



SCIENCE LETTER

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Diagnosing FA and FA 101

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Fanconi anemia (FA) is the most common of the rare inherited bone marrow failure syndromes, with ~1000 cases reported in the literature. It is an autosomal recessive disorder, in which parents are carriers, and children have a 25% chance of being affected. Birth defects and aplastic anemia are common, but leukemia and solid tumors emerge with age.

Patients with FA have a DNA repair defect, with at least 8 different genes involved, and chromosomal instability provides the best current diagnostic test. FA is diagnosed at a median age of 9 years, but the range is from birth to the 50s. Patients may be seen by many subspecialists, yet only be diagnosed after their blood counts decline below normal levels. An early indication may be large red blood cells, and declining blood counts even within the normal range. FA should be thought of in children (and adults) with characteristic birth defects (e.g., abnormal thumbs or kidneys, poor growth), aplastic anemia, myelodysplastic syndrome, acute myeloid leukemia,

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Cloning of a New FA Gene and the Cancer/Leukemia Connection

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Complementation analysis: back to seven FA subtypes

Complementation analysis by cell fusion had indicated at least eight complementation groups, each group supposedly being connected to a distinct FA gene. Group H was unique in that it was represented by a single patient, whose cell line corrected the defect in fusion products with cell lines from all other groups. Since Dr. D'Andrea and Dr. Hanenberg found correction of the defect with a retroviral vector containing the *FANCA* cDNA, which suggested that the FA-H cell line actually belonged to group A, we reexamined the assignment and were able to demonstrate pathogenic mutations in the *FANCA* gene, confirming that the patient was, in fact, in group A. Apparently, when fused with the reference A cell line, the resulting hybrid was "complemented," due to genetic reversion rather than to genuine complementation. This pitfall, which may happen in a minority of cases, can be

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Gene Therapy for Group A Fanconi Anemia Patients

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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, 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 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, seven 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 *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 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. This is to ensure the safety of our patients during this study.

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 FA-A 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 study 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.

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 transfusions and/or androgen support. CD34+ cells were obtained following G-CSF mobilization and either apheresis or bone marrow har-



vest. 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. The stage of disease (the severity of the peripheral blood counts and marrow cellularity) correlates with the number of CD 34+ cells that can be harvested. Following transduction (the process where the gene enters the cell and functions), 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 is ongoing. One patient died due to a malignant head and neck tumor that was refractory to surgery and irradiation. There is no evidence that the tumor development or growth was related to gene transfer or the procedure.

Patients are monitored for blood counts and marrow cellularity at 3, 6 and 12 months. Patients may undergo the gene transfer procedure three times. We plan to test new vectors and procedures to enrich for stem

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Low-risk Allogeneic Transplantation and Autologous Stem Cell Banking in Fanconi Anemia

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Ninety-eight patients with genotype and clinical data have been reported to the German FA Registry in Berlin. On the basis of these data, a nationwide study will investigate diagnostic and therapeutic approaches to this disease, in close cooperation with other European FA projects and the German FA support group. In a pilot study, we have tested allogeneic transplantation and an autologous stem cell banking program.

We attempted low-risk fludarabine-based conditioning regimens for transplantation in order to reduce the risk of toxicity and secondary tumors by avoiding high dose chemotherapy and especially radiation, while trying to achieve engraftment by high stem cell doses and T-cell antibodies. Current protocols are based on our experience with eight Fanconi anemia patients.

The characteristics of these children were as follows: three patients were in aplastic phase, three suffered from MDS with multiple cytogenetic abnormalities; and two had developed AML. In addition, there were critical complications like liver adenomas, perianal ulcers, chronic renal failure and dialysis. Six out of these eight patients are currently surviving with functioning grafts between one and 18 months post-transplant. One patient died from progression of AML in spite of three transplant attempts. One patient died due to transplant related complications. Two of the six surviving patients required a second graft. Three of the eight patients had matched sibling donors. Five had alternative donors, either 5/6 family donors (N=2) or 6/6 matched unrelated donors (N=3). Both deaths occurred in the alternate donor group.

Considering the degree of toxicity and the risk of graft failure seen in these patients, the following protocols are now being used:

1. Protocol for matched related donors

fludarabine (4 x 40 mg/m²), rabbit-ATG Merieux (4 x 7, 5 mg/kg), no T cell depletion;

2. Protocol for partially matched related donors/unrelated donors and clonal disease

fludarabine (6 x 30 mg/m²), busulfan (2 x 0.5 mg/kg), rabbit-ATG Merieux (4 x 7, 5 mg/kg) and OKT3 (0.1 mg/kg, day +1 until +12);

3. Protocol for retransplantation

fludarabine (5 x 30 mg/m²), cyclophosphamide (2 x 10 mg/kg), rabbit-ATG Fresenius (4 x 20 mg/kg) and OKT3 (0.1 mg/kg, day +1 until +12). The first option of escalation is the increase of busulfan in protocol #2. The very little toxicity of these protocols is impressive, even in critical children. The main risk is graft failure. This requires a close follow-up of the chimerism, but also a good viral surveillance.

One option in the circumstance of graft failure would be the reinfusion of autologous stem cells, or autologous stem cells corrected by gene transfer. In order to use such an approach with or without a preceding transplant attempt, it is necessary to harvest cells early in the course of the disease. For this reason, we harvested autologous stem cells in 13 cases to get an idea about the feasibility and the quality of cells. Bone marrow was harvested in 12 patients,



containing 59.6 x 10⁸ TNC (range: 15.8 - 378 x 10⁸); 22.0 x 10⁶ CD 34 cells (range: 1.0 - 62.1 x 10⁶); and a viability after thawing of 72.4% (range: 47 - 94). Cord blood was harvested in one patient, containing 5.2 x 10⁸ TNC; and 2 x 10⁶ CD34 cells. Clearly the quantity and quality of remaining cells as well as the absence of clonal aberrations are dependent on the interval between time of diagnosis and cell harvest.

There is concern and experimental evidence that Fanconi anemia stem cells bear a poor re-homing capacity. To study this, cryopreserved autologous bone marrow cells were reinfused in one patient with severe infectious complications in a pancytopenic, preleukemic phase. This resulted in a significant leucocyte increase, solving the infectious problems, and finally allowing an unrelated transplant. Although in the short run this cell infusion was helpful in this individual patient, nothing can be said about the long-term reconstitution potential.

Epidemiology of Cancer in a High-risk Population: Fanconi Anemia

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Patients with Fanconi anemia are at a much increased risk of cancer compared to the general population. The highest risk is for leukemia, followed by solid tumors, and then liver cancer. The solid tumors are primarily oropharyngeal and esophageal, cervical and vulvar in females, and a scattering of other types. Oropharyngeal cancers are also a risk after bone marrow transplant.

Liver cancers include hepatomas and adenomas, and are usually found in association with other problems; they do not usually lead to death by themselves.

The leukemias are usually of the acute myeloid type, and are difficult to treat. In the literature there is an excess of females among those with solid tumors, even after exclusion of gynecologic malignancies.

The risk for leukemia, after exclusion of all other outcomes, reached a plateau of ~35% at age 25 years, while the risk for myelodysplastic syndrome reached 50% by age 40.

The risk for solid tumors was 75% by age 40, and for liver tumors it was 50% by age 50. The most significant risk association was for females and solid tumors, according to survival analyses. Literature reports are subject to many publication biases, and thus conclusions from these analyses may be inadequate.

In January 2000 a survey was sent to 284 FA individuals whose families belong to the Fanconi Anemia Research Fund. By July 2000 127 responses were received (45%). Data from this survey are self-reported, with no validation to date. The absolute prevalence of cancer was ~20%, of which one-third were leukemia, and one-third oropharyngeal cancer. The excess of females among solid tumor patients in the

literature was not seen in the current cohort. The individuals with the mildest physical phenotypes were at higher risk of cancer, since they had a lower risk for aplastic anemia and its complications. The cumulative risk of leukemia was ~25% and plateaued at age 25, while the risk of solid tumors

did not level off, and was >90% by age 45. This survey requires medical record validation, followed by further epidemiology and biologic studies of the tumors in these patients. FA patients are at very high risk of cancer, and can serve as a model for cancer in the general population.

Diagnosing FA and FA 101

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early characteristic solid tumors, or decreased fertility.

Hematologic treatment includes stem cell transplant, androgens, or hematopoietic cytokines. Gene therapy is hopeful, but not yet of clinical use. Treatment should begin according to symptoms, or when hemoglobin is < 8 g/dl, platelets <30,000/fl, or neutrophils <1000/fl. Leukemia is diagnosed when there are blasts in the peripheral blood, or >30% blasts in the marrow. Myelodysplastic syndrome (MDS) depends on morphologic criteria (see below), and not just the presence of a cytogenetic clone.

Transplant can be from bone marrow, peripheral blood stem cells, or cord blood. HLA-related donors can be used when blood counts begin to warrant medical intervention. Unrelated donors should be reserved for leukemia, morphologic MDS, or for patients who have failed treatment with androgens or cytokines, due to the higher risk of poor outcome.

Medical treatment includes oxymetholone, prednisone (see Editors' note on page 5), as well as folic acid. G-CSF may help for neutropenia. Supportive care includes transfusions of red cells or platelets; both should be leuko-poor and irradiated,

and not from family members. FA patients who do not succumb to aplastic anemia have a high risk for leukemia and solid tumors (see "Epidemiology of Cancer in a High-risk Population: Fanconi Anemia" above). Monitoring for bone marrow disease should include complete blood counts every 4 months or more often as needed, and annual bone marrow aspirates for morphology and cytogenetics, and biopsies for cellularity. More frequent studies may be indicated.

Hematology 101

Peripheral blood cells include red blood cells with hemoglobin to carry oxygen, white blood cells to fight infection, and platelets to stop bleeding. These cells are derived from multipotent bone marrow stem cells. In aplastic anemia there is decreased or absent blood cell production, and the bone marrow biopsy shows empty spaces of fat rather than large numbers of cells of all lineages, with the few remaining cells lymphocytes or macrophages. Cultures of blood progenitor cells in the laboratory show very reduced numbers of blood colonies. Bone marrow criteria for MDS should be objective: major = overt dysplasia including 2 or more

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Understanding Cytogenetic Clonal Abnormalities in Fanconi Anemia

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Cytogenetic evaluations of the blood and marrow are important in the care of all Fanconi anemia patients. The diagnosis of Fanconi anemia is based on the extreme hypersensitivity of FA lymphocytes

to diepoxybutane (DEB) and/or mitomycin C (MMC). After the diagnosis is made, all children with FA should have yearly marrow examinations with cytogenetics in order to optimally evaluate the existing stem cell pool in terms of propensity for malignant transformation.

The underlying premise is that clonal cytogenetic abnormalities are never found in healthy individuals and the clones herald the development of a premalignant or malignant disorder. While it is yet unproven whether all clonal cytogenetic abnormalities in FA patients portend myelodysplastic syndrome (MDS) or leukemia, they bear close monitoring.

Definitions:

Cytogenetic clone: the presence of two or more cells with the same chromosomal abnormality.

Clonal abnormalities may be numerical, i.e., the gain or loss of genetic material (e.g., trisomy 21 [Down syndrome] or monosomy 7). Clonal abnormalities may also be structural which includes a rearrangement of chromosomal material. If the rearrangement does not result in a net loss or gain, it is called balanced; if the rearrangement does result in a net loss or gain, it is called unbalanced. In FA, clones are frequently "complex" with multiple numerical and structural abnormalities present simultaneously.

Importantly, the cytogenetic evaluation needs to be performed on > 20 banded metaphases with sufficient resolution to detect the structural abnormalities. Different laboratories have different experiences, especially with complex karyotypes. Importantly, suboptimal evaluations may be secondary to inadequate mar-

row collections or delayed handling. Frequently, too little sample is obtained, particularly with FA patients with bone marrow failure. Therefore, the absence of abnormality does not truly indicate its absence if an inadequate analysis was performed.

FISH stands for fluorescent in situ hybridization. It is a technique for evaluating large numbers of non-dividing cells. FISH is indicated when there is an inadequate specimen for metaphase analysis (e.g., when there are too few dividing cells), G banding is suboptimal, and structural abnormalities are very complex. In the latter instance, multi-color FISH or chromosomal painting can aid in the evaluation. This technique requires a cocktail of probes that each recognizes a specific chromosome. In the evaluation of unrecognizable genetic material by G banding, it can be a powerful technique for identifying the origin of the material.

Conclusions:

1. Cytogenetic evaluation should be performed in every marrow without exception.

2. The appearance of a clone should be monitored for clonal progression (i.e., increasing proportions of cells with the clone) and evolution (i.e., increasing numbers of cytogenetic abnormalities) over time.

3. Clones frequently herald the development of MDS and leukemia in FA patients, but not always.

4. All patients with FA should have an annual bone marrow examination with cytogenetic evaluation. Once a clonal abnormality is identified, more frequent examinations are indicated (every 3 to 4 months).

Diagnosing FA and FA 101

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lineages with >20% dysplasia, or clonal cytogenetics involving at least 2 cells; intermediate = suggestive dysplasia involving 1 lineage; minor = myeloperoxidase deficiency, dual esterase positive, PAS positive erythroblasts, or ring sideroblasts. Diagnosis of MDS requires 1 major, or 1 intermediate and 1 minor criteria. Additional studies include flow cytometry for surface marker abnormalities, and aberrant expression of oncogenes. Serial monitoring of blood and bone marrow from FA patients will lead to more specific criteria for aplastic anemia, MDS, and leukemia, and may distinguish predictors of leukemia from those of nonmalignant MDS.

Editors' note: In May, 1998, a conference was held in Portland, OR to develop clinical care guidelines for FA patients. Nineteen treating physicians or researchers were in attendance. Attendees discussed the question of including prednisone, along with oxymetholone, in the treatment protocol for aplastic FA patients. After considerable debate, a vote of the physicians in attendance supported eliminating prednisone from the protocol. The group's consensus was explained in Chapter 2, page 14, of *Fanconi Anemia: Standards for Clinical Care*, 1999.

Progress Report

Alan D. D'Andrea, MD

The Dana-Farber Cancer Institute/Children's Hospital Comprehensive Fanconi Anemia Center, Boston, Massachusetts

Our laboratory is dedicated to applying the latest basic science advances in Fanconi anemia (FA) research to the diagnosis and treatment of the disease. Our laboratory has recently determined that the five cloned FA genes (for subtypes A, C, D, E, and G) interact in a novel cellular pathway, which regulates DNA repair and ultimately regulates normal blood cell production. Disruption of this pathway leads to the common clinical and cellular phenotype observed in FA. While FA is a rare disease, it provides a model system for the molecular understanding of cancer and the most common form of adult leukemia, acute myelogenous leukemia (AML).

We aim to establish a diagnostic laboratory for FA with state-of-the-art molecular diagnostics, subtyping, carrier detection, and FA mutational screening. To this end, we have performed the systematic evaluation of over 150 patient-derived cells and cell lines from FA patients and have begun to establish a new diagnostic test, the FANCD western blot, for FA. All of these studies have been performed in close collaboration with Dr. Markus Grompe at the Oregon Health Sciences University (OHSU). In the process of setting up this diagnostic center, we have established an outpatient bone marrow failure clinic which provides up-to-date services for Fanconi anemia families and provides a useful training setting for medical students and fellows pursuing clinical investigation.

In addition, we have begun to establish a gene therapy program for FA. Through gene therapy, FA bone marrow cells transduced with retroviruses carrying the FA genes will have a selective growth advantage

over non-transduced cells *in vitro*. Accordingly, we anticipate a selective growth advantage of modified FA cells *in vivo*, allowing the opportunity for a successful autologous transplant program. We now plan to optimize retroviral gene transfer, to scale-up retroviral infection of large numbers of bone marrow cells from FA patients, and to launch a Phase 1 clinical trial for FA complementation group A gene therapy.

At the same time, we strive to provide a rich training environment for pediatric and adult hematologists and oncologists pursuing careers in clinical investigation in Fanconi anemia and other related blood diseases. At the family meeting at Lake Geneva this year, I presented a progress report on several ongoing activities in our Comprehensive Cancer Center at the Dana-Farber Cancer Institute, Children's Hospital, Harvard Medical School, Boston.

Basic Science Activities

In collaboration with Dr. Markus Grompe at the Oregon Health Sciences University, our laboratory has recently begun the systematic characterization of the newly-cloned *FANCD2* (Fanconi Type D2) gene and protein. I presented evidence suggesting that the FANCD2 protein functions downstream in the FA pathway. These studies further support a unifying model in which all FA proteins function cooperatively, in a common pathway, to regulate chromosome stability. Disruption of this pathway, by genetic mutation of any one of the FA genes, leads to the common clinical abnormalities observed in the disease in all subtypes. We believe that understanding the cellular function of the FA path-



way will have important implications for new approaches to FA diagnostics and novel FA therapeutic agents.

Diagnostic Activities/New Testing

Our laboratory at Dana Farber has begun to develop a new rapid diagnostic test for FA, which appears to have the same sensitivity and reproducibility as the DEB test. This test involves a molecular analysis of the newly-cloned FANCD2 protein. Thanks to the generosity of several FA patients and family members, I collected several blood samples at the Family Meeting. These blood samples will allow us to perform a side-by-side comparison of the new diagnostic test with the standard DEB test.

Diagnostic Activities/Subtyping

Dr. Grompe and I have recently reviewed our subtyping studies of FA patient-derived cell lines. We have subtyped 62 FA families. Among these FA patients, we identified the following subtype frequency: forty-six FA-A, 74%; six FA-G, 10%; eight FA-C, 13%; two non-A/C/G, 3%. This subtype frequency is similar to the expected frequency, based on reports by other investigators. We

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Preimplantation Genetic Diagnosis

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Preimplantation Genetic Diagnosis (PGD) was developed for couples at high risk for conceiving children with genetic diseases. PGD involves “hyperstimulation” with hormones which cause women to make several mature eggs (6-30). These eggs are then removed from the ovaries and fertilized with the husband’s sperm. As the resulting embryos develop in the test tube, a single cell can be removed and its genetic content determined by a technique known as the polymerase chain reaction. Genetic diseases such as Fanconi anemia can be diagnosed. Embryos known to be unaffected (normal or carriers) can be transferred back to the woman’s uterus. Any fetus that results from such a transfer will not suffer from FA.

At the request of Lisa, Jack, and Molly Nash, we expanded PGD to include HLA typing, so they could conceive a child who would not only be free from FA, but be an HLA identical sibling cord blood donor. Using normal conception, only 3 out of every 16 (19% or approximately 1 out of 5) babies would be free from FA and HLA identical to the patient. Using PGD, we create multiple embryos and select the appropriate embryos for transfer.

On her fifth try, Lisa Nash’s ovaries were stimulated by Dr. William Schoolcroft in Denver, Colorado. Sixteen eggs were successfully fertilized. Dr. Viktor Ivakhehko from our center flew to Denver and performed the biopsies. There were 2 embryos affected with FA, nine carriers, and three normal embryos. The diagnosis was not successful for two embryos. Only one embryo was both HLA identical and free from Fanconi anemia. This embryo was transferred and a pregnancy developed. The

unaffected embryos that were not HLA matched to Molly were frozen for future pregnancies.

We are currently working with couples who have children with thalassemia for the same purpose. PGD provides a way for couples to “beat the odds” and conceive children who are free from genetic disease and who can help save the life of their older siblings.



Cloning the *FANCD2* Gene

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In 1995 a group of researchers in Portland, Oregon mapped the Fanconi anemia group D gene to human chromosome 3p. Very recently, the region containing the gene was narrowed down to a very small area, which contained only 3 genes. All 3 genes were analyzed in detail and mutations were found in 2 patients with group D Fanconi anemia. The mutations proved that the *FANCD* gene had indeed been cloned. Interestingly, however, not all complementation group D patients had mutations in this gene. For example, the HSC62 cell line, used to define FA group D, does not have mutations in the new gene. It is therefore likely that FA complementation group D is heterogeneous and represents at least 2 distinct genes. Because the HSC62 cell is the reference cell line for group D, we termed the new gene *FANCD2*.

Interestingly, many other multicellular organisms including plants, fruit flies, and worms have a

FANCD2 homologue. This is in contrast to other FA genes isolated to date, which seem to exist only in vertebrates. The finding of a *FANCD2* gene in these other organisms is potentially very important for the future study of the FA pathway, since these other organisms are comparatively easy to study genetically. The *FANCD2* gene is very large and has 44 exons. The protein is also large and has 1,451 amino acids.

Dr. D’Andrea’s lab in Boston has produced an antibody to *FANCD2*. The *FANCD2* protein resides in the nucleus of cells and exists in 2 forms, termed *FANCD2-S* (short) and *FANCD2-L* (long). Interestingly, cells from most (>95%) FA patients contain only the *FANCD2-S* form of the protein. Therefore, FA can be readily diagnosed by a rapid antibody test which determines whether both forms of the protein are present or not. Chromosome breakage analysis is not required for this test.

The Use of Fludarabine for Patients with Fanconi Anemia Undergoing Bone Marrow Transplantation

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Over the past 10 years the success of bone marrow transplantation in patients with Fanconi anemia has gradually improved. Adjustments to preparative therapies and manipulation of stem cells (i.e. lymphocyte depletion) have greatly reduced the incidence of severe regimen-related toxicity and graft-versus-host disease (GVHD), respectively. More recently, the major obstacles to successful transplantation have been graft failure (the failure of the transplanted bone marrow to take and to grow new cells), infections and late effects, particularly late malignancies. At the University of Minnesota, we are addressing each of these issues in an effort to improve upon the success of bone marrow transplantation in patients with FA.

In a previous study at the University of Minnesota we determined that graft failure might be partly due to insufficient suppression of the immune system. Therefore, we developed a new preparative regimen which includes an immunosuppressive agent, fludarabine, added to the commonly used preparative regimen of cyclophosphamide, total body irradiation and anti-thymocyte globulin for unrelated donor transplantation. To date, twelve patients have been enrolled on our new fludarabine protocol at the University of Minnesota. Of the eleven evaluable patients, all have successfully engrafted. No patient developed severe toxicity to the preparative therapy or severe GVHD. Thus far six patients are alive, two months to 18 months post-transplant.

FA patients undergoing transplantation are at a high risk for developing serious infections, especially fungal infections. These infections

tend to occur extraordinarily early after transplantation, likely because patients have had a weakened immune system for some time prior to transplantation with prolonged low white blood cell counts and the use of steroid therapy. In an effort to reduce the risk of fungal infections, patients should be referred to a bone marrow transplant center at the earliest sign of medical management failure as prolonged periods of neutropenia may increase the risk of developing fungal infections. As well, we suggest that all FA patients receive an antifungal drug called itraconazole at least one month prior to transplantation.

In an effort to identify patients at particular high risk of developing fungal infections, we perform CT examinations of the chest and sinuses prior to transplantation. If any suspicious areas are noted, patients are examined by an otolaryngologist and/or pulmonologist. As well, all patients are seen prior to transplantation by an infectious disease physician who specializes in fungal disease in bone marrow transplant patients. We are hoping that our efforts to identify patients at risk for fungal disease and treat them early will enable us to prevent and/or detect and treat early fungal disease before it is overwhelming.

Although bone marrow transplantation cures the hematological abnormalities in FA patients, unfortunately these patients remain at a high risk for cancers, especially of the head and neck, and cervix in females. Two factors which appear to be associated with risk of late malignancy in FA patients are the use of irradiation and the development of chronic GVHD.



In an attempt to decrease the risk of malignancy, we have developed a regimen that does not include irradiation for patients receiving a matched related donor transplantation.

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Progress Report

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have begun to report this information systematically to the FA families and plan to pursue carrier detection studies in families where a specific mutation is known.

For individuals interested in the activities of our Comprehensive FA Center in Boston, please contact us for further information.

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Cloning of a New FA Gene

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avoided if more stringent criteria for new complementation groups are maintained: A new group should be based on: (1) at least two FA patients whose cell lines are excluded from all known groups and that fail to complement each other in fusion hybrids, or, (2) if only one such cell line is present, on a new complementing gene that carries pathogenic mutations in this cell line. Based on these criteria the current number of complementation groups is seven.

New FA gene isolated

Using the well known method of complementation cloning we were able to identify the gene that is defective in FA-E patients (*FANCE*). The gene, which localizes on the short arm of chromosome 6—close to the HLA region—encodes a novel protein that does not look like any other known protein. So, like previously cloned FA genes, *FANCE* has not provided us with any new clues about how FA proteins operate at the molecular level. However, the protein carries a nuclear localization signal, which means that it is most likely active in the nucleus of cells. Special about the E group patients is that they have a very low probability for having a matched sibling donor, because of the close linkage between the E gene and the HLA genes on chromosome 6 (which means that any completely matching sibling is very likely to be also affected by FA). With the cloning of the E gene these patients have become prime candidates for gene therapy trials.

The cancer/leukemia connection: FA and Acute Myelogenous Leukemia (AML)

Since AMLs encountered in FA patients do not seem to be different from those seen in non-FA patients,

we wondered whether AML cells occurring in non-FA individuals perhaps arise through the acquisition of an FA phenotype first. Rather than looking at FA genes and possible mutations, we used antibodies to look at the various FA proteins. We found aberrant FA protein patterns in 5 out of 10 AML cell lines and 11 out of 15 fresh AMLs, a very significant proportion. This raises the hypothesis that a disturbance of the FA pathway may be a necessary early step in the development of AML in non-FA individuals, which would explain why FA patients get AML so early in life.

FA and squamous cell carcinoma

Our results on 2 oral tumors from FA patients revealed that they were karyotypically as complex as similar tumors encountered in the non-FA population. They also shared a high proportion of chromosomal breakage events being whole-arm translocations. One tumor had 33% and the other had 69% of all chromosomal break events in this category. Non-FA oral tumors have in the order of 50% whole-arm translocations, so the FA tumors fit in that picture. This is typical for squamous cell carcinomas and in contrast to non-squamous cell cancers, where these translocations are relatively rare. Thus FA oral tumors are in this respect very similar to non-FA oral tumors. We are now looking for FA protein profiles in non-FA tumor samples to see if a similar situation may exist as in the AMLs.

In non-FA oral cancer patients there is a clear link with environmental factors. Therefore, one could extrapolate that environmental cross-linkers may be important causative agents in oral carcinogenesis in FA patients. Numerous studies have implicated a beneficial effect of certain dietary factors on the occurrence of various tumors, oral cancer being one of these. A diet rich in fresh fruit

and vegetables is well accepted to reduce risks. Some scientists believe that tomatoes and tomato-based products are particularly effective. Such claims refer to the general population and may not hold for FA patients. On the other hand, in the absence of any concrete data, it is plausible that similar effects may be achieved in FA patients.

The Use of Fludarabine

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Patients receive fludarabine, cyclophosphamide and anti-thymocyte globulin, and the marrow will be T-cell depleted to reduce the risk of chronic GVHD. At present three patients have received this form of preparative therapy followed by a matched related donor transplantation. All patients achieved successful engraftment. One patient who received maternal bone marrow required a second infusion of cells with successful engraftment after the standard preparative therapy of cyclophosphamide and limited field irradiation. Overall, no patient experienced severe regimen related toxicity, GVHD or serious infections. Currently, we are using this approach only in patients who have a matched related donor as we believe irradiation is required to promote engraftment in the unrelated donor setting.

In summary, bone marrow transplantation remains the only treatment with curative potential for the hematological complications in FA patients. Fludarabine has been shown to promote engraftment in the unrelated setting and may allow for the elimination of irradiation in the matched sibling setting. Novel approaches to reducing the risks of infections and late malignancies are currently being investigated.

Endocrine Issues with FA Patients

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In a joint project with Dr Auerbach of The Rockefeller University, we are evaluating individuals with Fanconi anemia for endocrine disorders in an NIH-funded study. Subjects are admitted (at no cost to them) for up to five days and undergo studies evaluating the status of their growth, thyroid, adrenal, pubertal and glucose control. Additionally, we perform a host of non-medical studies for the benefit of the patients, including evaluations by ENT/Hearing, Ophthalmology/Vision, Neurology, Cardiology, Genetics, Reproductive Endocrinology and Bone Marrow Transplant Service, where appropriate. We welcome anyone with the diagnosis of FA to be studied: young and old, on treatment or off and pre- or post-transplant. Interested families should contact either me or Dr. Auerbach for details. I can be reached as follows:

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To date we have studied 70 patients and have found that endocrinopathies are a common finding in children with FA, and may manifest as short stature, GH deficiency, hypothyroidism with or without TBG (a protein) deficiency and abnormal glucose/insulin metabolism (leading to impaired glucose tolerance or diabetes mellitus). Both the basic underlying disease and its treatment impact the endocrine systems of these individuals. For these reasons, a thorough endocrine evaluation should be performed in every subject, when clinically stable.

In a recent study of a group of children enrolled in the International Fanconi Anemia Registry, we found that short stature was less severe than previously thought, with a mean slightly below the third percentile for the general population. In that study, endocrinopathy was a frequent finding, with over 80% of subjects demonstrating at least one abnormal finding. Seventy-two percent of subjects tested had evidence of excess insulin, most likely due to insulin resistance, while thirty percent of individuals had hypothyroidism. Importantly, 20% of subjects had abnormal thyroid testing as a result of a protein deficiency (TBG). This condition (TBG deficiency) is not a clinical disease, but is often mistaken for true hypothyroidism.

The mean height of those individuals with no demonstrable endocrinopathy was also below the third percentile, demonstrating that significant short stature is inherent to Fanconi anemia. Although the control of statural growth is a complex process regulated by many endocrine and non-endocrine processes, these results suggest that GH insufficiency and hypothyroidism, superimposed on a baseline of short stature, may further contribute to the evolution of short stature in FA.

The question of whether to treat short children with FA was also discussed at the Fanconi Anemia Family Meeting. The global experience with growth hormone (GH) treatment of non-FA subjects indicates that for the general public, GH treatment is safe. The total experience of treating FA patients with GH is 38 people throughout the world. Of these, three died, one from leukemia, one from a severe infection, and the



third from an undetermined cause. None of these three deaths was directly attributed to the GH treatment. An additional two GH-treated FA patients reported problems with glucose intolerance. Thus, GH appears to be a relatively safe treatment in Fanconi anemia, but there are only minimal data on which to make this assumption.

Gene Therapy for Group A Fanconi Anemia Patients

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cells for FA-C and FA-A patients in the future. Results obtained from this study will facilitate improved gene transfer for FA patients and other hematopoietic disorders.

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Gastrointestinal Problems and FA

Khalid Khan, MD, University of Minnesota, Minneapolis, Minnesota

Fanconi anemia (FA) is primarily a disorder of the hemopoietic system that is associated with physical abnormalities in up to 80% of cases. The gastrointestinal (GI) system is affected in only 4% of FA individuals. In these cases, the GI tract fails to form normally (atresia). The esophagus, duodenum and anus can be affected with more than one area being involved in some cases. Symptoms usually appear soon after birth. Surgical repair is possible for these abnormalities although multiple operations may be required in some individuals. Complications following surgical repair are not uncommon (for details see reference).

The GI tract and nutritional status may be affected by treatments given for the primary hematological problems in FA. The most notable example of this is bone marrow transplantation. Nutritional support is often required around the time of transplant, and gastrointestinal symptoms occur with graft-versus-host disease or opportunistic infection in the months after transplantation. Intestinal biopsies following an endoscopic procedure are required for diagnosis in these cases.

Gastroesophageal reflux and constipation are common disorders in the pediatric population. Chronic or recurrent abdominal pain and poor weight gain are symptoms for which children are frequently referred to pediatric gastroenterologists. These problems may, therefore, be expected to occur in some FA children. Some parents of FA children report decreased food intake (poor enteral intake) in their child.

Growth failure occurs in 57% of FA children and in a proportion of these an endocrine disorder is identified. Growth failure can potentially result from poor enteral intake espe-

cially if poor weight gain is the major clinical finding. Nutritional insufficiency alone, however, does not account for the pattern of growth failure commonly seen in FA children. The true prevalence of poor enteral intake, with associated growth failure in FA children has not been documented and neither has there been a systematic exploration of the possible causes. This is likely related to FA being a rare problem with no more than half a dozen individuals from any given state. Gathering this type of data may be suitable for future endeavors of the FA research fund.

Management strategies for common gastrointestinal problems faced by FA parents cannot be easily formulated without the above data. It is likely that a multidisciplinary approach is required. Apart from a pediatric gastroenterologist, a dietitian and other sub-specialists (depending on organ systems involved) need to have an input as non-gastrointestinal disorders often give rise to gastrointestinal symptoms. Small children with upper gastrointestinal problems resulting in pain and/or reflux perhaps with prolonged periods of nasogastric tube feeding will develop food aversion which can manifest as poor enteral intake. A speech pathologist can help to identify and rehabilitate these individuals. Other experts who may be relevant include child psychologists, as behavior problems are common amongst children with chronic disorders requiring multiple hospitalizations.

I would postulate that at present the best format to deal with gastrointestinal problems would be to have a gastroenterologist involved early on when symptoms are perceived by parents, so that an appropriate management strategy and follow-up can be established.



Achieving adequate nutritional growth is perhaps the most important issue from a gastrointestinal point of view. Some children have undergone supplementary feeds through periods of their childhood to achieve this. The methods of feeding have been discussed previously (see reference). The proportion of FA children who need supplementary nutritional support and the period of time that such feeding is required is not known. It is likely to vary between individuals and depend on the presence or absence of other serious conditions.

In the management of FA the primary hematological problem has taken center stage with regards to the development of and advances in treatment. As a multisystem disorder, FA impacts the individual in many ways and this is now being appreciated, although there is little data on some of these issues. The gastrointestinal tract and nutrition are such areas. With the efforts of the FA research fund they will receive more focused attention in the future.

Reference: SJ Schwarzenberg. "Fanconi anemia and the GI tract." *FA Science Letter*. Fall 1999; No. 26: 5-6



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