i.e., generally not recommended without close monitoring, if at all), if the patient concurrently has diabetes, pre-existing liver, heart, or kidney disease, history of increased calcium levels, or is pregnant.

If androgens are either not effective or toxicity ensues, it may necessitate altering the drug dose (decreasing drug if there are elevations of liver enzymes or increasing drug if decreased effectiveness). In some cases, physicians might add hematopoietic growth factor therapy if the neutrophils decline in number; in other cases, switch to alternate form of androgen drug. The benefit of this option is unknown. Further, it is unknown whether oxymethalone is the most effective or least toxic option. Currently, some patients are being treated with other forms of androgens (e.g., halotestin/Danazol) with the hope of having fewer masculinization side effects. It should be clear, however, that such a treatment plan has as yet unproven efficacy and less known toxicity profile in patients with FA.

While it is clear that androgen therapy can stimulate marrow function, albeit temporarily in most cases, a negative effect on transplant outcome has long been suspected. In at least two retrospective analyses by Harris et al. (in Sibling Donor Bone Marrow Transplantation for Fanconi Anemia) and Gluckman et al. (in Unrelated Bone Marrow Transplantation for Fanconi Anemia), use of androgens has been associated with lower rates of survival. The reasons for poor outcome are unclear and are probably multiple.

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Long Term Complications in Patients with FA

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Fanconi anemia (FA) is a complex disease that includes numerous congenital malformations, most commonly of the bones, kidneys and gastrointestinal tract, bone marrow failure and predispositions to diabetes and other hormonal deficiencies, infections and cancer. In addition, there are a number of late effects associated with various treatments common in patients with FA (i.e., androgens, prior surgeries and bone marrow transplantation). Although not addressed in detail, the long term psychological impact of chronic disease and short stature must also be considered a long term complication. With substantial improvements in long term survival after bone marrow transplantation, emphasis must now be placed on finding new strategies for reducing the risks of these long term complications of FA and treatment-related late side-effects and maximizing quality of life.

Long term medical complications of FA

- Short stature/poor growth
- Chronic food intolerance/early satiety/abdominal pain/poor nutrition
- Immune deficiency/infections
- Diabetes
- Hormone deficiencies (particularly, thyroid hormone, gonadal hormones and growth hormone)
- Infertility
- Cataracts
- Cancer (leukemia and particularly cancers of the head and neck, skin, and cervix)

Long term neuropsychological complications of FA

- Learning disabilities
- Behavioral problems (may be related to the use of androgens and steroids)
- Depression (may be related to chronic disease and need for chronic long term therapy)

Late effects of treatment

Surgery

- Adhesions/contractures (e.g., esophageal narrowing, gastric outlet obstructions, abdominal pain, dumping syndrome, biliary obstruction and chronic inflammation of the gallbladder)
- Persistent functional impairments (e.g., thumbs, curvature of spine)
- Blood transfusions
- Viral hepatitis, HIV, other
- Allosensitization
- Androgens (see associated article on complications of androgen therapy)
- Hepatomas/hepatocellular carcinoma
- Peliosis hepatis
- Long term masculinization effects (lower voice, early closure of bone growth plates and exacerbation of shortened stature)
- Prednisone
- Avascular necrosis of bones
- Infection

Bone Marrow Transplantation

- Hormonal deficiencies
- Chronic graft-versus-host disease
- Sterility
- Cancer

FA patients are at high risk of a number of late complications that are either a manifestation of the disease itself or its treatment. In some instances, it is not yet known whether or how much a treatment exacerbates the underlying risk of a complication, like diabetes and cancer. Nonetheless, new strategies for reducing these risks or treatment itself are being investigated.

In most cases, the long term survivor of FA today will have undergone multiple treatments that included prior androgen therapy with or without prednisone, transfusions, and bone marrow transplantation after cyclophosphamide and radiation. With rare exceptions, bone

marrow failure, myelodysplastic syndrome and leukemia and need for further therapy with androgens, growth factor and transfusions, have essentially been eliminated by marrow transplantation. Risks of complications related to the disease itself or prior non-transplant therapies may or may not be modified by transplantation. Although there are a number of potential long term complications of FA and treatment-related late effects, the most important plan is early detection and treatment intervention. Hormone deficiencies are easily treated with replacement therapy. If not managed by diet, diabetes can be treated with insulin under the guidance of an endocrinologist. Short stature is frequently the effect of inadequate growth hormone at least in part. Growth hormone replacement should be considered since height may impact psychosocial development.

Cancers of the head and neck, skin, and cervix are a major concern, particularly as FA patients enter their teens and twenties. Other than avoidance of UV light exposure to prevent skin cancer, careful monitoring of the mouth (dental visits every 6 months, particularly in patients >20 years) and cervix (gynecological exams every year after menarche) and early detection are the most significant effective strategies to date. While there are some reports suggestive of a viral etiology in cancers of the head and neck and cervix, it has yet to be proven. It is possible that the immune deficiency characteristic of patients with FA with or without transplant may play a role in the increased risk of these cancers. For patients with a history of chronic graft-versus-host disease after transplantation, particularly close follow-up is required since this disease is associated with prolonged immune deficiency.

New Directions

- Voice reconstruction, a relatively new surgical procedure to remodel

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Hematopoietic Cell Transplantation for Patients with FA: An Update

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Unrelated Donor HCT

Until recently, unrelated donor hematopoietic cell transplantation (HCT) in patients with FA has been limited by excessive rates of graft failure and toxicity from the preparative therapy. In an attempt to improve results, we have added fludarabine to the standard regimen of cyclophosphamide, total body irradiation and ATG. Between April 1999 and July 2001, 23 patients with FA underwent unrelated HCT using this fludarabine protocol. All evaluable patients engrafted, including 7 patients with somatic mosaicism, a potential risk factor for graft failure. No patient developed severe graft versus-host disease (GVHD). Survival rates greatly varied according to the risk of patients at time of transplant, as defined below.

Standard Risk

- 1) Aplastic anemia ± clonal cytogenetic abnormalities
- 2) Early myelodysplasia
- 3) No history of major infections

High Risk

- 1) Advanced myelodysplasia or AML
- 2) Prior gram negative infections
- 3) Proven or probable fungal infection

The probability of 1 year survival after BMT in the standard risk group is 86%, a rate which for the first time is comparable to that of matched sibling donor recipients. On the other hand, survival in the high risk group is only 13%, most patients dying from infections which have likely been dormant for some time before transplant. These infections are occurring in the high risk patients despite our efforts to identify patients most susceptible for developing opportunistic infections. Prior to transplantation, CT examinations of the chest and sinuses are obtained on all patients. If any suspicious areas are noted, patients are examined by an otolaryngologist and/or

pulmonologist. All patients are seen prior to transplantation by an infectious disease physician who specializes in fungal disease in bone marrow transplant patients. Clearly, patients should come to transplant earlier before they develop high risk characteristics.

Matched Related Donor HCT

Low dose cyclophosphamide and limited field irradiation is the standard preparative therapy for patients with FA undergoing related donor HCT. However, long-term follow-up studies have demonstrated a high risk of malignancy, particularly of the head and neck. Reported risk factors include the use of radiation and chronic GVHD. Therefore, in an attempt to reduce the risk of malignancy, we have replaced radiation with fludarabine and ATG. Between November 1996 and June 2001, 12 patients with FA underwent related donor transplantation using this fludarabine regimen. All patients engrafted and no patient developed severe acute GVHD or chronic GVHD. Two patients

(one with leukemia and the other who received bone marrow from his mother) later lost their grafts, and yet were successfully transplanted a second time. One patient died from a malignancy 2 years after transplantation. The remaining patients are alive and well. These early results demonstrate comparable engraftment rates using fludarabine in lieu of radiation. Long term follow up is required before determining whether this preparative therapy reduces late effects, particularly the risk of malignancy.

New Strategies in Unrelated Donor BMT for FA

- 1) HSV-tk ('suicide gene') transduced lymphocytes added back to the T cell depleted marrow graft to improve immune recovery and anti-MDS effect without risk of uncontrollable GVHD.
- 2) Shielding to thymus and mouth to improve immune recovery and reduce complications in the mouth, respectively.

FA and the Gastrointestinal Tract

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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 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.

Some researchers have speculated that FA is an oxidant stress disease. Diets high in vegetables and fruits may reduce the risk of some cancers. Fruits and vegetables contain a broad spectrum of antioxidants. Controlled trials with preparations of individual vitamins do not show similar results. In addition, some vitamins are toxic in excess, including:

- Vitamin A
- Vitamin D
- Vitamin C
- Niacin

Controlled clinical trials of antioxidants will be essential to avoid unnecessary toxicity and demonstrate efficacy. In the meantime, a diet containing a broad-spectrum of fruits and vegetables is a prudent course.

FA is an autosomal recessive disorder, in which parents are carriers (heterozygotes), with one normal and one mutant FA gene. There is a 1 in 4 random chance in each pregnancy of having a child who receives a mutant FA gene from both parents (1 in 2 chance from mother x 1 in 2 chance from father). Cells from FA patients are sensitive to DNA cross-linking agents, which lead to aberrations in the chromosomes which can be detected under the microscope.

There are more cases of FA reported in the medical literature than any of the other inherited bone marrow diseases (Diamond-Blackfan anemia, Shwachman-Diamond syndrome, dyskeratosis congenita, severe congenital neutropenia, thrombocytopenia absent radii, amegakaryocytic thrombocytopenia, and Pearson's syndrome). The median age at diagnosis of FA is ~8 years of age, with a range from birth to over 50. Approximately 75% of FA patients in the literature have physical findings, including pigmented skin, café-au-lait spots, short stature, thumb and radial anomalies, abnormal gonads, and structural kidney abnormalities.

There are at least 7 or 8 different genes involved in FA, although a given patient has mutations in both copies of only one of the genes. The "complementation groups" which define the genes were assigned by identifying cells from patients which, when cultured with cells from other patients, corrected the sensitivity to DNA cross-linkers. They thus complemented each other, or provided the factor which was missing. If cells fail to complement, they belong to the same group. The protein products of 5 of the groups (A, C, E, F, G) form a complex in the nucleus, which permits the D2 protein to accept a small molecule called ubiquitin. This modified D2 then may interact with other proteins which form complexes in response to DNA damage. See Dr. Shimamura's report.

Somatic mosaicism is the term used to describe the appearance in the blood

of some cells which are not sensitive to DNA cross-linkers. At the level of the blood stem cell, a molecular event (most often presumably a cross-over) occurred, in which both mutations ended up on one chromosome, and the other was repaired. This is most easily explained in cases where the parental mutations were in different positions on the same gene.

A bone marrow clone is defined as cells derived from a single stem cell, identified because they have an abnormal and identical chromosome pattern, such as missing one chromosome (e.g., monosomy 7; the normal pattern would be 2 or disomy 7), or mixing up pieces (translocation) of chromosomes. If at least 2 cells have the same translocation, or at least 3 the same deletion, the patient has a clone. Since only 20 cells are normally examined, the proportion or even presence of a clone may vary from time to time. The significance of a clone is under study, but in general it is the appearance of the bone marrow cells under the microscope (morphology), rather than the clone itself that is most important. Myelodysplastic syndrome (MDS) is defined when the marrow looks myelodysplastic, with many abnormal cells, in a patient who has clinical problems because the marrow function is impaired, independent of the type or presence or amount of a clone. If the clone increases to 100% of cells, or if it is specifically monosomy 7, there may be a reason for concern regarding evolution to leukemia, but further data are needed.

Treatment of FA will be described in the summaries of several other speakers, and cancer surveillance in the FA Cancer summary.

Based on these observations (i.e., androgens as a negative risk factor for transplant recipients) and the marked improvements in survival observed after unrelated donor BMT with modern therapy incorporating fludarabine, we advocate an amendment to the "conventional" practice of prescribing androgen therapy to all patients not having an HLA-matched sibling donor. Based on current statistics at the University of Minnesota and elsewhere, patients with bone marrow failure and with an HLA-matched unrelated donor should not be treated with androgens but should be offered early transplant therapy exactly as prescribed for those with an HLA-matched sibling donor. However, androgens should be considered for those patients at higher risk for problems with unrelated donor BMT, for example, 1) adult groups, 2) HLA mismatched donor, or 3) pre-existing significant organ dysfunction. While it is possible that these patients could do well with BMT, these are known risk factors in non-FA patients in general undergoing unrelated donor transplantation. Hence, a delay in BMT may be beneficial to the patient with the hope that alternative treatment strategies may be discovered (e.g., gene therapy, novel BMT regimens).

There is currently one clear exception to the above plan where androgens may be used as a measure to delay BMT. This exception would be parents who are attempting *in vitro* fertilization and preimplantation genetic diagnosis (PGD) in order to have a healthy child to serve as a HLA matched donor. While transfusion therapy and use of hematopoietic growth factor therapy remain alternatives to androgen therapy, it is not yet clear if one option is better than the other. Further consideration is required.

Gene Therapy for Group A FA Patients – Current Update and Future Trials

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FA is an inherited disorder characterized by pancytopenia, and a predisposition to malignancy. Current therapy for FA patients is allogeneic bone marrow transplantation from a histocompatible donor. However, most patients lack a suitable donor and usually die from bone marrow aplasia or acute leukemia. Thus, alternative therapies must be investigated.

The hallmark of FA is the hypersensitivity of FA cells to DNA cross linking agents such as mitomycin C. This observation suggests that FA cells are defective in DNA repair. To date, seven different complementation subtypes of FA have been identified from somatic cell hybridization studies. Of these, the FANCA group is the most prevalent, comprising up to 70% of FA cases.

After the *FANCA* gene was cloned, a retroviral vector carrying the *FANCA* cDNA was constructed (FAA5.5 clone 27) and the vector tested for its ability to transduce CD34+ hematopoietic cells obtained from FA patients. Retroviral-mediated transduction of lymphoblastoid cells from four different FAA patients resulted in phenotypic correction, i.e., expression of the *FANCA* transgene normalized cell growth, cell-cycle kinetics, and chromosomal breakage in the presence of mitomycin C. These experiments were an early indication of the feasibility of treating the bone marrow failure of FA patients through transfer of the *FANCA* gene into hematopoietic stem/progenitor cells.

This clinical protocol is designed to test whether a competitive growth advantage in gene-transduced cells will allow for hematopoietic reconstitution. The clinical protocol uses a retroviral vector carrying the *FANCA* gene to transduce CD34+-selected hematopoietic stem/progenitor cell populations obtained from FANCA patients. In this protocol there is no preconditioning of the bone marrow prior to cell infusion due to the sensitivity of patients to standard induction drugs. Patients are monitored for blood counts and marrow cellularity at 3, 6 and 12 months. Patients may

undergo the gene transfer procedure three times.

A gene therapy protocol testing for retroviral-mediated gene transfer as a potential treatment for FA has begun at the University of North Carolina at Chapel Hill. This protocol is currently investigating gene transfer for Group A FA patients.

The criteria for entry include:

1. patients diagnosed by DEB breakage analysis;
2. group A diagnosed by mutation analysis or complementation studies;
3. a bone marrow biopsy/aspirate and cytogenetics studies without evidence of malignancy;
4. no acute infection or medical problem;
5. lack of an HLA sibling donor for bone marrow transplantation.

Expenses for the patient and family include only travel and lodging costs. There are no medical costs paid by the patient or family.

FANCA Gene Transfer Trial

Four patients have enrolled in the trial ranging in ages from 11-48 years old. Three of the four patients have severe pancytopenia requiring blood transfusion and/or androgen support. CD34+ were obtained following G-CSF mobilization and either apheresis or bone marrow harvest. In the four patients tested, none mobilized significant numbers of CD34 cells into the blood. It now appears that bone marrow harvest yields far more CD34+ cells for gene transfer in the group A patients. However the number of CD34+ cells collected from the marrow is 1/10-1/100 that expected from a normal individual. The stage of disease (the severity of the peripheral blood counts and marrow cellularity) correlates with the number of CD34+ cells that can be harvested.

Following transduction (the process where the gene enters the cell and functions) in a specially designed clean room, cells were reinfused into the patients. All

four patients tolerated the procedure without complications. Gene transfer was positive in the CD34 cells at the time of cell reinfusion in all patients. The genetic analysis of blood and bone marrow samples revealed that only 2 of the 4 patients tested had significant long-term gene transfer. One patient had a significant increase over time in the number of peripheral blood cells carrying the *FANCA* gene. That patient's blood counts have stabilized without the need for transfusions or androgens/cytokines. This is extremely encouraging and suggests that we pursue this avenue of treatment vigorously.

Future Prospects

We have developed new vectors based on the lentiviral virus system and the results from testing patient CD34+ cells suggest that they may be superior to the current vector we are using in our trial. Further testing of patient marrow samples is required. We have used these new vectors to correct the hematologic defect in FA knockout mice and have sufficient data to initiate discussions with the FDA/NIH to begin a new trial for FA patients. Results obtained from this study will facilitate improved gene transfer for FA patients and other hematopoietic disorders.

We thank all the physicians and patients who have contributed time, effort and samples for our studies. We appreciate the support of the FARF and FA patient groups. We would greatly appreciate the opportunity to test marrow samples at the time of yearly marrow examination. Peripheral blood may also be sent for complementation testing.

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Decision-Making Guidelines for Treatment and Therapy of FA

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transplant will give the best chance of a cure of your child's bone marrow failure. With current approaches to transplant for FA, the chance of a cure of the aplastic anemia is about 85% if a matched sibling donor transplant is done at a center experienced at such transplants. But only about 25% of patients will have a matched sibling available. If your child is early in the disease process and still has good blood counts, another consideration might be to give birth to a child who you know will be a match and not have FA. This can be done by pre-implantation genetic testing of embryos in the early stages of development and then implantation into the mother's uterus of a suitable embryo. This process, termed PGD/IVF, is very expensive and may not be covered by insurance, and is very demanding on the mother. Also, a family may have to try several times before an embryo implants successfully and grows to term. Often, this process does not result in a pregnancy or live birth. The ability to wait a minimum of one year and adequate financial resources (insurance or personal) are prerequisites. A less involved approach might be to become pregnant, then have testing done from tissue obtained from a biopsy of the placenta or from amniotic fluid cells to see whether the baby is a match and does not have FA. If the fetus turns out to have FA, then aborting the fetus may be an option.

Other options for therapy of children without a matched sibling donor include androgen therapy, cytokine therapy, and an unrelated donor transplant. Androgen therapy has been used for decades in children with FA. The advantages are that androgens often work for years and there is a low risk of early death. The disadvantages are that the androgens can cause serious side effects and reduce the chance of a good outcome of a later transplant. Also, androgens are not curative; they only delay curative therapy. The most serious side effects relate to damage to the liver with development of adenomas, adenocarcinoma, and lakes of dilated

blood vessels in the liver. These problems can later lead to an increased risk of toxicity, infection, or bleeding after a transplant. The most commonly used androgen is Anadrol. However, there are other available androgens which might work as well but with less toxicity. One of these, oxandrolone, is currently going through trials in patients with FA.

Cytokines are drugs which stimulate the bone marrow to produce more cells. G-CSF stimulates white cell production and erythropoietin stimulates red cell production. These two drugs may help the marrow function for a longer period of time, but again are not curative. They require frequent shots or intravenous infusions. There also is a concern that G-CSF might cause a clone to progress into MDS or leukemia. The platelet stimulating drug, interleukin-11, seems to have a high risk of toxicity in FA patients, but sometime in the future, a better platelet-stimulating drug may become available (thrombopoietin).

Unrelated donor transplant in past years did not work very well. Only about 30% or so of patients survived such a transplant for FA. But much has changed in the past couple of years. The addition of fludarabine to the preparative regimen for the transplant has all but eliminated the risk of graft rejection, and the use of lymphocyte-depleted peripheral blood stem cells has allowed large numbers of stem cells to be given (increasing the rapidity of engraftment) with a much lower risk of graft-vs-host disease, since the lymphocytes have been taken out of the stem cell graft. Current results are about 60-70% survival with this approach, but not many patients have been transplanted so far with this approach and the follow-up is not very long. So, it is not yet known what the final outcome of these patients will be. Transplant has some major disadvantages: long hospitalization and a high risk of dying within the first three months of transplant, perhaps as high as about 1 in 3. The primary advantage of transplant is that it is curative of the aplastic anemia.

Gene therapy is still in its infancy, though this option has been under investigation for several years. The concept is

that cells removed from the patient's own marrow can be fixed by inserting a normal copy of the gene into the stem cells. Then these cells can be given back to the patient with the hope that they will take over the blood cell production and crowd out the sick, poorly-functioning cells. A major problem with this approach is that it is difficult to collect enough cells in a patient who already has a weakened marrow. Also, the insertion of the gene into the stem cells and keeping those cells growing is still not very reliable. Only a few of the stem cells may get corrected, and these cells may gradually disappear once they are given back to the patient. Better methods may soon be available to get around these problems.

In summary, FA is a very serious disease. The bone marrow failure associated with FA can be cured reliably only by transplant at the present time. Androgens and cytokines are temporizing approaches. Matched sibling donor transplant works very well, but most patients are not lucky enough to have such a donor. Unrelated donor transplants for FA are only recently starting to give good results, but still carry a high up-front risk of death. Producing a baby who is free from FA and is an HLA match is very involved and costly and takes a long time, time which many patients don't have. Gene therapy is not currently routinely available and the methodologies are still in development. But, in spite of all the negatives, the prospects for cure of the bone marrow of a child with FA born today is remarkable, especially compared to what was available in the past.

Cancer: Risks, Screening, and Prevention

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The risk of leukemia appears to reach a plateau of 35% at approximately age 30, although the risk of MDS continues to a plateau of 50% at age 45. This is consistent with the hypothesis that "MDS" in FA may not be the same as MDS in non-FA, where it has preleukemic connotations. The risk of liver tumors continues to rise to 50% at age 50, and the risk of solid tumors reaches 75% by age 40. It must be emphasized that these risks are calculated based on cases being withdrawn from the denominator if they die from other causes, or at their current age at the time of the report, without the occurrence of the event being evaluated.

Since the literature cases were published because of interest, it is impossible to determine the true risk of cancer, factors associated with increased risk, or the total spectrum of the types of cancer in FA in this retrospective manner. A better study design is a prospective cohort, in which all FA patients are enrolled as soon as they are diagnosed, and followed forward to development of events of interest. As a first step, a survey to determine the prevalence of cancer was sent to the US FA patients by the FARF in January 2000, and to the Canadian patients by FA Canada in September 2000. The returns from the US survey were 127/284 (45%), and from the Canadian survey 18/34 (53%) (differences not significant). The combined results for the 145 patients whose surveys were returned were then confirmed by medical records for the 23 patients (16%) who self-reported cancer. Those with solid tumors were older at diagnosis of FA than those without cancer, consistent with the possibility that FA patients who develop cancer have a milder phenotype, without severe birth defects and early onset of aplastic anemia.

The reports in this Pilot Survey include acute myeloid leukemia (AML), head and neck cancer, esophageal cancer, liver tumors, vulvar and cervical cancers, sarcomas, and brain tumors.

The observed/expected frequencies were calculated by using the age and sex structure of the survey population to statistically model the baseline cancer risks determined from the Connecticut Cancer Registry. This analysis indicates that in FA the relative risks of AML and of head and neck cancer are approximately 700-fold, while esophageal is more than 2000-fold, and vulvar more than 4000-fold that in the general population. Although an earlier publication suggested that the relative risks were more than 15,000-fold, it did not appropriately model the age and sex of the cases. Whatever the number, the data do support the conclusion that cancer risk is markedly increased in FA. The cumulative risks were remarkably similar to the literature results, with a 40% risk of MDS by age 45, 20% risk of AML by the plateau at age 20, and a 75% risk of solid tumor by age 45. Data following bone marrow transplant are sparse, although there were 3 patients who reported cancers, among 50 transplanted patients, of whom only 17 survived more than 3 years.

The next step will be to develop a truly prospective FA cohort, in which the risk of cancer and the types of cancer can be more clearly defined. Cancer surveillance is critical for FA, since cancers that are detected early are more easily treated. In particular, patients with cancers for which surgery provides a cure have a major advantage over those for whom chemotherapy and/or radiation are needed, because of potential toxic side effects.

Recommendations for cancer screening are:

MDS or leukemia: For all FA patients, complete blood counts at 3-4 month intervals (or more often as needed). Bone marrow aspirate and biopsy annually, including chromosome analysis.

Gynecology: Gynecological exam, Pap smear, and HPV exam annually, for those who are age 16, or have begun their periods. Breast exam by doctor and self-exam. Consider breast MRI on a research basis. Since mammography provides radiation exposure, its role in screening FA patients is not clear.

Liver Tumors: For patients on or going on androgens. Physical exam for size, tenderness, masses. Liver enzyme tests every 3-4 months. Ultrasound every 6 to 12 months.

Head and neck: Annual exam by otolaryngologist, and immediately if pain, swallowing problem, change in voice, weight loss, etc. Examine oral cavity, oropharynx, and hypopharynx. Biopsy of suspicious areas. Dental exam for leukoplakia at each visit, and immediate biopsies. Do not "watch and wait." Start at age 10 or so, or within the first year after a transplant.

Esophageal: At this time the only method is endoscopy, which requires sedation, and is performed by a gastroenterologist. This may be considered by FA patients who have reached adolescence or young adulthood.

There are a few ongoing cancer prevention trials for leukoplakia, including a COX-2-inhibitor, and a virus which is anti-p53. The results are very early, and the numbers of treated patients (mostly non-FA) too small for definitive conclusions. It is very important that these be performed in an unbiased manner, in which patients with leukoplakia randomly and blindly receive the experimental treatment or a placebo (an inert treatment), in order to determine the role of the treatment in the prevention of the development of cancer.

Long Term Complications

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- the vocal cords, to reduce the androgen effect;
- New methods of monitoring for oral pre-cancerous lesions and cancer;
- New agents and clinical trials in FA patients to prevent recurrent head and neck cancer;
- New BMT strategies for improving immune recovery without GVHD (i.e., add back of genetically modified T cells to T cell depleted marrow grafts and thymic shielding) and reducing/eliminating the need for radiation, particularly to the mouth and neck.



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