



SCIENCE LETTER

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Hormones and FA

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Children with FA have distinct medical needs. Many have hormone problems. At least 40% of children with FA have short stature, and many develop high blood sugar or diabetes mellitus.

Hormones are a message system, like the postal service, within the body. Messages such as "make that bone grow longer" or "keep that blood sugar normal" are made in one part of the body, and are sent to another part of the body. Endocrinology is the study of the way hormone messages interact. Hormones affect growth, development, stamina, general health, and ability to cope with medical illness.

Prior endocrine studies in FA have included several small studies (fewer than 8 children), but there has been only one large study (Wajnrajch MP et al. *Pediatrics* 2001; 107: 744). In this study, endocrine function was evaluated in 54 patients (47 between the ages of 2 years and 16 years): 72% of the persons with FA had resistance to insulin action, while 25% had glucose intolerance, 46% had low growth hormone peak, and 36% had low thyroid. Wajnrajch suggested performing "endocrine evaluation in all FA children because correction of these endocrinopathies may improve growth, final height, and overall quality of life."

Factors in Normal Growth

Understanding of factors controlling
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Cancer in FA

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The majority of FA patients survive into adulthood, but unfortunately have an increased risk of leukemia and solid tumors. Among 1300 reports of FA in the medical literature, there are more than 100 cases of leukemia, and more than 100 with solid tumors. The most frequent tumors are liver adenomas (considered to be benign) and hepatomas (both primarily in patients on androgens), and cancer of the head and neck, esophagus, and gynecologic areas. The median age for leukemia is 14, liver tumors 13, and other solid tumors 25 years. There are a dozen reports of oral cancer following bone marrow transplant, and the median age is 21, significantly younger than the median of 28 years in the 26 untransplanted FA patients with oral cancer. The type of leukemia in FA is predominantly acute myeloid leukemia (AML), whereas the usual childhood leukemia is lymphoid. The cumulative probability of a solid tumor in FA is predicted from the literature reports to be 75% by age 45.

Analysis of the literature may be biased by the publication of the most interesting cases, and thus the denominator may be underestimated. A cohort analysis should be more reliable, although there is still the possibility of selection biases, due to volunteerism (those with cancer all participate), survival bias (those who die of cancer without being diagnosed with FA are missed), and surveillance bias (those

who participate undergo more cancer screening).

The Pilot Study done with the help of the FARF and Fanconi Canada was a cross-sectional survey to determine cancer types and rates, using increasingly more sophisticated statistical analyses. Among 145 responders (45% of those who were mailed a survey), there were 14 with tumors and 9 with leukemia prior to transplant, as well as 3 with cancer

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

Christopher Walsh, MD, PhD,

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Fanconi anemia (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, there are risks and toxicity associated with transplantation, and so alternative therapies must be investigated. The hallmark of the FA disease 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, at least eight different complementation subtypes of FA have been identified from somatic cell hybridization studies. Of these, the FA-A 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 FA-A 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 *FAA* 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 FA-A 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 a 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.

Current *FANCA* Gene Transfer Trial

Four patients have enrolled in the trial, ranging in ages from 11-48 years old. Three of the four patients had severe pancytopenia requiring blood transfusion and/or androgen support. CD34+ cells were obtained following G-CSF mobilization and either apheresis or bone marrow harvest. In the four patients tested, none of the four patients mobilized significant numbers of CD34+ cells into the blood. It now appears that bone marrow harvest yields far greater 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 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.

What have we learned? That all FA patients, despite age and blood counts, have significantly fewer stem cells (at least 10-100 times fewer) than the normal population. This observation is extremely important in developing new avenues to treat the blood defect in FA. This suggests that either we improve the efficiency of correcting stem cells, i.e., build better gene vectors that can correct the few remaining cells, or find alternate sources of stem cells in the marrow or from other organs. Our lab has undertaken both these approaches by the development of better vectors (see below) and the isolation of new cell types that function as hematopoietic stem cells.

This study also suggests that young patients should consider bone marrow harvest and cryopreservation early on in their disease, when we believe the CD34+ count may be at its highest. Although this recommendation seems reasonable, unfortunately we have no clinical data on the CD34+ content of patients over time.

Proposed *FANCA* Lentiviral Trial

We have developed new vectors based on the lentiviral virus system. The results from testing FA-A patient CD34+ cells suggest that they may be superior to the vector we are using in our current trial. The lentiviral vector is derived from the

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Hand and Arm Differences in FA

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Hand and arm differences in FA primarily involve the radial border of the arm. A deficiency of part of or this entire border creates considerable variation in presentation. The presence of a deficiency along the radial side of the limb may be an early clue to the diagnosis of FA. The anomalies involve the entire arm and can include bones and muscles around the shoulder girdle. The forearm is most affected and the radius can be slightly smaller, considerably smaller, or absent. Complete absence of the radius is the most common type of radial deficiency. The remaining ulna is frequently thickened and bowed toward the absent radius. In addition to the deficiencies in the forearm, the thumb ray is also commonly affected. The thumb can be smaller than normal or completely absent.

The diagnosis is made by a careful examination and supplemented by x-rays. Associated syndromes can involve the heart, platelets, kidneys, spine, and bone marrow. Appropriate referral is necessary for further evaluation of these potential problems. The initial treatment for the absent radius is stretching, both by the therapist and the caregiver. Splints are used to maintain the hand in a straight alignment. If no treatment is rendered, the hand will develop a perpendicular relationship to the forearm. Stretching is usually recommended every diaper change and is paramount to the overall success of treatment.

Surgical treatment of the forearm involves placing the wrist on top of the ulna, which is the only substantial bone within the forearm. The procedure is known as a "centralization" or "radialization." The procedure is typically performed at about 1 year of age, and the initial correction is impressive. Unfortunately, the ability to maintain the correction and prevent recurrence has not been completely solved. In many patients, the wrists tend to deviate over time, which has led to a search for other options and treatments. Recent advances in treatment strategies have included the application of an external fixator, or Ilizarov device, to stretch the tissues prior

to centralization. This allows complete placement of the wrist on top of the end of the ulna, which was often not obtainable when the radial structures were extremely tight. In addition, the external fixator has been used to lengthen the forearm when the child becomes older, usually between 8 and 15 years of age. The application and process of soft tissue and bone lengthening is difficult. A team approach is utilized which consists of physician, nurse, therapist, and social worker. The process is arduous and requires considerable participation from the patient and family. The outcomes from this type of procedure are still evolving and more information will be available in the future.

The thumb deficiency is usually addressed as a separate entity. A thumb that is slightly smaller than the normal thumb can be reconstructed, or augmented, by tendon transfers to improve its motion and use. A thumb without a stable base is removed, and in this case and the case of a missing thumb, the

index finger is moved to the thumb position. This procedure is known as a pollicization and involves transfer of the index into the thumb position along with its nerves, arteries, tendons, and muscles. The procedure is performed any time between 6 months and 2 years of age, and requires meticulous technique to position the index finger in a thumb position. The index finger must be shortened, rotated, and rebalanced to give the appearance of an innate thumb. The outcomes of pollicization are directly related to the degree of index finger mobility and use prior to transfer. A mobile index finger will provide an excellent digit when transferred to the thumb position. In contrast, a stiff index finger will function more as a post for grasping of large objects. The decision to remove a thumb without a base is often a difficult process for parents and caregivers. Lengthy discussions with the surgeon and conversations with families who have undergone similar procedures are often helpful.

FA 101

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This session was designed for the newly diagnosed families, and provided a brief overview of FA.

FA (pancytopenia with physical abnormalities) was described first in 1927, and should not be confused with Fanconi's Syndrome (defect in kidney function). The appearance ranges from patients with striking birth differences to those whose appearance is entirely normal; the latter group are probably underdiagnosed. The inheritance is autosomal recessive: each parent has a normal and an abnormal FA gene, and the chance of having an affected child (with 2 abnormal FA genes) is 1 in 4 with each pregnancy. Among the rare inherited bone marrow failure syndromes, FA is the one most frequently reported in the medical literature, with more than 1300

cases reported from 1927 through 2001. The median age at diagnosis is around 8 years, but FA patients have been diagnosed from birth (or even prenatally) to 48 years. The hallmark of diagnosis is the presence of chromosomal aberrations in cultured lymphocytes to which DNA crosslinking agents have been added (DEB, diepoxybutane, and MMC, mitomycin C).

There are more than 8 different complementation groups, meaning at least 8 different genes are responsible for FA. The groups are defined by correction of the DNA crosslink sensitivity of one FA patient's cells following fusion with the cells of another FA patient, or by correction following addition of a retrovirus that contains the normal version of the

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Recent Advances in FA Research: Consequences For Diagnosis

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Only about one in five to ten patients suspected of having FA (on the basis of clinical symptoms) in fact does appear to have the disease. FA is confirmed if blood lymphocytes from the patient show an extremely sensitive response to chromosomal breakage or cell cycle arrest when challenged with a cross-linking agent such as mitomycin C (MMC) or diepoxybutane (DEB). Distinction between FA and non-FA is important, because the treatment of FA patients is different—most importantly, the conditioning treatment for a bone marrow transplant.

At present FA has at least 8 complementation groups (genetic subtypes), 7 of which have been connected to a distinct gene: groups A, C, D1, D2, E, F and G. The corresponding genes are named *FANCA*, *FANCC*, *FANCD1(BRCA2)*, *FANCD2*, *FANCE*, *FANCF* and *FANCG*.

An estimated 95% or more of all FA patients have mutations in one of these genes.

Molecular diagnosis (that is, the determination of the defective gene and the disease-causing mutations in that gene) is important, as this allows researchers to distinguish FA from similar syndromes that also score positive in a chromosomal breakage test. Moreover, detection of the disease-causing mutations is important because some mutations are known to be more severe than others and may dictate a different clinical management, such as aiming at BMT at an earlier age. Finally, a molecular diagnosis allows for an earlier and more reliable prenatal diagnosis, preimplantation genetic diagnosis, carrier detection, and access to gene therapy trials.

However, whenever a molecular diagnosis is used as a basis for further

clinical decision-making the diagnosis has to be carried out in a certified clinical diagnostic laboratory that meets certain standards of good laboratory practice. The clinical diagnostic laboratory of Dr. Gerard Pals (g.pals@vumc.nl) in our department in Amsterdam has recently implemented all methodology to carry out molecular diagnosis of FA, which covers all currently known FA genes. This service is offered at a reasonable price, with a turnaround time of 1-3 months. Patients in whom no mutations can be found are referred to our research division, where we attempt to identify the defective gene at no further cost.

Thus, as a result of the rapid progress in the identification of FA genes, a clinically certified molecular diagnosis is now available for the majority (over 95%) of FA patients.

FA 101

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gene that is mutated in that patient. In response to DNA damage, most of the normal products (proteins) of the known FA genes combine in a complex, which then interacts with the product of a downstream gene (called *FANCD2*), and this further interacts with other proteins to repair DNA damage. This pathway is defective in any of the FA groups, which in part explains the similarities between FA patients with different mutant genes.

Testing for FA should be offered to anyone with the characteristic physical findings of radial ray abnormalities (in particular), aplastic anemia, myelodysplastic syndrome, acute myeloid leukemia, typical cancers seen in FA patients, and decreased fertility with any of the previous features.

Treatment for FA depends on the findings. *The Standards for Clinical Care* guidelines, published in 1999, recommended treatment for cytopenias when the hemoglobin was consistently below

8 g/dl, platelets below 30,000/mm³, and absolute neutrophil count below 1000/mm³. Leukemia was defined by blast cells in the peripheral blood, or more than 30% blasts in the marrow. Myelodysplastic syndrome (MDS) was defined as cytopenias with characteristic bone marrow cellular morphology, and not solely based on an abnormal cytogenetic clone. Treatment includes stem/progenitor cell transplant, androgens, hematopoietic growth factors (mostly G-CSF, granulocyte-colony stimulating factor, or Ep, erythropoietin), and gene therapy in the future. Family members should not donate blood products to the patient, since this might jeopardize a future transplant because of sensitization to minor tissue antigens.

Surveillance for hematologic disease includes blood counts at least every 4 months or more often, and an annual bone marrow aspirate, biopsy, and cytogenetics. Head and neck cancer screening includes an examination of the oral pharynx, the throat, and the region down to the vocal cords (this requires a thin

fiberoptic tube). We suggest doing this annually starting at age 10 in untransplanted patients, and within the year after transplant at any age. Monitoring for gynecologic cancer should begin at age 16 or earlier. Skin examination should be done at least annually. Because of the risk of so many types of cancer, pain, sores, or lesions that persist for more than a brief time should be brought to the attention of a physician.

The NCI Inherited Bone Marrow Failure Syndrome study was developed (see *FA Family Newsletter* from Spring, 2002), with an emphasis on collection of epidemiologic data, in order to answer many of the obvious questions with regard to the natural history of FA, the risks of cancer, and genotype/phenotype/cancer susceptibility (www.marrowsfailure.cancer.gov). It is hoped that this study will collect information on large numbers of patients, and that comprehensive clinical evaluation, consultation, and cancer screening can be obtained at several places in North America.

Hematopoietic Stem Cell Transplantation for the Treatment of FA

John E. Wagner, MD and Margaret MacMillan, MD, University of Minnesota School of Medicine

Over the past decade tremendous improvements have been made in the treatment of patients with Fanconi anemia. In contrast to the results in 1995 when the incidence of graft failure exceeded 10% in those with a matched sibling donor, and 35% in those with an unrelated donor, graft failure is now a rare complication. Further, survival in 1995 was 85% in those with a sibling donor and less than 20% in those with an unrelated donor. Today, FA patients without leukemia, advanced myelodysplastic syndrome, or infection can expect excellent survival rates (Figures 1 and 2 below).

Because of promising results in FA patients with HLA identical sibling donors and unrelated donors, the timing of transplant is considered at an earlier point well before the development of leukemia, advanced myelodysplastic syndrome, or infection. While it was previously recommended that patients fail androgen therapy prior to *unrelated donor* hematopoietic stem cell transplantation, patients less than 18 years of age with standard risk disease and an HLA matched donor should seriously consider transplant prior to a trial of androgen therapy. In contrast, patients with high-risk disease, as evidenced by recurrent infections, poor organ function or older age, should delay transplant since survival is poorer. Use of androgens, growth factor, and transfusions in these high-risk patients is recommended with the hope that newer alternative therapies may become available. For patients with advanced myelodysplastic

syndrome and acute myeloid leukemia, however, there is no good treatment option. The best course, at this point, may still be transplant but perhaps with a novel regimen (see below). However, survival is only approximately 20%.

Current research in bone marrow transplantation at the University of Minnesota is focused on the development of new methods for improving immune reconstitution and reducing the risk of infection after transplant, particularly in high-risk patients. New strategies include the use of TK-transduced donor T cells. In a study being performed by Dr. Paul Orchard at the University of Minnesota, FA patients receive the "standard" preparative therapy of fludarabine, cyclophosphamide and total body irradiation 450 cGy (without ATG) followed by the infusion of T cell depleted bone marrow stem cells. TK-transduced donor T cells are then added to the marrow to potentially 1) improve immune function in order to reduce the risk of viral and fungal infections and 2) prevent relapse in patients with acute myeloid leukemia and myelodysplastic syndrome. Because TK will make T cells exquisitely sensitive to Ganciclovir (an anti-viral drug), GVHD that cannot be easily controlled with methoprednisone could now be eradicated by treatment with Ganciclovir. Ultimately, this could be a strategy to allow elimination of total body irradiation since donor T cells themselves can eliminate the patient's diseased marrow.

In another study performed by Dr. Margaret MacMillan, FA patients with

acute myeloid leukemia or advanced myelodysplastic syndrome will be treated with fludarabine, cyclophosphamide and busulfan, rather than total body irradiation. While busulfan is a poor agent for suppressing the immune system, it kills MDS and leukemia progenitor cells, making it a reasonable choice when patients have advanced disease. It is hoped that engraftment will not be impaired and toxicity will be minimal. If this is proven, only then will such therapy be considered for standard-risk patients in the future since survival already exceeds 80%. Dr. MacMillan also leads studies evaluating immune function of FA patients before and after transplant in order to determine 1) if some patients have an immune defect (e.g., FANC-D2 group), putting them at higher risk of infection prior to transplant, and 2) if new approaches in transplant therapy are effective in speeding immune recovery.

Thus far, no therapy has focused on the treatment of the non-hematopoietic tissues (*i.e.* tissues other than marrow and blood) of the FA patient. In collaboration with Drs. Catherine Verfaillie and Bruce Blazar at the University of Minnesota, multipotent adult stem cells (MASC) are now being evaluated as a completely novel treatment in patients with FA. These cells have the capacity to become many different tissues: for example, islet cells for the treatment of diabetes, liver cells for the treatment of liver disease, muscle cells for the treatment

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Figure 1. Survival in FA Patients after Unrelated Donor Transplantation (FLU-CY-TBI)

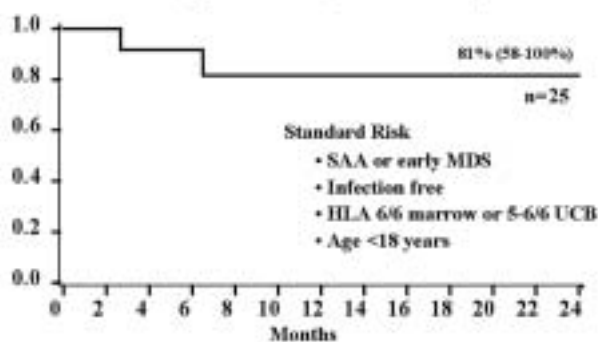
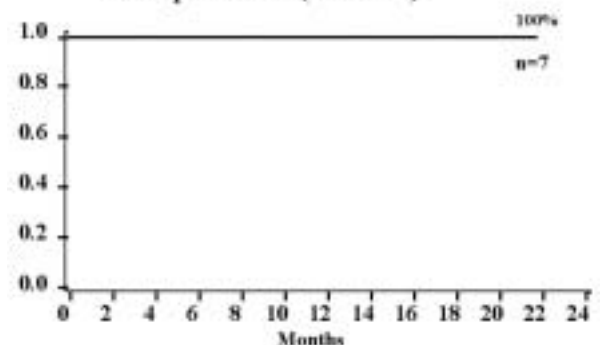


Figure 2. Survival in FA Patients after Sibling Donor Transplantation (FLU-CY)



Hormones and FA

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normal growth is important if we want to help children grow better. The factors that control normal growth are not the same at all ages. In infancy, nutrition and insulin action drive growth. Thyroid hormone is important, while growth hormone is less important. Childhood growth is driven by all these factors but thyroid hormone, nutrition, and insulin are most important, followed by growth hormone. Pubertal growth is governed by sex steroids (estrogen, testosterone) and growth hormone, followed by thyroid hormone, nutrition, and insulin action.

Other ingredients are also important in normal growth, including intake of calories, protein, calcium, minerals, and vitamins. In addition, the child needs to get adequate sleep and exercise. In order to grow optimally, the child needs a positive attitude and self-esteem, and a sense of security and of being loved.

Health problems may contribute to poor growth in FA, such as tracheal fistula, kidney problems, heart problems, reduced oxygenation, and low blood counts. Androgen therapy may improve health by increasing bone marrow output and blood counts. Androgen therapy may increase growth rate, but at the same time the androgen may mature the bones more rapidly and thus lead to a shorter adult height. Bone marrow transplant, though necessary to save the child's life, may itself have hormone side effects that can be detected through hormone monitoring each year.

Hormone Evaluation

At Cincinnati Children's Hospital Medical Center, the goals of our endocrine testing in children with FA include providing optimal care for each child, better understanding of the causes of poor growth, and better understanding of the causes of high blood sugar. In addition, we hope to see how each child's hormones relate to FA complementation group.

To determine why a child is not growing well, we can test each part of the hormone message system. The pituitary

makes growth hormone (GH), thyroid stimulating hormone (TSH), adrenal stimulating hormone (ACTH), and puberty stimulating hormones (LH and FSH). GH acts on the bones and muscles to produce growth. TSH acts on the thyroid gland to produce thyroxine (T4), which controls growth and rate of body processes (energy, bowel movements, dryness of skin, hair growth). ACTH acts on the adrenal glands to produce cortisol, a hormone that helps a person to better tolerate medically stressful illness. LH and FSH causes the ovaries and testes to go into puberty by making estrogen or testosterone. The pancreas makes insulin when a person eats or drinks, in order to keep the blood sugar or glucose normal, and in order to get the body cells to take in nutrients.

Endocrine testing involves giving a medication that raises a hormone level in the blood stream. Then a blood sample is drawn to measure the level in the blood. Standard endocrine testing for GH may involve giving the medications arginine and clonidine, which are known to raise the GH level in a person with a normal ability to make GH. Standard endocrine testing for thyroid may involve measuring the changes in TSH from the afternoon to the middle of the night (the TSH surge) and giving TSH releasing hormone, known to raise the TSH level to a certain normal range. Standard endocrine testing for cortisol involves giving a tiny dose of ACTH and measuring the rise in cortisol. Evaluation for physical signs of puberty is done during the physical examination and by first morning measurement of LH, FSH, and the sex steroid level in the blood. Standard endocrine testing for insulin is done by giving an oral glucose drink and measuring changes in both glucose and insulin levels.

During the past five months at Cincinnati Children's Hospital Medical Center, we have evaluated 12 children with FA, 67% prior to transplant. Ages range from 4 months to 16.5 years. Of these children, 63% were born small for gestational age birth. Short stature was identified in 58% of them.

Of this small group of children with FA, every child has had an endocrine

deficiency. Glucose intolerance or diabetes has been identified in each of them. Insulin levels have been elevated in 63% of those who were old enough to do the oral glucose tolerance test. Abnormal thyroid tests have been found in 83% of them, including a Free T4 in the lowest third of normal in 63%, elevated TSH in 50%, and a low TSH surge in 17%. A low peak stimulated GH has been found in 62% of them, and a low stimulated cortisol in 10%. Puberty has started at a normal age in all children who have started puberty, but sometimes the start of puberty has occurred at a very short height.

Summary and Recommendations

We have found frequent thyroid insufficiency and inadequate insulin effect in the FA patients we have tested. These may be at least as important as GH in the short stature of children with FA.

Therapy for insulin resistance in children with short stature must include adequate nutrition with adequate insulin. In a person with a high blood sugar, calories are lost in the urine. If the child has inadequate calories staying in the body, there will be poor growth. Therefore, insulin therapy may promote growth.

Therapy for hypothyroidism in children with short stature should be started in order to bring the TSH to normal (the goal should be a TSH value of 1 or 2) to optimize growth. Low GH measures may be an artifact of low thyroid levels.

I want to emphasize that thyroid and insulin are quite important in childhood growth and health. I recommend that children with FA undergo a careful and systematic baseline evaluation. In addition, there should be a yearly review of their growth. If growth rate continues to be slow, there should be endocrine retesting as needed to uncover any subtle abnormalities in the hormones. I recommend an early start of thyroid or insulin hormone therapy in order to optimize growth and health.

Cancer in FA

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after transplant. The results were compared with data from the Connecticut Cancer Registry after adjustment for age, sex, and birth cohort. The relative risk of any cancer in FA was 50-fold, and for solid tumors it was 48-fold. Significant increased risks were 800-fold for myeloid leukemia, 700-fold for head and neck cancer, 2000-fold for esophageal cancer, 4000-fold for vulvar cancer, 400-fold for liver tumors, and 200-fold for cervical cancer. Despite the small numbers, the cancer rate after transplant was 2.8-fold higher than the rate without transplant.

We separated death (from bone marrow failure), marrow transplant (for bone marrow failure), leukemia, and solid tumors as independent first outcomes. The outcome-specific annual hazard rates for death from bone marrow failure or leukemia rose from infancy, peaked during adolescence, and then leveled off or declined in early adulthood. One-third of the patients had received a transplant, with a peak age of 7 years followed by a decline. The hazard rate for solid tumors rose slowly during childhood and then increased in a more than linear manner, reaching 10% by age 45, with no sign of decreasing. The cumulative incidence of each outcome in the presence of the competing risks was approximately 10% for leukemia, 10% for death from marrow failure, 30% for solid tumor, and 40% of having a marrow transplant for marrow failure. If the competing risks were eliminated, the risk of a solid tumor by age 45 was 75%, the same as seen in the literature review.

We conclude that 1) FA patients are at increased risk of cancer; 2) cancer occurs much earlier in FA patients; 3) the maximum risk for AML is at 16 years of age; 4) the risk for solid tumors rises steeply from childhood and does not plateau; 5) the types of cancer seen in FA are specific and unusual; 6) the carcinogenic role of germline FA mutations may be different in the AML pathway than in the pathways of solid tumors.

To further investigate cancer epidemiology in FA, we have initiated a prospective cohort, The NCI Inherited Bone Marrow Failure Syndrome study.

The hypotheses are: 1) A prospective cohort of IBMFS families will provide new information regarding cancer rates and types; 2) mutations in IBMFS genes are relevant to carcinogenesis in sporadic cancers; 3) patients with IBMFS who develop cancer differ from patients with IBMFS who do not develop cancer. [These differences may include genotype/phenotype/cancer susceptibility differences, modifier genes, and environmental risk factors.]; 4) carriers of IBMFS gene mutations (i.e., heterozygotes) are at increased risk of cancer.

All participants are entered into the "Field Cohort," and complete detailed questionnaires regarding their family and

Hematopoietic Stem Cell Transplantation for the Treatment of FA

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of damaged muscles, lung cells for the treatment of damaged lung, as well as gut and bronchi (lungs). Blood vessels and other tissues may also be repaired using such cells. It is now conceivable that new treatments might be applied with bone marrow stem cells to correct both the diseased marrow and diseased organs. This could be an effective strategy to induce rapid regeneration after tissue injury, which often occurs in patients who receive chemotherapy with or without radiation. Although only speculative at this point, it may be a strategy to help prevent or treat patients with cancer. This work is still in the laboratory research stage.

In collaboration with Drs. Francesco Galimi and Inder Verma at the Salk Institute, Drs. Verfaillie, Blazer and Wagner are investigating lentiviral vectors and other novel approaches that will improve the transfer of FA genes into marrow stem cells and MASC. This work is also in the laboratory research stage at this point.

Conclusions

- Unrelated hematopoietic stem cell transplantation can be associated with a high probability of survival, particularly in patients transplanted prior to the development of advanced myelodysplastic syndrome and leukemia.

personal medical histories. A small subset will become the "Clinical Center Cohort," who will undergo comprehensive evaluations at the NIH Clinical Center, while most of the participants will be evaluated at other centers. We recommend complete medical histories, evaluations, and laboratory tests for FA patients as well as for their siblings, parents, or children, in order to understand more about medical risks in FA heterozygotes.

Information about the protocol is available on the web site www.marrow-failure.cancer.gov, or call 800-518-8474 and ask about "The NCI Inherited Bone Marrow Failure Syndrome Study".

- New investigations are underway to reduce the risk of infection and improve tissue repair.
- Timing of transplant is critical to success.

Other Issues

The importance of T cell mosaicism, number of transfusions and prior use of androgens on transplant outcome is currently under investigation. In the era of fludarabine-based therapy, these risk factors may be less important.

The optimal stem cell source (peripheral blood vs. umbilical cord blood vs. bone marrow) is currently unknown. Patient selection, treatments, and HLA matching currently make comparisons and conclusions impossible. However, it is clear that the use of haploidentical (HLA 2 or 3 antigen mismatched) related donors is NOT a first line therapy for standard risk patients with FA. Considerable work on the use of haploidentical related donors needs to be done first in non-FA patients prior to its general application in FA.

Finally, laboratory research is critical to the development of new clinical trials. This research depends on the availability of patient marrow and blood. Contact Dr. John Wagner at 612-626-2961 or by email, wagne002@umn.edu if you or your child is having a marrow or blood examination. Shipment of research tests will not result in any charges. A consent form will be mailed to you at your convenience.



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Gene Therapy for Group A FA Patients

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HIV-1 virus, the causative agent of AIDS. The viral vector has been modified dramatically by the removal of all elements known to cause manifestation of AIDS. Several laboratories including our own have demonstrated the superior effectiveness of this vector. We have used these lentiviral vectors to correct the hematologic defect in FA knockout mice. Based on this study, we received approval from the National Gene Vector Lab to manufacture the vector for a clinical trial. We are now ready 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 those with other hematopoietic disorders.

In concert with vector development, we have isolated several population of non-CD34+ cells that act as hematopoi-

etic stem cells and that, using the newer vector, correct the defect in FA knockout mice. Further study of these cells and their expansion after gene-correction is ongoing.

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 from patients at the time of their yearly marrow examinations. Peripheral blood may also be sent for complementation testing.

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