



FA SCIENCE LETTER

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Management of Patients with FA

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Fanconi anemia (FA) is a complex disorder characterized by congenital malformations, progressive marrow failure and marked predisposition to malignancy. The nature and course of the disease is different for each patient necessitating individual tailoring of investigations and long term follow-up management.

Investigations at Diagnosis

Patients require a thorough investigation at diagnosis including a bone marrow aspirate and biopsy with cytogenetic evaluation, complementation group determination, and major organ function evaluation. HLA-typing should be performed on the patient, siblings and parents. In addition, all siblings must be tested for FA, even in the absence of FA features. All patients with FA should be followed routinely by an experienced hematologist even prior to the onset of marrow failure, a genetic counselor and an endocrinologist. Other specialists depend on individual needs.

Bone Marrow Failure

Blood counts should be checked at a minimum of every 3 months. To better understand the natural history of this

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Hematology 101

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A brief introduction to the terminology of hematology is provided here. Blood cells are produced in the bone marrow, and enter the circulation after they are sufficiently mature. Blood contains plasma (the liquid), and cells. The cells consist of red cells, white cells, and platelets. Red cells are filled with hemoglobin, which carries oxygen from the lungs to the tissues. Red cells do not have a nucleus; it is lost before the maturing cell leaves the bone marrow. Reticulocytes are the newest red cells. They fail to increase in number if anemia is due to bone marrow failure, because of the inability of the marrow to respond. There are many different types of white blood cells. Lymphocytes form the immune system and consist of T-cells, which direct other cells and are responsible for cell-mediated immunity, and B-cells, which produce antibodies. The myeloid white cells are primarily phagocytes, which kill and digest bacteria. The most important are the neutrophils, also called granulocytes (because the cytoplasm contains granules which have enzymes which destroy bacteria), or polymorphonuclear or segmented cells (because the nucleus has several segments). Monocytes are another type of phagocyte. Eosinophils and basophils are important during allergic events. Platelets are particles derived from the cytoplasm of their

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Fanconi Anemia 101

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FA is so named because the first patients were described by Professor Guido Fanconi in Switzerland in 1927. His name is also associated with Fanconi Syndrome, a defect in renal tubular function, totally unrelated to Fanconi anemia. The inheritance pattern is autosomal recessive—each parent has one normal and one mutated FA gene. The offspring receive one FA gene from each parent. The risk of receiving two mutated FA genes is 25%. FA patients have a range of physical appearances, ranging from quite normal, to severe birth defects. Approximately half of the known patients have pigmented skin and/or *café au lait* spots, short stature, and upper limb (thumb and forearm) anomalies. Other physical findings which occur relatively frequently include abnormal gonads, small head, small eyes, and abnormal kidney structures. The most common complication in FA is aplastic anemia, which occurs at a median of about 7 years of age, with a range from birth to 40s. There are high risks of leukemia and solid tumors as well.

FA is a disorder associated with a defect in the response to DNA damage. There are more than 10 FA genes. A defect in any one of them may lead to FA. Cells from FA patients show increased chromosome breakage and decreased growth with increasing concentrations of DNA cross-linkers, such as diepoxybutane (DEB) or mitomycin C (MMC).

Gene complementation groups were originally identified by co-culturing cells from two different patients to determine whether they correct (“complement”) the sensitivity to MMC. The group assignment is now done by culturing FA cells in the presence of retroviruses which contain each of the cloned FA genes, and similar determination of the gene which corrects the MMC sensitivity. *FANCA* is mutated in ~70% of FA patients, and the other genes are implicated less commonly.

When DNA damage occurs in normal individuals, several of the FA

proteins (so far we know about A, C, E, F, G) form a complex in the cytoplasm, which interacts with the FANCD2 protein and facilitates the addition of a ubiquitin molecule. The modified D2 then relocates to DNA damage response foci, along with other non-FA proteins.

A minority of FA patients have hematopoietic somatic mosaicism. One of the bone marrow stem cells has undergone a gene correction, for example by a cross-over between two FA genes in which the parental mutations are at different positions within the FA molecules. This stem cell produces progeny which may have a selective survival advantage over the uncorrected FA cells and, thus, a varying proportion of the blood cells may have normal FA proteins. The patients are “mosaics,” because they have a mixture of FA and corrected cells. The correction can occur in a pluripotent stem cell, or in a later cell, such as the progenitor for T lymphocytes. T cells are the ones usually examined in chromosome breakage analysis. Mosaicism is usually defined by the observation of more than 10% cells that are no longer sensitive to DEB or MMC.

About half of FA patients have abnormal thumbs and/or forearms. They are often seen by hand surgeons prior to the diagnosis of FA. We suggest

that those physicians pay attention to the Hb (hemoglobin) and platelet level of patients, and to the MCV (mean red cell volume), which may be increased in the absence of any other hematologic signs. Downward trends in Hb or platelets might be clues that the patient has an underlying bone marrow failure syndrome.

Treatment of FA should be considered if the Hb is <8 g/dl, ANC <1500/mm³, or platelets <30,000/mm³, or if there is clinically significant MDS or AML. One treatment which may cure the bone marrow is a stem cell transplant (using related or unrelated bone marrow, peripheral blood, or cord blood). Medical treatment involves androgens, folic acid, or G-CSF, as needed. Supportive care includes transfusions of red cells or platelets. Family members should not be donors, because they might sensitize the patient and jeopardize a future transplant.

Surveillance of FA patients for potential complications includes blood counts at least every 4 months and annual bone marrow examinations to monitor for MDS or AML. Examination of the oral cavity and pharynx should be done to monitor for early head and neck cancer, and of the gynecologic areas for vulvar or cervical cancer. ♦

Squamous Cell Carcinoma of the Head and Neck

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Patients with FA have an increased risk for developing aggressive head and neck cancer, especially of the oral cavity. Until new therapeutic and preventive measures are available, strict abstinence from tobacco and alcohol, avoidance of second-hand smoke, maintenance of oral hygiene and aggressive routine screening are the most immediate ways to reduce the development and morbidity of head and neck cancer in this patient population. Early and frequent head and neck examinations, including careful oral cavity evaluations and flexible fiberoptic laryngoscopy are important surveillance measures. Appropriate surgical resection remains the mainstay of treatment for FA patients, since radiation and chemotherapy are poorly tolerated. If radiation and chemotherapy are required for advanced tumors, they should be used with caution and by physicians who have experience in identifying, preventing, and treating associated sequela. ♦

2003 Cincinnati Update on Matched Sibling Donor BMT for FA and Approach to Patients Who Present with MDS or Leukemia

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Matched sibling donor transplant

To date 34 patients have undergone matched sibling donor BMT for Fanconi anemia at Cincinnati Children's Hospital utilizing a preparative regimen of low dose cyclophosphamide (5 mg/kg x4 days), low dose thoraco-abdominal irradiation (400 cGy as a single dose) and anti-thymocyte globulin (ATG) both pre and post-BMT. None of the donor bone marrows (N=31) or matched sibling donor cord bloods (N=3) were T-cell depleted.

Overall survival is 85% with a median follow-up of 7.4 years (range 3 months to 15.6 years). All patients but one engrafted and have remained permanently engrafted. The non-engrafting patient rejected the marrow of both his matched siblings.

The median day to an absolute neutrophil count (ANC) of 500 was 13, and the median day to a platelet count of 50,000 was 24. The incidence of grade I-IV acute graft-vs-host disease was 9% (1 grade I, 2 grade II, no grade III or IV). Two patients developed chronic GVHD. One resolved her cGVHD but later developed a squamous cell carcinoma (SCC) of the mouth 15 years post BMT and subsequently died of progressive disease. The other patient has long term effects of now inactive cGVHD but is alive off immunosuppressive therapy and without SCC. One other patient without cGVHD developed a genito-urinary SCC 6 years after BMT, and is still alive 3 years later. One patient with chronic myelomonocytic leukemia (CMML) relapsed 7 months post-BMT and died. One other patient who had a monosomy 7 clone without MDS or leukemia pre-BMT developed monosomy 7 acute myeloblastic leukemia (AML) 12 years post-BMT in her original host cells and subsequently died. No other secondary cancers have been seen.

Organ toxicity and infectious complications from the transplant preparative

therapy have been minimal. One toxic death occurred; a teenager who had been on androgens and was heavily transfused for several years died of multi-organ failure 49 days post BMT. Another patient who was successfully engrafted and had returned home died unexpectedly of cytomegaloviral pneumonia 5 months post BMT at the local hospital. Overall, 29 of the 34 patients are alive and well.

Mini-FLAG regimen for MDS or leukemia

A major issue facing transplant physicians is how to manage the FA patient who has developed myelodysplastic syndrome (MDS) or leukemia pre-BMT. Should such patients undergo a standard BMT prep, an altered/intensified BMT prep, or a pre-BMT course of induction chemotherapy? At our institution, we have tried several approaches and have settled on an approach of a mild course of chemotherapy induction given 2-3 weeks prior to the start of the BMT preparative therapy. Also, the BMT prep is modified in that the radiation is given as total body irradiation rather than as thoraco-abdominal irradiation. The induction chemotherapy is not started until the donor is confirmed so that the induction chemotherapy can be timed to occur 2-3 weeks prior to the BMT. Thus, some patients may need to be observed only, or treated with very mild oral chemotherapy such as with hydroxyurea or oral etoposide (VP-16) to prevent the leukemia from progressing, while the donor search is being finalized.

The standardized induction chemotherapy being utilized at Cincinnati Children's is a modified low-dose "FLAG" regimen, which we have termed "Mini-FLAG for FA." Patients receive G-CSF 5 µg/kg on days 0-4, fludarabine 30 mg/m² at hour 0 over 30 minutes on days 1-3, and cytosine arabinoside (Ara-C) 300 mg/m² as a four hour infusion starting at hour 4 on days 1-3. The total

fludarabine dose is 90 mg/m² and the total AraC dose is 900 mg/m².

Patients eligible for this approach are those with leukemia or advanced MDS. This approach has now been used in 4 FA patients. One patient presented with AML with a WBC over 40,000, one with CMML and a WBC over 30,000, one with MDS with 6% blasts in the bone marrow, monosomy 7 and a 3q26q29 gain, and one with MDS and an XXX clone involving 100% of her bone marrow and peripheral blood cells. The patient with CMML had to be maintained first with hydroxyurea, then oral VP-16, until the donor could be confirmed, then received the mini-FLAG.

The induction chemotherapy was well tolerated in all patients, all achieved remarkable cytoreduction pre-BMT, and all but one are currently alive free of disease. The one patient who died did so after 2 failed unrelated donor cord blood transplants and one successful partially matched PBSC transplant, but died of pulmonary infection 5 weeks post transplant. She was in complete remission and fully engrafted at the time of her death. In our experience, this approach has been the best tolerated and most successful when compared to previous attempts to treat such patients. ♦

Hormones and FA

Susan R. Rose, MD

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Children with Fanconi anemia 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 are made in one part of the body, and are sent to another part of the body. A message may be "make that bone grow longer," or "keep that blood sugar normal." 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 2y and 16y). In this study, 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 hypothyroidism. The authors recommended that "more care should be taken to exclude these problems in individuals with (FA)." 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 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 patients, 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 his or her FA complementation group.

When 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 cause the ovaries and testes to go into puberty by making estrogen or testosterone. The pancreas makes insulin when a person eats or drinks; this keeps the blood sugar or glucose normal, and helps 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 in order 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 18 months at Cincinnati Children's Hospital Medical Center, we have evaluated 25 children with FA, 67% prior to transplant. Ages range from 4 months to 16.5 years. Of these children, 27% were born small for gestational age birth. Short stature was identified in 64% of them.

Of this group of children with FA, nearly every child has had an endocrine deficiency. Glucose intolerance or diabetes has been identified in 78% of them. Insulin levels have been elevated in 28% of those who were old enough to do the

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Bone Marrow Transplantation for the Treatment of FA

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Hematological complications including bone marrow failure, myelodysplastic syndrome (MDS) and acute myelocytic leukemia (AML) are part of the natural history of the disease in patients with Fanconi anemia (FA). Hematopoietic stem cell transplantation (HSCT) offers the only curative potential for the marrow complications of this disorder. However, high complication rates are experienced, in part because of an underlying DNA repair defect. Therefore, we have developed new therapies geared toward reducing the risk of major complications, improving survival and enhancing long term quality of life.

Results with HLA identical sibling donor HSCT

To attempt to reduce the late effects of malignancy, chronic graft-versus-host disease (GVHD), endocrinopathies (hormonal disorders) and infertility in patients with FA undergoing HLA-matched related donor HSCT, we developed a new non-irradiation based regimen using fludarabine, cyclophosphamide and anti-thymocyte globulin followed by the infusion of T-cell depleted bone marrow or unmanipulated umbilical cord blood. Between April 2000 and February 2003, 11 patients (10 with aplastic anemia, 1 with MDS) underwent HSCT using this regimen. Stem cell sources were marrow and umbilical cord blood in 8 and 3 patients, respectively. All patients engrafted and none experienced severe regimen-related toxicity. Median days to neutrophil and platelet engraftment were 11 days (range 9-21) and 39 days (range 18-381) respectively. No patient developed GVHD. The patient with MDS relapsed and another with maternal donor experienced secondary graft failure. Both patients, however, are alive and well (3.1 years and 2.9 years later) after repeat HSCT. With a median follow-up of 15.1

months (range 2.2-34.3), the probability of survival at 2 years after HSCT is 100%.

In summary, this fludarabine-based approach in combination with T cell depletion of the sibling donor marrow is effective for FA patients and is associated with excellent survival and quality of life.

Results with HLA matched and mismatched unrelated donor HSCT

To attempt to reduce the risk of unrelated donor transplantation and improve survival in patients with FA undergoing HLA-matched or mismatched unrelated donor HSCT, we developed a new regimen using total body irradiation (TBI), fludarabine, cyclophosphamide and anti-thymocyte globulin followed by the infusion of T cell depleted bone marrow or unmanipulated umbilical cord blood. Between April 1999 and July 2003, 42 patients with FA underwent HSCT using this regimen. Stem cell sources were marrow and umbilical cord blood in 37 and 6 patients, respectively. All but one patient engrafted. The incidence of neutrophil recovery and engraftment was 98%. Incidences of grade 2-4 acute GVHD and chronic GVHD were 19% and 16%, respectively. With a median follow-up of 27 months, probability of survival at 2 years after HSCT is 76% for recipients with standard risk disease and 34% for those with high risk disease (patients >18 years of age, presence of advanced MDS or leukemia, history of gram negative or fungal infections before HSCT, or recipients of HLA-mismatched BM or >2-antigen HLA-mismatched umbilical cord blood).

In summary, the addition of fludarabine to the standard regimen of cyclophosphamide and TBI is associated with superior engraftment and survival in patients with FA undergoing alternate donor HCT as compared to all other regimens previously reported.



Margaret L. MacMillan, MD

New treatment protocols

Since January 2003, three new transplant protocols have been opened for FA patients. The purpose of these studies is to further reduce risks of transplant related toxicity and infection, particularly in high risk patients with unrelated donors. The first study uses the same fludarabine and cyclophosphamide and replaces TBI with busulfan. Thus far, two patients with leukemia have been enrolled. Both had prompt neutrophil engraftment and no severe regimen-related toxicity. Neither has had GVHD thus far, and both are alive and well at 1 month and 7 months after transplantation, respectively. The other 2 protocols have not yet accrued patients and will be discussed. ♦

Stem Cell Transplantation for FA: The MSKCC Experience

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Since 1998, we have performed stem cell transplants from donors other than HLA-matched siblings for 11 patients with FA. These patients ranged in age from 5 to 24 years of age. Complementations Groups included Group A for 7 patients, Group C for 4 patients and undetermined group for 1 patient. Mosaicism for DEB-induced chromosome breakage included 75-78% for 3 patients, 95-98% for 2 patients and 100% for 6 patients.

Four patients had aplastic anemia (AA), 5 patients had advanced forms of myelodysplastic syndrome (with excess blasts for 4 patients and in transformation for 1 patient) and 2 patients had AML. High-risk features included (1) significant transfusion history for 9 patients, (2) prior androgen therapy for 8 patients and (3) significant infectious history for 7 patients. Donors were HLA-mismatched related donors for 5 patients with 4/6 (2 patients) or 5/6 matching (3 patients) or unrelated donors for 6 patients with 5/6 matching (4 patients) or 6/6 matching (2 patients).

Our transplant cytoreductive regimen included: TBI (450 cGy), fludarabine (30 mg/m²/day x 5), cyclophosphamide (10 mg/Kg/d x 4) and Rabbit ATG (Thymoglobulin) (2.5 mg/Kg/d x 4). Tacrolimus (FK506) was used for GVHD prophylaxis for all patients. Steroids were also used for GVHD/rejection prophylaxis for the first 4 patients and were deleted from the cytoreduction (successfully) for the last 7 patients. We used G-CSF-mobilized peripheral blood stem cell (PBSC) grafts for 8 patients and bone marrow (BM) grafts for 3 patients (based on donors' choice). All grafts were (aggressively) T-cell depleted with soybean agglutinin and E-rosetting for the BM grafts and CD34 selection and E-rosetting for the PBSC grafts. The stem cell doses were significantly higher, and the T-cell doses significantly lower for the PBSC grafts.

All 11 patients engrafted, and none developed GVHD. Nine patients are alive

disease-free, 0.5-5 years post-transplant. One patient died early post-transplant from the consequences of infection. One patient had started recovering and then developed a viral pneumonia 6 months post-transplant and died from pulmonary failure. One patient (with MDS in RAEB) relapsed 7 months post-transplant, developed AML and received a second transplant from the same donor after busulfan and fludarabine. This patient is now alive disease-free.

All patients have had normal hematologic reconstitution. The average time for T-cell immune reconstitution post-transplant was 8 months (range 6-12 months). One patient who is now 3 years post-transplant has had normal T and B-cell reconstitution, is doing well, but remains with hypogammaglobulinemia (low antibody production).

In summary, the combination of TBI, fludarabine and cyclophosphamide with ATG and tacrolimus and T-cell depleted grafts has resulted in consistent engraftment with minimal GVHD in high-risk patients with FA. Our effort needs to focus now on the prevention of further infections and organ toxicity. This could possibly be achieved by performing transplants earlier in the course of the disease and by substituting TBI with agents such as busulfan or thiotepa.

In order to attempt to decrease secondary malignancies, future plans include a similar approach of T-cell depleted transplants for patients with matched related donors using non-TBI cytoreductive regimens. One high-risk 34-year-old FA patient with MDS and pulmonary nodules received cyclophosphamide, fludarabine and ATG followed by a T-cell depleted PBSC from her HLA-matched sister. She has engrafted with no GVHD and is alive and disease-free 6 months post-transplant.

In addition, based on the finding of the association of solid tumors with Human Papilloma Virus (HPV) in patients with FA, we are actively pursuing a multi-center trial of the use of an

HPV vaccine pre- or post-transplant to decrease the risk of solid tumors in patients with FA. ♦

Hematology 101

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bone marrow precursor, megakaryocytes. Red cells survive 120 days, platelets 7-10 days, lymphocytes months to years, and neutrophils only 6-12 hours.

Normal blood counts are age-dependent for Hb (hemoglobin) and MCV (mean cell volume of the red cells). In general, Hb <8 g/dl, ANC <1500/mm³, or platelets <30,000/mm³ lead to consideration of therapeutic intervention, either stem cell transplantation, or medical treatment. ANC stands for absolute neutrophil count, and is obtained by multiplying the total white blood count by the % neutrophils. To evaluate the status of the bone marrow, we recommend an annual bone marrow aspirate to evaluate the types of blood cell precursors in the marrow, a biopsy to assess cellularity (the proportion of the marrow that contains cells rather than fat), and cytogenetics for evidence of clonal disease.

Aplastic anemia is defined as pancytopenia (decreased red cells, white cells, and platelets) with hypocellular bone marrow. Leukemia is defined as a malignant proliferation of immature cells, called "blasts." Myelodysplastic syndrome (MDS) is cytopenia with a hypercellular marrow, but with less than 20% blast cells. Significant MDS is associated with clinically important cytopenias, associated with dysplastic erythroid, myeloid, or megakaryocytes; the dysplastic features are characteristic of each cell lineage. Cytogenetic clones, while often seen in MDS and in acute leukemia, may not independently predict leukemia or an adverse outcome. ♦

Modified Transplant Regimen

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The life expectancies of children and teenagers with Fanconi anemia have greatly improved by better management over the last 30 years. It is not clear yet whether the transplant of hematopoietic stem cells has contributed to this development. The German Fanconi Anemia Register with more than 160 patients at the age of 1 to 49 years does not show a long-term survival advantage for transplanted versus non-transplanted patients yet. This is due, on the one hand, to the early risks of transplant and, on the other hand, to the unchanged high prevalence of tumors, particularly at a later age. Numerous attempts are therefore made worldwide to minimize these risks by modification of transplant methods. These risks are much higher for Fanconi anemia patients—like graft-versus-host disease (GVHD), graft rejection, dangerous infections, toxicity and late tumors—and result in numerous questions. Is irradiation required for conditioning, or is chemotherapy sufficient to ensure engraftment? Can critical substances (for example, alkylating agents) be omitted or further reduced in dose? What is the ideal method of GVHD prevention (*ex vivo* T-cell depletion in the

transplant or *in vivo* T-cell antibodies given after transplant)? What are the methods of the future to cope with the high infectious risks? Many of these questions remain unanswered and require larger patient populations and longer observation times.

Our own attempt combines a chemotherapeutic conditioning with fludarabine (180 mg/m²), busulfan (1-2 mg/kg) and the pre/post-transplant immunosuppression (GEFA protocol). Twenty-two patients have been transplanted according to this protocol in aplastic phase (N=12), myelodysplastic syndrome (N=7) or AML (N=3). The overall survival is 64%. For 13 unrelated transplants the probability of event-free-survival (pEFS) is 61%, which is satisfying in respect to historical results and the specific risk factors of that group. To further improve the results, the following questions are currently addressed: role of mosaicism and donor chimerism of distinct cell populations after transplantation, management of viral reactivations, pharmacokinetics of chemotherapeutic drugs used within the conditioning regimen, type and quality of stem cell sources, and the early detection of clonal diseases.



Wolfram Ebell, MD

There are already some trends, which are worth following carefully. This concerns the type of transplant (bone marrow vs. peripheral blood stem cells), age of the patient, androgen pre-treatment, and the progress to advanced types of MDS or AML. Cytogenetic aberrations alone do not seem to indicate a poorer transplant outcome. Whether all these transplant modifications will indeed result in a lower secondary tumor risk in the future remains to be seen. ♦

Hormones and FA

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oral glucose tolerance test. Abnormal thyroid tests have been found in 60% of them. A low peak stimulated GH has been found in 47% of them, but many of those with low GH have a normal height. 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. In 76% of boys, testes have been small for age.

Summary and Recommendations

We have found frequent thyroid insufficiency and inadequate insulin effect. These may be at least as impor-

tant 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 mea-

asures 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. ♦

Hand and Arm Differences in FA

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Hand and arm differences in Fanconi anemia primarily affect the radial border of the arm. A deficiency of some or this entire border creates considerable variation in presentation. The presence of a deficiency along the radial side of the arm may be an early clue to the diagnosis of Fanconi anemia. The anomalies involve the entire limb 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 is difficult and 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. Many of 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. However, the outcome following this surgical approach is not yet known. The external fixator has also 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 after lengthening are still pending and more information will be presented 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. An absent thumb or a thumb without a stable base is removed and the index finger 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 anywhere between 6 months and 2 years of



Scott Kozin, MD

age and requires meticulous technique to position the index 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 ablate 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. ♦

Editors' Note and Disclaimer

Statements and opinions expressed in this newsletter are those of the authors and not necessarily those of the editors or the Fanconi Anemia Research Fund. Information provided in this newsletter about medications, treatments or products should not be construed as medical instruction or scientific endorsement. *Always consult your physician before taking any action based on this information.*

Feeding Intolerance in Fanconi Anemia

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Many parents of children with FA find that their child has poor oral intake. Causes of poor oral intake in FA include gastrointestinal causes, such as nausea or abdominal pain. Other causes include chronic inflammation or infection, medication side effects, and neurologic or behavioral problems.

Nausea in patients with FA can be a very difficult symptom to diagnose and manage. It may result from infections, including urinary tract infections or sinusitis, from medication side-effects, from complication of anatomic gastrointestinal abnormalities, from gastric emptying delay, and from behavioral problems. Some patients may have more than one cause of their nausea.

Gastroesophageal reflux (heartburn) may also reduce oral intake. While any patient may have reflux, it is commonly associated with esophageal atresia. Reflux may become more common with age. Medical management is essential to reduce complications, such as bleeding or stricture formation. Fortunately, we now have excellent medications to suppress gastric acid.

Some FA patients are born with congenital anomalies of the gastrointestinal tract. Repair of these conditions may result in long-term complications. Complications of duodenal atresia are the most significant. In children who have had repair of duodenal atresia, more than 25% have abdominal pain. This may result from chronic alkaline reflux or blind loop syndrome (which causes large quantities of bowel gas). There may be poor duodenal motility above the repair site. Children may experience recurrent obstruction-like episodes. Newer forms of management of duodenal atresia, such as duodenal tapering, may lessen the complications.

Abdominal pain may have many causes, including mechanical obstruction of the bowel, abnormal gut motility, small bowel overgrowth, and gallbladder disease. Evaluation of gastrointestinal symptoms may include many different

modalities. The basis for all evaluation is a good history and physical exam. Many patients can be diagnosed from history and physical exam alone. Certainly, a well-taken history can dramatically reduce the number of procedures and tests needed for diagnosis. It may take 45 minutes to take a thorough history and physical. If parents come with notes on the problem, or even a crude diary of pain and nausea symptoms, it may greatly facilitate the process.

Despite all efforts to diagnose and manage poor oral intake, some children cannot take in enough food to grow normally. Adequate nutrition is that amount that leads to normal growth for the child's genetic potential and enough energy for the child to do normal activities. In addition, there should be adequate reserve to face short-term malnourishment during acute illness.

Supplemental feedings may be indicated if the child is persistently less than 85% of the expected weight for height or if he/she failed to gain weight over a 3-6 month period. Whenever possible, supplementation should be given into the gut, rather than as IV nutrition support. This is safer and more likely to allow normal function of the gut. Most children requiring supplementation cannot simply drink extra calories through special formulas. They need to have extra calories delivered in a way that does not depend upon their appetite.

Families may need help choosing a route for supplemental nutrition. Supplementation can be given into the stomach (gastric feedings) or into the small intestine (jejunal feedings). Optimal supplementation is usually done at night, when the child ordinarily would not be eating. This allows daytime appetite to continue undiminished. Although nightly supplementation produces the best results, children should get a "night off" if they are going on an overnight trip or to a friend's house for a sleep-over.

A small flexible feeding tube can be passed through the nose into the stomach

or small intestine for feedings. Gastric feedings are simpler. Larger volumes can be given at any one time. If the tube into the stomach is dislodged, it can easily be replaced, often by the parent or child. The risks associated with this tube include the chance it could dislodge at night leading to aspiration of food or the risk of sinusitis from blocking the nasal drainage of the sinuses.

Jejunal feedings reduce the risk of reflux, but must be given by drip (small volumes continuously on an IV pump). If a jejunal tube is dislodged, a radiologist must replace it, leading to a trip to the hospital.

If feedings will be needed for a long time, discussion of a gastrostomy tube is appropriate. This is a soft, flexible tube surgically placed through the abdominal wall into the stomach. Before choosing such an approach, the needs of the child and family should be discussed, as well as the child's current health status.

Finally, it should be noted that families with children with chronic disease are targets of individuals peddling appetite stimulants and special food supplements, herbs, and vitamins that are said to provide miraculous cures. Rarely have these products been subjected to rigorous medical investigation. Families should be wary of such claims. No appetite stimulant has been shown to be very effective in well-designed controlled clinical trials; all such medications tested thus far have significant side-effects. Supplemental formulas and vitamins, if necessary, can usually be purchased quite cheaply. Special vitamins, derived from exotic sources, are not necessary. Herbs and "natural products" at best are often ineffective and at worst, dangerous. If a family feels strongly that some or all of these unproven therapies might benefit their child, they should discuss it with their primary physician to insure the safety of the products used. ♦

Cancer Epidemiology

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The life expectancy of FA patients has improved dramatically in recent years, due to better supportive care and stem cell transplants for bone marrow failure. In addition, physicians are now recognizing patients with milder phenotypes, who often are diagnosed at older ages.

Cumulative incidence analyses still indicate that bone marrow failure is the most frequent complication. However, the potential problems of the FA adult are of increasing importance, and the major concern is cancer. FA patients develop specific and unusual cancers at earlier ages and without the risk factors usually seen in the general population. The age range is from the teens to the 40s. The major cancer types include acute myeloblastic leukemia, liver tumors (usually related to treatment with androgens), and cancer of the head, neck, and esophagus, as well as the vulva, vagina, and cervix. In literature reports of FA patients with cancer, about 25% were diagnosed with FA only after they had developed cancer. This suggests that those patients were milder in their physical appearance, and that their bone marrow function was better than is expected in FA.

In our pilot North American Survey (NAS) of FA, we found that the patients who had not had a bone marrow transplant but had solid tumors were older at the diagnosis of FA. The relative risk of specific cancers in FA was compared to the risk in the general population after adjustment for age, sex, and birth cohort. The relative risk of AML was 800-fold, head and neck cancer 700-fold, esophagus 2300-fold, vulvar cancer 4300-fold, and cervical cancer 200-fold. Cause-specific hazard rate analyses suggest that the rate of leukemia levels off in teenage years at <1% per year. The rate of solid tumors rises more than linearly, and reaches 10% per year by age 45. In the presence of competing risks, the cumulative incidence of the first adverse event is 53% for bone marrow failure, 29% for solid tumor, and 10% for acute leukemia.

The impact of bone marrow transplant on solid tumors is still unresolved.

More than a dozen patients have been reported to have oral cancer, primarily tongue, at 3 to 15 years after transplant, and at 11 to 33 years of age. In the NAS study, the crude rate for tumor development in patients who did not have a BMT was 0.7%, while it was 2% per year in the patients who had a BMT. The rate in the BMT group was 2.8-fold the rate in the untransplanted group.

It is clear that FA patients are at increased risk of cancer, and that cancer occurs much earlier in FA. The

maximum risk for solid tumors rises steeply from childhood, and the types of cancer are specific and unusual. Further studies are needed to understand the natural history of cancer in FA, the role of viruses in cancer etiology, the importance of cancer screening, and methods for cancer prevention. We recommend blood counts every 3-4 months; and an annual bone marrow aspirate, biopsy, and cytogenetics; head and neck cancer screening with laryngoscopy; and gynecologic exam. ♦

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A Novel Ubiquitin Ligase is Deficient in Fanconi anemia

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Fanconi anemia is a recessively inherited disease characterized by congenital defects, bone marrow failure and cancer susceptibility. Cells from individuals with Fanconi anemia are highly sensitive to DNA-crosslinking drugs, such as mitomycin C (MMC). Fanconi anemia proteins function in a DNA damage response pathway involving breast cancer susceptibility gene products, BRCA1 and BRCA2. A key step in this pathway is monoubiquitination of FANCD2, resulting in the redistribution of FANCD2 to nuclear foci containing BRCA1. The underlying mechanism is unclear because the five Fanconi anemia proteins known to be required for this ubiquitination have no recognizable ubiquitin ligase motifs. Here we report a new component of a Fanconi anemia protein complex, called PHF9, which possesses E3 ubiquitin ligase activity *in vitro* and is essential for FANCD2 monoubiquitination *in vivo*. Because PHF9 is defective in a cell line derived from an individual with Fanconi anemia, we conclude that PHF9 (also called FANCL) represents a novel Fanconi anemia complementation group (FA-L). Our data suggest that PHF9 has a crucial role in the Fanconi anemia pathway as the likely catalytic subunit required for monoubiquitination of FANCD2.

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Management of Patients with FA

continued from page 1

disease and the significance of cytogenetic clonal abnormalities, patients should have marrow examinations annually, and more frequently after the onset of marrow failure and/or development of a clonal cytogenetic abnormality. Soon after diagnosis patients should have a consultation at a transplant center experienced in FA. This consultation will assist in management of the patient and help determine the optimal timing of transplantation, as the role of androgens, growth factors and antioxidants is changing with improved outcomes with transplantation. Epsilon-aminocaproic acid (amicar) is useful in patients with thrombocytopenia and bleeding, and hematopoietic growth factor therapy and antibiotics are often used in patients with neutropenia and fever. Risk of iron overload should be minimized by early chelation therapy, keeping in mind that many patients even without a history of red cell transfusion will often have elevated iron levels. For the patient considering allogeneic HCT, use of red cell and platelet transfusions should be minimized to reduce the risk of alloimmunization. If transfusions are required, an effort should be made to transfuse only irradiated, cytomegalovirus (CMV) negative (or filtered) red cell or single donor platelet products from unrelated volunteer donors (*i.e.*, not family members).

Malignancy

Because older FA patients age ≥ 20 years are at high risk for malignancies, they require routine dental examinations every 6 months, annual gynecological examinations for women after menarche, ENT evaluations and dermatological examinations for suspicious lesions. Sun and cigarette smoke exposure should be minimized.

Endocrinopathies

Endocrinopathies including short stature, diabetes mellitus and hypothyroidism are common. All patients should receive a thorough endocrine evaluation at diagnosis regardless of height and weight, during puberty, and after



Blanche Alter, MD; Richard Harris, MD; Margaret MacMillan, MD answer questions at FA Family Meeting.

transplantation, with appropriate follow-up thereafter. In adults, patients should be monitored and treated for osteoporosis.

Failure to Thrive

Poor appetite and gastrointestinal disorders, including reflux and endocrinopathies, can lead to failure to thrive in FA patients. Patients should be evaluated by a gastroenterologist and/or nutritionist to assist in optimizing nutrition and growth.

Reproductive Issues

The majority of males with FA are infertile. Fertility can be tested after puberty by semen analysis. Approximately one third of FA women are infertile. Women with FA who become pregnant may experience a rapid decline in bone marrow function. In addition, there are many potential obstetrical complications. Pregnancies should be carefully monitored by an obstetrician who specializes in high risk pregnancy and by a hematologist. Hormonal replacement may be necessary in both males and females, and should be assessed by an endocrinologist.

Organ Dysfunction

Patients with FA may have a number of major organ anomalies. At diagnosis, all patients should have a cardiac echocardiogram, kidney ultrasound, hearing test and vision test. Additional tests may also be warranted. Patients

should be referred to specialists if abnormalities are noted.

Infectious Diseases

Opportunistic infections are the major cause of morbidity and mortality for FA patients. The risk for developing life threatening infections increases during periods of neutropenia and for at least a year after transplantation until the immune system has recovered. In addition, a subset of FA patients who have not been transplanted appear to be at higher risk for infections suggesting they have an inherent impaired immune system. Research is underway to examine this further. All FA patients should receive the routine childhood immunizations including hepatitis B, Varivax (for chickenpox) and influenza (annually each autumn). These immunizations need to be repeated after transplantation, once the immune system has recovered, which takes at least a year. The risk of infection can be reduced with vigilant hand-washing and avoidance of potential sources of infectious disease, including reptiles, raw meat, and fungal spores released into the air during building demolitions. Prior to transplantation at the University of Minnesota, patients are urged to receive at least a month of antifungal therapy to eradicate any undetectable fungal infection. In addition, patients must receive an extensive work-up for evidence of occult infection, which must be thoroughly treated before transplantation. ♦



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