Fanconi Anemia 101

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Questions

1. How many are new to the FA Adult meeting?
2. How many were diagnosed after age 18 (i.e. as adults)?
Attendees as of 3/1/14

<table>
<thead>
<tr>
<th>Feature</th>
<th>Data</th>
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<tbody>
<tr>
<td>Number</td>
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</tr>
<tr>
<td>Male:Female</td>
<td>12:29</td>
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<tr>
<td>Age</td>
<td>28 (18-61)</td>
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<tr>
<td>USA</td>
<td>27, from 16 states</td>
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<tr>
<td>Other Countries</td>
<td>14, from 11 countries</td>
</tr>
<tr>
<td>“New”</td>
<td>21</td>
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</table>

More females
My Tasks

1. FA101 (advanced FA) for those who have not heard it.
2. Update on what is new in FA research.
3. Address specific concerns of people with FA who are adults.

All in 50 minutes…..(+ questions)
<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Hematology</th>
<th>Leukemia</th>
<th>Solid Tumors</th>
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<tbody>
<tr>
<td>Fanconi Anemia (FA)</td>
<td>Aplastic</td>
<td>AML</td>
<td>SCC</td>
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<tr>
<td>Dyskeratosis Congenita (DC)</td>
<td>Aplastic</td>
<td>AML</td>
<td>SCC</td>
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<td>Diamond-Blackfan Anemia (DBA)</td>
<td>Pure anemia</td>
<td>AML</td>
<td>Sarcomas</td>
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<td>Severe Congenital Neutropenia (SCN)</td>
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<td>AML</td>
<td>-</td>
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<td>Amegakaryocytic Thrombocytopenia</td>
<td>Thrombocytopenia</td>
<td>AML</td>
<td>-</td>
</tr>
<tr>
<td>Thrombocytopenia Absent Radii (TAR)</td>
<td>Thrombocytopenia</td>
<td>AML</td>
<td>-</td>
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These disorders are the “Inherited Bone Marrow Failure Syndromes” (IBMFS).
History: Guido Fanconi

- **Fanconi Anemia** (Fanconi pancytopenia syndrome): 1927, 3 brothers with pancytopenia and physical abnormalities, “perniziosiforme”
- **Fanconi Syndrome** (renal Fanconi syndrome): 1936, proteinuria, glucosuria, phosphaturia, aminoaciduria, citraturia, and proximal renal tubular acidosis
Fanconi Anemia: Definition

- Autosomal recessive
  - 1 X-linked recessive gene
- Physical findings
- Aplastic anemia
- Leukemia
- Solid tumors
- Chromosome instability
- DNA repair defect
- >16 genes
FA Child

All photos with permission
FA Literature: Physical Findings, 60%

- Low Birth Weight
- Short
- Thumbs
- Skin hyperpigmented
- Gonads male
- Microcephaly
- Renal
- Eyes
- Skin café au lait
- Developmental delay
- Ears, deaf
- Radii
- Cardiopulmonary
- Legs, hips, feet
- Gastrointestinal tract
- Brain/pituitary
- Gonads female
- Short or skin only

M:F 1.2:1

Shimamura and Alter, Blood Reviews 2010
Characteristics of People with FA

- Physical findings described in the literature may not be found in all people with FA
  - 11% had short stature and skin findings only
  - At least 25% of those reported had no other (or none) physical findings
- Some people without physical findings may be diagnosed with FA at a later age
Fanconi Anemia: Adult Examples

- Diagnosed at birth due to absent radius
- Aplastic anemia, age 5; BMT age 9
- Aplastic anemia, age 16; BMT age 18
- Aplastic anemia, age 42
- Tongue cancer, age 30
- BMT donor, age 55

- Look around you....
FA Literature: Age at Diagnosis

Shimamura and Alter, Blood Reviews 2010
Laboratory Findings in FA

- Low blood counts (pancytopenia)
- Large red cells (macrocytosis)
- Increased fetal hemoglobin (Hb F)
- Chromosome breakage in lymphocytes or fibroblasts cultured with a DNA crosslinker, e.g. diepoxybutane (DEB) or mitomycin C (MMC)
FA Inheritance

- People with FA:
  - Unaffected parents: one FA and one normal gene (carriers)
  - Affected offspring: one FA gene from each parent

- Children of FA:
  - Each has one FA gene (carriers); chance of FA marrying an FA carrier of the same genotype is ~1/200 or less
1:300 in New York State in 1971 (Swift, 1971)

- “Taking only the families I know, twelve definitive cases have been born in New York State in that period [1956 until 1967] among a total of $4.185 \times 10^6$ live births, providing an estimated birth incidence of 1 in $3.48 \times 10^5$. If the Normal and FA alleles follow the Hardy-Weinberg law, the expected heterozygote frequency is about 1 in 300.”

- ~1:100 in Ashkenazi Jews, Afrikaaners, Spanish Gypsies, black sub-Saharan Africans

- Birth rate of FA (known to FARF) ~60% ascertainment, general birth rate, Hardy-Weinberg equation

- 1:181 in US in 2010 (birth incidence 1/130,000)

- 1:93 in Israel in 2008 (birth incidence 1/35,000)

- *FA is more common than we think*

Rosenberg, Tamary, Alter: AJMG 2010
Thenar Muscle Hypoplasia
### FA: Complementation Groups/Genes

<table>
<thead>
<tr>
<th>Group</th>
<th>Locus</th>
<th>cDNA</th>
<th>Exons</th>
<th>AA</th>
<th>%</th>
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<td>43</td>
<td>1455</td>
<td>~70</td>
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<td>Xp22.31</td>
<td>2.8</td>
<td>10</td>
<td>859</td>
<td>Rare</td>
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<tr>
<td>C</td>
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<td>4.6</td>
<td>14</td>
<td>558</td>
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<td>D1/BRCA2*</td>
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<td>10</td>
<td>536</td>
<td>~5</td>
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<td>L/PHF9/POG</td>
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<td>1.7</td>
<td>14</td>
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<tr>
<td>M/HeF</td>
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<td>6.5</td>
<td>22</td>
<td>2014</td>
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<tr>
<td>N/PALB2*</td>
<td>16p12.1</td>
<td>3.5</td>
<td>13</td>
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<td>Rare</td>
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<tr>
<td>O/RAD51C*</td>
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<td>9</td>
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<td>P/SLX4*</td>
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<td>11</td>
<td>916</td>
<td>Rare</td>
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</table>

*Breast cancer genes
16 FA Genes

*Breast cancer in carriers

Adapted from Leiden Open Variation Database, http://chromium.liacs.nl/LOVD2/FANC/home.php
FA/BRCA DNA Repair Pathway

DNA damage
Oxidative stress
Cytokines

Fanconi Anemia
Core Complex

ubiquitin

Adapted from Shimamura and Alter, Blood Reviews 2010
Who Should be Tested for FA?

- Characteristic birth defects (eg thumbs, kidneys, poor growth, etc, especially VACTERL)
- Aplastic Anemia (AA)
- Myelodysplastic Syndrome (MDS)
- Acute Myeloid Leukemia (AML)
- Decreased fertility
- Early characteristic cancer
- Siblings of people with FA
What are the FA Tests?

- Chromosome breakage, DEB or MMC
- D2 ubiquitination (Western blot)
- BRCA2 (Western blot)
- Complementation with cell lines
- Complementation with retroviruses
- Sequencing of candidate genes (eg, $FANCC$ IVS4+4 A->T)
- Sequencing of all cloned genes
- NextGen sequencing: exome, GWAS, etc

*Blood lymphocytes, skin fibroblasts*
Chromosomes

Shimamura and Alter, Blood Reviews, 2010, courtesy of Lisa Moreau, Dana Farber Cancer Institute
FA: D2 Ubiquitination

Shimamura et al, Blood, 2002

Also called a “Western” or “D2” “blot

Green and Kupfer, HemOnc Clin NA, 2009
Complementation Analysis, Retroviruses

- FA cells are sensitive to DEB or MMC
- Transfect retroviruses containing cloned FA genes
- Transfected cells no longer sensitive
  - Normal gene ‘complemented’ FA cells, defining the complementation group
- Transfected cells still sensitive
  - Normal gene not identified for FA cells
Retrovirus-mediated Correction of FA Cells

Retrovirus-mediated Correction of TA 0252's T-cells analyzed by flow cytometry after five days of MMC-Incubation

- S11EG
- SFA
- S11FCIEG
- S11FEIEG2
- S11FFIEG
- S11FG

FANCA
But, welcome to the modern era
DNA: the molecule of life

Trillions of cells

Each cell:
- 46 human chromosomes
- 2 meters of DNA
- 3 billion DNA subunits (the bases: A, T, C, G)
- Approximately 30,000 genes code for proteins that perform most life functions
Gene Finding by Exome Sequencing

Exome sequencing cases → Coding variants → Exome sequencing controls → Exclude common variants → Candidate genes

Genetic variation databases
Blood Production (Hematopoiesis)

- **Pluripotent Stem Cell**
  - **Myeloid Stem Cell**
    - Neutrophils
    - Monocytes
    - Eosinophils
    - Basophils
    - Red Cells
  - **Platelets**
  - **Pluripotent Stem Cell**
    - **Lymphoid Stem Cell**
      - **T Lymphocytes**
      - **B Lymphocytes**
      - Lymphocytes
Bone Marrow Biopsy

Normal

Aplastic
Proof of Mosaicism in FA

- Peripheral blood lymphocyte chromosome breakage test normal
- Skin fibroblast chromosome breakage test abnormal
Mosaicism from Recombination
Complications

- Aplastic anemia
- Myelodysplastic syndrome
- Acute leukemia
- Solid tumors
- Liver tumors
Definitions

- Aplastic Anemia (AA)
  - Pancytopenia
  - Hypocellular bone marrow
- Myelodysplastic Syndrome (MDS)
  - Cytopenias with hypercellular (or hypocellular) bone marrow
- Acute Leukemia (AL)
  - Malignant proliferation of immature cells
Blood Cytopenias: Signs and Symptoms

- Thrombocytopenia
  - bruises, petechiae
- Anemia
  - fatigue, lassitude, dyspnea
- Neutropenia
  - infections
When to Treat Bone Marrow

- **Cytopenias:**
  - Hb <8 g/dL, Platelets <30,000/mm$^3$, ANC <500/mm$^3$

- **Leukemia:**
  - Blasts in blood; >20% blasts in marrow

- **MDS:**
  - Morphologic + cytopenias: Not for clone alone

- **Pre-emptive:**
  - ????
FA: Treatment for Bone Marrow

- Transplant
- Androgen
- Hematopoietic growth factors
- Gene therapy?
FA: Medical Treatment

- Oxymetholone
  - 2-5 mg/kg/day oral
- Danazol
  - ~200-400 mg/day oral
- Folic acid
  - 1 mg/day oral
FA: Supportive Care

- Red blood cells: Hb <8 g/dl or symptoms
- Platelets: <10,000/mm$^3$ or symptoms
- Blood products
  - No family member donors
  - Leukopoor (filtered), preferably irradiated
- Antibiotics
  - Only as needed for infections
FA: Treatment with Transplant

- Bone marrow, cord blood, or peripheral blood stem cells
- HLA-related donor
  - when meet any treatment criteria
- Alternate donor (mismatched unrelated [MUD], partial match family member)
  - Leukemia or clinical MDS (not clone alone)
  - Refractory aplastic anemia
- Preemptive?
FA Risk of Adverse Events

Alter et al, BJH, 2010
Stem Cell Transplant (SCT)

- How many?
  - 40-50% of people with FA

- Survival?
  - Half are more than 5-10 years beyond SCT
  - Overall 80-90% with current methods

- Does age at SCT matter?
  - Need to do at whatever age meet criteria for SCT.
  - Consider seriously “Preemptive” SCT, because half of those with FA may never need SCT
There is no single answer, perhaps no “right” answer. It depends.

On what does it depend?
Medical Information Sources

The Internet (sometimes)

What you want to hear

Second opinions

Whose advice to follow

Mom

Who to trust

* A good doctor

NY Times Oct 20, 2013
Shared Decision re: SCT

- Communication between physicians and families
- Education by (and of) physicians

Zierhut, Bartels: J Genet Couns 2012
Uncertainty and Medical Decisions

- Subjective perception of ignorance
- Expert opinions conflict
- Evidence is limited
- Risk estimates are imprecise

Survey of NCI FA and SCT

- 178 parents of 126 FA patients
- Sources of uncertainty associated with decision outcomes:
  - **Probability** (future outcome may be random or indeterminate):
    - lower likelihood of SCT
  - **Ambiguity** (due to conflicting expert opinions):
    - greater decision-making difficulty

Decision Analysis

- **Payoff**: What is the patient’s subjective view of the quality of life offered by each outcome?
  - E.g. death = quality 100% undesired.
- **Probability**: What is the likelihood of a specific outcome?
- **Payoff x probability**: Level of risk aversion
188 leukemias and 286 solid tumors in 413/2190 people with FA; 47 had 2-4 cancers.
Relative Risk of Cancer in FA

Data from North American Survey, Germany, Israel, and NCI
Solid tumor or leukemia preceded the diagnosis of FA in 35% of those with these events.
Diagnosis of FA before Cancer

- Aplastic anemia
- Birth defects
- Family history
Diagnosis of FA after Cancer

- Clinical suspicion based on appearance
- Family history
- FA-type cancers, atypically young, no risk factors
- Unrecognized marrow failure
- Absence of marrow involvement (e.g. somatic mosaicism)
Possible Causal Factors for Cancer in FA

- Genetics
- Stem cell transplant - GVHD, XRT
- HPV
- Immunodeficiency
- Tobacco
- Alcohol
- Dental X Rays
- Oral trauma (braces)
Cancer Populations

- FANCD1/BRCA2
- Other FA, HNSCC and Gyn SCC
Hypothetical cumulative incidence curves for SCC expected if the competing risks of non-SCC death could be removed. BMT would increase risk of HNSCC 4-fold, and 16 years earlier. All had GVHD.

Rosenberg et al, Blood 2005
HPV and FA Tumors: Background

- Kutler, JNCI 2003
  - 21 of 25 tumors were HPV16/18+ (19 were HPV16+)
  - 6/7 vulvar; 15/18 HNSCC

- Van Zeeburg, JNCI 2008
  - Two of 21 tumors were HPV16+
  - 0/16 HNSCC, 0/2 esophagus, 2/3 anogenital

- Alter, Intl J Ca 2013
  - One of 9 tumors was HPV16+
  - 0/5 HNSCC, 1/4 Gyn
HPV16/18 Antibodies, Vaccinated FA

- *Prior BMT. ¹, ² 1 or 2, not 3 doses.
- Lines are geometric means of levels in healthy females
- **One male, 12 years post-BMT, had antibody to HPV16 but not 18, 1.2 yrs post-vaccination.
- People with FA achieved levels similar to healthy females.

Alter et al, Vaccine 2014
Role of HPV?

1. None of 5 FA HNSCC tumors had HPV DNA.
2. One of 4 FA Gyn SCC had HPV16 DNA.
3. Antibody levels in unvaccinated people with FA or other IBMFS resembled levels in healthy women.
4. Antibody levels in vaccinated people with FA were generally in the range seen in vaccinated healthy women.
5. People with FA should follow standard HPV vaccine recommendations.
FA: Adult Females

- Late onset of menses (14-16)
- Heavy periods if platelets low
- Early onset of menopause (30s)
- Decreased fertility, but possible
- Increased need for Caesarean sections (due to pre-eclampsia)
- Worsening bone marrow function during pregnancy
- Endocrine problems: cholesterol, thyroid, growth hormone, metabolic syndrome, small pituitary, osteopenia
- Cancer: AML, HNSCC, Vulva, vagina, cervix
FA: Adult Males

- Short stature
- Infertility associated with low sperm count
- Endocrine problems: cholesterol, thyroid, growth hormone, metabolic syndrome, small pituitary, osteopenia
- Cancer
  - AML
  - HNSCC
FA Surveillance/Management

- Every 4-6 months (or more as needed): CBC
- Annual:
  - BM aspirate/biopsy/chromosomes
  - Liver enzymes, chemistries, lipids, thyroid; ultrasound
  - Dental
  - Head and neck exam with nasolaryngoscopy
  - Gyn exam
  - Skin exam
  - Consider esophageal endoscopy?
- HPV vaccine
Transition from Pediatric to Adult Care

- When?
  - Age 18
  - Age 21
  - When leave home for work or college

- Who decides?
  - Those with FA
  - Parents
  - Doctors

- How?
Inherited bone marrow failure syndromes (IBMFS) are rare disorders in which there is usually some form of aplastic anemia (failure of the bone marrow to produce blood), associated with a family history of the same disorder. Some of these conditions have typical changes in physical appearance or in laboratory findings which suggest a specific diagnosis. There are several well-described syndromes, which can be recognized by health care experts. There are also patients who are harder to classify, but who appear to belong in this category.

Patients with these syndromes have a very high risk of development of cancer (either leukemia or certain solid tumors). At the moment, we cannot predict which specific patient with an IBMFS is going to develop cancer. The NCI IBMFS Study will enroll North American families in which at least one member has or had an IBMFS. We plan to:

- include individuals known to have an IBMFS as well as their first degree relatives (brothers, sisters, parents, and children);
- collect clinical information from study participants and their physicians;
- perform detailed physical examinations, x-rays and routine laboratory tests on those who are interested in traveling to the NIH to be seen in person by our team;
- attempt (on a research basis) identification of the specific genetic mutation that is associated with each family's disease;
- screen participants for early changes related to the specific cancers that occur in each syndrome;
- perform detailed research laboratory studies on blood and tumors collected from study participants, in an effort to understand the process by which cancers develop;
- monitor study participants in an ongoing fashion to determine the rate at which complications develop related to each disease, and to identify those complications more precisely;
- provide suggestions to study participants and their physicians regarding how to best take care of family members who are affected with a particular IBMFS, and offer genetic counseling, and an opportunity to learn the results of mutation testing, for those persons who decide that this information will be of use to them.

The Principal Investigator responsible for this study is Blanche P. Alter, MD, MPH. For further information regarding her credentials and experience, please see http://cancer.gov/peopleandorganizers/Alter.html.

Our overall goal is to reach a better understanding of how cancers develop in persons with IBMFS, so that we may improve the health care which can be offered to persons with these disorders.

Clinical Genetics Branch: Neelam Giri, Sharon Savage
Westat: Lisa Leathwood, Maureen Risch, Ann Carr
All the Patients