Registry Protocol

Fanconi Anemia Registry 01

Principal Investigator:

Christian Kratz, M.D.
Hannover Medical School
Pediatric Hematology and Oncology
Carl-Neuberg-Str. 1
30625 Hannover
Germany
Phone: +49 511 532 6712
Fax: +49 511 532 9120
Email: kratz.christian@mh-hannover.de

List of abbreviations

AA	*Aplastic Anemia*
ACIP	*Advisory Committee on Immunization Practices*
AD	*Alternative Donor*
ANC	*Absolute Neutrophil Count*
AL	*Acute Leukemia*
ALL	*Acute Lymphoblastic Leukemia*
AML	*Acute Myeloid Leukemia*
ATG	*Anti-Thymocyte Globulin*
BM	*Bone Marrow*
BMF	*Bone Marrow Failure*
BMFS	*Bone Marrow Failure Syndrome*
CBC	*Complete Blood Count*
CMV	*Cytomegalovirus*
CNS	*Central Nervous System*
CP	*Cisplatin*
CRC	*Coordinating Registry Center*
DEB	*Diepoxybutane*
EFS	*Event-Free Survival*
ENT	*Ears, Nose, and Throat (Otolaryngology)*
FA	*Fanconi Anemia*
FDA	*Food and Drug Administration*
GCP	*Good Clinical Practice*
G-CSF	*Granulocyte Colony-Stimulating Factor*
GEFA02	*German Fanconi Anemia 02*
GEFA03	*German Fanconi Anemia 03*
GH	*Growth Hormone*
GVHD	*Graft Versus Host Disease*
aGVHD	*Acute Graft Versus Host Disease*
cGVHD	*Chronic Graft Versus Host Disease*
HHV6	*Human Herpesvirus 6*
HLA	*Human Leukocyte Antigen*
HPV	*Human Papillomavirus*
HSCT	*Hematopoietic Stem Cell Transplantation*
ICH	*International Conference on Harmonisation*
ID2	*FANCI-FANCD2 Dimer*
IEC	*Independent Ethics Committee*
IFAR	*(NorthAmerican) International FA Registry*
IRB	*Institutional Review Board*
LFTs	*Liver Function Tests*
MCV	*Mean Corpuscular Volume*
MMC	*Mitomycin C*
MDS	*Myelodysplastic Syndrome*
MMRD	*Mismatched Related Donor*
MRD	*Matched Related Donor*
MRI	*Magnetic Resonance Imaging*
MSD	*Matched Sibling Donor*
MUD	*Matched Unrelated Donor*
OS	*Overall Survival*
PBSC	*Peripheral Blood Stem Cell*
RAEB	*Refractory Anemia with Excess Blasts*
RBC	*Red Blood Cell*
SAS	*Statistical Analysis System*
SCC	*Squamous Cell Carcinoma*
SD	*Standard Deviation*
SPSS	*Statistical Package for Social Sciences*
TBI	*Total Body Irradiation*
TRM	*Transplant Related Mortality*
TS	*Thymic Shield*
TSH	*Thyroid Stimulating Hormone*
UCB	*Umbilical Cord Blood*
UCBT	*Umbilical Cord Blood Transplantation*

Table of Contents

Synopsis

Title of the registry	Fanconi Anemia (FA) Registry 01
Protocol No.	FAR01
Design	Natural History Study
Objectives	• To describe the clinical course of FA • To define genetic and environmental modifiers
Registry Population	300-450 patients are expected to enroll
Inclusion Criteria	• Written informed consent • Confirmed diagnosis of FA
Methodology	Central review of diagnostic procedures Regular follow-up (annual)
Statistical Methods	The analysis of survival times and other quantitative and qualitative variables will be completed using suitable descriptive methods. Confidence intervals for all estimates will be computed.
Timetable	Start of registry: January 2014
Principal Investigator	Christian Kratz, M.D.

Flow-chart For details see section 21 (page 37) and section 22 (page 41)

	Registry entry	**Annual follow-up**	**Prior to HSCT**	**Day 100 post HSCT**	**Annually post HSCT**
Medical history	X	X	X	X	X
Physical exam	X	X	X	X	X
Genetic counseling	X				
Chromosomal breakage / G2 arrest	X				
Mutation analysis	X				
ENT	X	X	X		X
Gynecology	X	X	X		X
Endocrinology	X	X	X	X	X
Hematology	X	X	X	X	X
Chemistry	X	X	X	X	X
Virus serology	X		X	X	(X)
Malignancy history	X	X	X	X	X
Transfusion history	X	X	X	X	X
Therapy history	X	X	X	X	X
BM morphology	X	X	X	X[1]	X[1]
BM or PB cytogenetics	X	X	X	X[1]	X[1]
BM FISH	X	X	X	X[1]	X[1]
BM arrayCGH *(optional)*	X	X	X	X[1]	X[1]
Somatic mutation testing (optional)	X	X	X	X	X
Research blood	X	X	X	X	X
Research skin biopsy *(optional)*	X	X			
Allograft data			X	X	X
GVHD			X	X	X
Chimerism			X	X	X

[1]not necessary >12 months after successful stem cell transplantation

Coordinating Registry Center

Principle Investigator and Morphology
Christian Kratz, M.D. Hannover Medical School Pediatric Hematology and Oncology Carl-Neuberg-Str. 1 30625 Hannover Germany Phone: +49 511 532 6712 Fax: +49 511 532 9120 Email: kratz.christian@mh-hannover.de
Registry Coordinator
Christina Dutzmann Cancer predisposition registry study physician Hannover Medical School Pediatric Hematology and Oncology Carl-Neuberg-Str. 1 30625 Hannover Germany Phone: +49 511 532 6738 Fax: +49 511 532 161026 Email: dutzmann.christina@mh-hannover.de Email: FA-GPOH1@mh-hannover.de
Data Manager
Beatrice Hoffmann Hannover Medical School Pediatric Hematology and Oncology Carl-Neuberg-Str. 1 30625 Hannover Germany Phone: +49 511 532 9408 Fax: +49 511 532 161026 Email: hoffmann.beatrice@mh-hannover.de Email: FA-GPOH1@mh-hannover.de
Birte Sänger Hannover Medical School Pediatric Hematology and Oncology Carl-Neuberg-Str. 1 30625 Hannover Germany Phone: +49 511 532 9408 Fax: +49 511 532 161026 Email: saenger.birte@mh-hannover.de Email: FA-GPOH1@mh-hannover.de

The FA Registry Committee

Deutsche Fanconi Anämie Hilfe e.V. / FA Experts
Eunike Velleuer, MD Pediatric Hematology Children's Hospital Neuwerk Dünner Strasse 214-216 41066 Mönchengladbach Germany Phone: +49 2161 668 2451 Email: E.Velleuer@kh-neuwerk.de
Ralf Dietrich, soz.paed.grad. Deutsche Fanconi-Anaemie-Hilfe e.V. Geschaeftsführung Boeckenweg 4 59427 Unna-Siddinghausen Germany Phone: +49 2308 2324 Email: ralf.dietrich@fanconi.de
Pediatric Hematology
Holger Cario, MD Pediatric Hematology/Oncology Department of Pediatrics and Adolescent Medicine University Medical Center Ulm Eythstrasse 24 89075 Ulm Germany Phone: +49 731 500 57219 Email: holger.cario@uniklinik-ulm.de
Pediatric Stem Cell Transplantation
Brigitte Strahm, MD Center of Pediatrics Pediatric Hematology and Oncology Mathildenstraße 1 79106 Freiburg im Breisgau Germany Phone: +49 761 270 46250 Email: brigitte.strahm@uniklinik-freiburg.de
Rita Beier, MD Pediatric Haematology and Oncology Pediatric stem cell transplantation University Hospital Essen Clinic for Pediatrics III Hufelandstr. 55 45122 Essen Germany Phone: +49 201 723 85190 Fax: +49 201 723 947 6775 Email: rita.beier@uk-essen.de

Martin Sauer, MD
Pediatric Haematology and Oncology
Pediatric stem cell transplantation
Hannover Medical School
Carl-Neuberg-Str. 1
30625 Hannover
Germany
Phone: +49 511 532 6716
Email: sauer.martin@mh-hannover.de

Adult Stem Cell Transplantation

Felicitas Thol, MD
Hematology, Hemostasis, Oncology and Stem Cell Transplantation
Hannover Medical School
Carl-Neuberg-Str. 1
30625 Hannover
Germany
Phone: +49 511 532 4018
Email: thol.felicitas@mh-hannover.de

Christian Koenecke, MD
Hematology, Hemostasis, Oncology and Stem Cell Transplantation
Hannover Medical School
Carl-Neuberg-Str. 1
30625 Hannover
Germany
Phone: +49 511 532 3696
Email: koenecke.christian@mh-hannover.de

Matthias Eder, MD
Hematology, Hemostasis, Oncology and Stem Cell Transplantation
Hannover Medical School
Carl-Neuberg-Str. 1
30171 Hannover
Germany
Phone: +49 511 532 9207
Email: eder.matthias@mh-hannover.de

Genetic Diagnosis of FA

Reinhard Kalb, PhD
Department of Human Genetics
University of Würzburg
Biozentrum
Am Hubland
97074 Würzburg
Germany
Phone: +49 931 31 84361
Email: R.Kalb@uni-wuerzburg.de

Detlev Schindler, MD
Department of Human Genetics
University of Würzburg
Biozentrum B362
Am Hubland
97074 Würzburg
Germany
Phone: +49 931 31 88075
Email: schindler@biozentrum.uni-wuerzburg.de

Cytogenetic Analyses
Gudrun Göhring, MD Department of Human Genetics Hannover Medical School Carl-Neuberg-Str. 1 30171 Hannover Germany Phone: +49 511 532 4517 Email: goehring.gudrun@mh-hannover.de
Radiation Therapy
Hans Christiansen, MD Radiation Oncology Hannover Medical School Carl-Neuberg-Str. 1 30171 Hannover Germany Phone: +49 511 532 2574 Email: christiansen.hans@mh-hannover.de
Internal Medicine Tumor Therapy
Philipp Ivanyi, MD Dep. for Hematology, Hemostasis, Oncology and Stem Cell Transplantation Hannover Medical School Carl-Neuberg. St. 1 30625 Hannover Phone: +49-511-532-3140 Email: Ivanyi.philipp@mh-hannover.de
Targeted Therapy
Stefan Fröhling, MD Division of Translational Medical Oncology National Center for Tumor Diseases Heidelberg German Cancer Research Center Im Neuenheimer Feld 460 69120 Heidelberg Germany Phone: +49 6221 56 6990 Email: stefan.froehling@nct-heidelberg.de
Simon Kreutzfeldt, M.D. National Center for Tumor Diseases (NCT) Heidelberg Department of Translational Medical Oncology German Cancer Research Center (DKFZ) Im Neuenheimer Feld 460 D-69120 Heidelberg Germany Phone: +49 6221 56-32990 Email: Simon.Kreutzfeldt@nct-heidelberg.de
Oral and Maxillofacial Surgery
Susanne Sehhati-Chafai-Leuwer, MD, DMD Oral and Maxillofacial Surgery Departement of Plastic Surgery, Hand Surgery Merheim Medical Center Kliniken der Stadt Köln gGmbH University of Witten / Herdecke Ostmerheimer Str. 200 51109 Köln Germany Phone: +49 221 890713561 Email: SehhatiChafaiLeuwerS@kliniken-koeln.de

Gregor Castrillón Oberndorfer, MD, DMD Maxillofacial and Oral Surgery Nuremberg Hospital Breslauer Str. 201 90471 Nürnberg Germany Phone: +49 911 398 5491 Email: Gregor.CastrillonOberndorfer@klinikum-nuernberg.de
Frank Tavassol Oral and Maxillofacial Surgery Hannover Medical School Carl-Neuberg-Str. 1 30171 Hannover Germany Phone: +49 511 532 4753 Email: Tavassol.Frank@MH-Hannover.de
Gynecology
Tjoung-Won Park-Simon Obsterics and Gynecology Hannover Medical School Carl-Neuberg-Strasse 1 30625 Hannover Germany Phone: +49 511 532 9546 E-mail: park-simon.tjoung-won@mh-hannover.de
Cordula Schippert, MD Obsterics and Gynecology Hannover Medical School Carl-Neuberg-Str. 1 30625 Hannover Germany Phone: +49 176-1532 3445 Email: Schippert.Cordula@mh-hannover.de
Sarah Schott, MD University of Heidelberg Im Neuenheimer Feld 440 69120 Heidelberg Germany Phone: +49 6221 56 7901 Email: sarah.schott@med.uni-heidelberg.de
Dermatology
Alexander Kreuter, MD Department of Dermatology, Venereology and Allergology HELIOS St. Elisabeth Hospital Oberhausen University of Witten/Herdecke Josefstr. 3 46045 Oberhausen Germany Phone: +49 208 8508 8001 Email: alexander.kreuter@helios-gesundheit.de
Cytopathology
Martin Schramm, MD Department of Cytopathology Heinrich Heine University Düsseldorf Moorenstr. 5 40225 Düsseldorf Germany Phone: +49 211 811 8346 Email: Martin.Schramm@uniklinik-duesseldorf.de

Hematopathology
Irith Baumann, MD Pathology Sindelfingen-Böblingen Medical Center Bunsenstr. 120 71032 Böblingen Germany Phone: +49 7031 668 22594 Email: I.Baumann@klinikverbund-suedwest.de
Endocrinology
Doris Franke, MD Department for Pediatric Kidney, Liver and Metabolic Diseases Hannover Medical School Carl-Neuberg-Str. 1 D - 30625 Hannover Germany Phone: +49 511 532 3276 Email: franke.doris@mh-hannover.de
Pediatric Surgery
Rainer Nustede, MD Pediatric Surgery Hannover Medical School Carl-Neuberg-Str.1 30171 Hannover Germany Phone: +49 511 532 9053 Email: nustede.rainer@mh-hannover.de
Basic Research / Young Investigators
Miriam Erlacher, MD PhD Division of Pediatric Hematology and Oncology University Medical Center Freiburg Mathildenstr. 1 79106 Freiburg Germany Phone: +49 761 270 43010 Email: miriam.erlacher@uniklinik-freiburg.de
Biobank
Thomas Illig, PhD Department of Human Genetics Hannover Medical School Carl-Neuberg-Str. 1 30625 Hannover Germany Phone: +49 511 532-4526 Mobile: +49 1761 532 7856 Email: Illig.thomas@mh-hannover.de
Clinical Studies
Marena R. Niewisch, MD Pediatric Oncology Hannover Medical School Carl-Neuberg-Str. 1 30625 Hannover Germany Phone: +49 511 532 6711 Email: Niewisch.marena@mh-hannover.de

The following information leans, at least in part, on findings and opinions from bone marrow failure syndrome (BMFS) experts summarized and released by the US parent support organization Fanconi Anemia Research Fund (FARF), Eugene, Oregon, U.S.A., in the Standards-of-Clinical Care, currently in 4th edition (www.fanconi.org).

1. Introduction

Fanconi anemia (FA) is a rare inherited BMFS characterized by congenital and endocrine anomalies, impaired hematopoiesis, and cancer predisposition.[1-6] Guido Fanconi, a Swiss pediatrician, was the first to describe this disorder in three brothers with pancytopenia, short stature, and hypopigmentation in 1927.[7] We now recognize that physical abnormalities are present in 60%-75% of affected individuals and include one or more of the following: short stature, abnormal skin pigmentation, malformations of the thumbs, forearms, skeletal system, eyes, urinary tract, ears, heart, gastrointestinal (GI) system, central nervous system (CNS), hypogonadism, and developmental delay. Progressive bone marrow failure (BMF) with pancytopenia typically presents in the first decade, often initially with thrombocytopenia or leukopenia. By 40 years of age, the estimated cumulative incidence of BMF is 90%.[1] The incidence of hematologic malignancies, primarily myelodysplasic syndrome, MDS/acute myeloid leukemia, AML; rarely acute lymphoblastic leukemia, ALL, or non-Hodgkin-Lymphoma, NHL, is 10%-30%; and of nonhematologic malignancies (particularly squamous cell carcinoma (SCC) of the head and neck, skin, GI tract, and genital tract) is 25%-30%.[1] FA can be clinically and genetically very heterogeneous. Comprehensive and expert care is advised for all patients and families affected by FA.

2. Epidemiology

FA is encountered in all ethnic groups and occurs nearly equally in males and females (ratio of 1.2 to 1).[1] The precise carrier frequency of FA mutations worldwide is unknown. According to recent estimates, the carrier frequency in the US is 1 per 181 persons (range, 156 to 209), leading to an expected birth rate of approximately 1 case per 131,000 individuals.[8] In certain subpopulations such as in sub-Saharan Blacks and South African Afrikaans,[9] the carrier frequency of FA is higher due to founder effects. Among Ashkenazi Jews in the US, the carrier frequency is approximately 1 per 77, leading to a projected birth rate of 1 per 30,000 people.[10]

Spanish gypsies have a carrier frequency of approximately 1 per 70, the highest identified so far in any human population.[11]

The median age at diagnosis in 754 FA patients in the North American International FA registry (IFAR) was 7.6 years.[12] FA individuals with birth defects are identified at a younger age than those without birth defects; most patients will have come to medical attention after development of hematopoietic complications. There is a small subset of FA patients who experience somatic reversion of one of their two germline mutations in at least one hematopoietic stem cell. Corrected progeny then repopulates the hematopoietic system resulting in normal blood and immune cells. Thus, these FA patients have a normal sensitivity of their hematopoietic cells to crosslinking agents and do not experience BMF, but will present in the second or third decade of life with solid tumors and also fertility problems.

3. Molecular pathophysiology

At the cellular level, hypersensitivity to DNA interstrand crosslinking agents (e.g. diepoxybutane, DEB; mitomycin C, MMC; cisplatin, CP) is the defining feature of FA cells.[15] Germline mutations in 23 distinct FA genes (*FANCA-Y*) have been shown to interfere with DNA-replication dependent repair of crosslinked DNA at stalled replication forks.[16] Initially, the FA pathway is activated by FANCM/FAAP24 and then the process amplified by signaling via ATR and the signal further amplified by CHEK1 and other kinases that phosphorylate FANCI, FANCA, FANCG, and others.[17-20]

Through binding of FANCF to FANCM and formation of other subcomplexes, a nuclear core complex of eight FA proteins (FANCA/B/C/E/F/G/L/M) is formed that functionally is a nuclear E3 ubiquitin ligase,[15] which monoubiquitinates FANCI and FANCD2. The two activated proteins FANCI-FANCD2 (ID2), as a dimer, co-localize with other DNA repair proteins such as BRCA1, BRCA2, or RAD51 in chromatin to form a repair focus.[16] Cells with mutations in the eight FA core complex genes and also in *FANCI* lack monoubiquitination of FANCD2, which can be readily detected by Western blot as a single FANCD2 band.[24] These eight genes are considered *early* genes. Other FA genes encode proteins, which are not involved in the monoubiquitination of FANCI and FANCD2. These FA genes include *FANCD1* (*BRCA2*)*, FANCN* (*PALB2*), *FANCJ* (*BRIP1*), *FANCO* (*RAD51C*)*, FANCP* (*SLX4*) *and*

FANCQ (*ERCC4=XPF*).[25] Cells with biallelic defects in these so called *late* genes have normal FANCD2 and FANCI monoubiquitination and FANCI phosphorylation. Although highly simplified as model, products of the *late* genes are thought to work downstream of the ID2 complex and are principally involved in the removal of the DNA crosslinks via the homologous recombination DNA repair pathway. Other FA-associated proteins such as FAAP16, FAAP20, FAAP24, FAAP100, the E2 ligase UBE2T, the de-ubiquitinating enzymes USP1 and UAP1, the nucleases FAN1, MUS81, and ERCC1 also interact with these *bona fide* FA proteins, however, germline mutations in these FA candidate genes have not been encountered thus far in FA patients.[26-28] In heterozygous germline mutation carriers, several late FA genes have also been established as cancers susceptibility genes: *FANCD1*, *FANCJ*, *FANCN*, and *FANCO*. This observation supports the importance of the normal function of these tumor suppressor genes as genomic caretaker genes.

4. Clinical features

The diagnosis of FA is sometimes made because of the presence and recognition of characteristic clinical features (Table 1). It is estimated that 60%-75% of patients with FA will have some congenital physical abnormality.[31] A wide range of malformations has been noted,[31] however, the most frequent physical abnormalities (50%-70%) include skeletal abnormalities, skin pigmentation irregularities, and short stature.[32] A smaller proportion of patients (10%-40%) have other abnormalities involving the head and CNS, eyes, ears, mental retardation, heart, GI tract, renal-urinary tract, and gonads.[32] In 25%-33% of FA patients, no physical anomalies are present at birth or in early childhood. In these patients, the diagnosis of FA is usually established because familial testing or development of BMF and/or other FA complications.[34] In FA patients who experienced a correction of the genetic defect in the hematopoietic system through naturally occurring back mutations leading to mosaicism, the BMF may be mild or absent.[35-37] The same is true for patients with mild phenotypes due to residual function of hypomorphic mutations in their affected FA gene. In these patients, the occurrence of unusually early malignancies and high toxicities after chemotherapy can lead to the diagnosis of FA.[35-37] Clinical manifestation of the disorders can be highly variable between affected individuals belonging to the same FA complementation group, having identical mutations, or even belonging to the same family. Thus, the diagnosis of FA is no longer confined to the pediatric

population but also to patients with mild phenotypes and young adults. Correct and timely diagnosis of FA is important to avoid costly and potentially detrimental medical procedures and treatments and to pursue appropriate care and family planning for both, the patients and their families.

Table 1. Frequency (%) of congenital abnormalities in FA[2]

Anomaly	**%**
Skeletal	71
Skin pigmentation	64
Short stature	63
Eyes	38
Renal and urinary tract	34
Mental retardation	16
Gastrointestinal	14
Cardiac	13
Hearing	11
CNS	8
No obvious abnormalities	29

5. Genotype-phenotype correlation

The majority of FA patients (~85%) have defects in the *early* FA genes (i.e. lacking FANCD2 monoubiqitination). The three most frequently affected genes, *FANCA*, *FANCC,* and *FANCG*, are often clinically indistinguishable, especially in the context of a genetically mixed European background. Genotype-phenotype correlations are especially difficult to identify for *FANCA* deficient patients, as only a few mutational hot spots have been defined in this large gene and the majority of mutations are isolated mutations, often specific to an individual family.[43-48] For *FANCC*, the second most frequently affected gene in North America, however, clear genotype-phenotype correlations have been identified. The IVS5 (formerly IVS4) splice site mutation in the Ashkenazi Jewish population and also exon 14 mutations are associated with more severe phenotypes including higher frequencies of congenital abnormalities, BMF before the age of 5 years, and an absolute need for hematopoietic stem cell transplantation (HSCT) in the first decade of life. In contrast, the European founder mutation in *FANCC*, c.67delG in exon 2, is less severe due to the presence of a second translational start site in exon 2, resulting in a shorter protein with residual function.[51] Mutations in *FANCB*, *FANCF*, and *FANCL* appear to be associated with a more severe phenotype, however, each of these defects has a frequency of 1% or

less.[52-54] Therefore, clear genotype-phenotype correlations for larger numbers of patients have not been established so far.

Patients with biallelic *FANCD2* mutations often have a more severe phenotype.[37] Kalb et al. demonstrated in a cohort of more than 30 FA patients that in every FA-D2 patient cell – independent of the mutation – infinitesimal levels of FANCD2 protein are present from one allele, suggesting that true germline null mutations on both alleles are embryonically lethal.[37] In Germany, a *FANCD2* founder mutation has been identified in individuals of Turkish descent, which appear to confer a more severe phenotype.[37] Mutations in *FANCI* are present in approximately 1-3% of FA patients and at least one of the *FANCI* germline mutations seemed to be hypomorphic, however, no apparent phenotype-genotype correlation has been described.[22]

Clinically, the most severe phenotype is found in FA patients with biallelic germline mutations in the two cancer-associated genes *FANCD1* (*BRCA2*) and its *partner-and-localizer-of-BRCA2*, *PALB2* (*FANCN*)[57-63] Children in these two complementation groups show a high frequency of severe congenital abnormalities and more than 90% of patients die before the age of 7 years due to the appearance of one to four malignancies, predominantly MDS/AML, CNS tumors, nephro- and/or neuroblastomas, often in parallel or in short succession. Due to the exquisite sensitivity of FA patients to alkylator chemotherapy, particularly crosslinking agents such as cisplatin, the prognosis in these patients is often dismal (although unpublished data from Minneapolis suggest that HSCT might be associated with longer survival in approximately 25% of FA patients with mutation of *FANCD1*). Biallelic mutations in the BRCA1-associated helicase gene *FANCJ* seems to be milder, however, very little clinical information is published.[65] Very few patients (1-4 per gene) have been identified so far worldwide with biallelic germline mutations in *FANCM*, *FANCO*, *FANCP*, and *FANCQ* (*ERCC4*).[66-69]

The clinical manifestations between FA patients with the same mutation can be dramatically different, suggesting that additional polymorphisms in modifier genes play a role in the natural course of the disease.[39] For example, the *FANCC* IVS5 splice site mutation is clinically severe in FA Ashkenazi Jews, however mild in Japanese FA patients.[38] Other modifier genes that may influence the FA clinical manifestations include glutathione s-transferase genes (*GSTT1*, *GSTM1*, *GSTP1*) or aldehyde detoxifying enzymes,[70-74].

6. Hematology

The vast majority of FA patients experience hematologic complication ranging from mild cytopenia to severe aplastic anemia (AA), MDS, or acute leukemia (AL). By the age of 40 years, the actuarial risk of developing BMF is 90% and MDS/AL is 10%-30%. The mean age for the onset of the hematological manifestations, predominantly BMF, was 7.6 years and 20% of the patients developed MDS/AL, typically during adolescence and young adulthood.[1] AML is the most common AL phenotype in FA patients. Rarely, cases of acute lymphoblastic leukemia (ALL) or lymphoma occur, usually only in patients with *FANCD1/BRCA2* mutations.

Approximately 17%-25% of FA patients undergo somatic reversion of one of their constitutional germline mutation in long-lived T-cells and more rarely (<5%) in immortal hematopoietic stem cells. This phenomenon leads to genetic mosaicism with corrected and uncorrected cells co-existing in the same individual. The corrected cell over time may repopulate the affected lineage or – in case of stem cells - the entire hematopoietic system with normal cells. In these situations, DNA crosslinker tests for FA in the PB usually are normal and FA diagnosis is obtained via skin fibroblast testing.[81] PB counts may be normal or only mildly depressed and the patients will manifest with other FA complications at an older age (up to 47 years). As described below, in the pre-fludarabine HSCT era, reverted T-cells were considered responsible for the high incidences of graft failure.

Thrombocytopenia and pancytopenia are the most common initial hematopoietic abnormalities encountered in FA patients with BMF. Infrequently, patients have isolated anemia or neutropenia.[84] Approximately 50% of patients with thrombocytopenia progressed to pancytopenia after a median of 3 years.[84] There is often evidence of stressed hematopoiesis with increased MCV, elevated hemoglobin F, and elevated “i” antigen on red blood cells (RBC). Comorbid conditions such as iron deficiency or thalassemia trait may complicate the laboratory findings.

Initial BM evaluation in FA patients generally demonstrates hypocellularity but may also show normal or increased cellularity, particularly in patients with MDS/AML.[84] On morphological evaluation, multilineage dysplasia is commonly observed. Dyserythropoiesis with irregular nuclear contours, budding nuclei, and/or karyorrhexis,

was found in 94% of patients and in 69% of patients, dyserythropoiesis was the only abnormality.[85] In addition, ringed sideroblasts (19%), dysmegakaryopoiesis characterized as small megakaryopoiesis with hypolobulated or nonlobulated nuclei (21%), and dysgranulopoiesis with hypogranular or agranular neutrophils and/or pseudo-Pelger-Huët anomaly (14%) were encountered in a substantial portion of FA patients.[85] In 8% of patients, blasts were observed.[85]

The frequent occurrence of BM dysplasia confounds the diagnosis of MDS in FA patients. The almost universal finding of dyserythropoiesis as the sole abnormality is insufficient for the diagnosis of MDS in FA patients. Likewise, bone marrow hypocellularity per se is not a suitable criterion for MDS in FA patients, especially because cytopenias are usually present at diagnosis.[85] The most reliable morphologic evidence of MDS in FA is an increased blast count.[85]

It is reported that 32%-48% of BM from FA patients harbor cytogenetic abnormalities. The clinical significance of some cytogenetic changes in FA is not clear, since some clones may be transient and some persist for many years without adverse clinical consequences.[87] Recent analysis, however, shows that certain FA associated cytogenetic abnormalities are nonrandom; in the majority of cases, these are unbalanced gains and losses of chromosomal material involving 1q+, 3q+, 7/7q-, and 11q-. Specifically, these abnormalities accounted for more than 75% of FA associated chromosomal aberrations. Gains of 3q were rather characteristic of FA and its presence indicated transformation to MDS/AML.[88] Often this aberration preceded other chromosomal changes.[88] Furthermore, the detection of 3q+ in seemingly sporadic cases of MDS/AML cases should indicate a possible diagnosis of FA.[88] Gain of 1q signified early stages of clonal evolution and often coexisted with 3q+ and other aberrations.[88] However, this aberration is also encountered in morphologically normal BM specimens and in non-FA patients. As in other non-FA patients, chromosomal abnormalities involving -7/7q- correlated with more advanced dysplasia, presence of more complex karyotype, and conferred a poor prognosis.[88] Likewise, 11q- was encountered in 14% of FA bone marrow samples and was associated with evolving MDS/AML and complex karyotype.[88] Recent gene expression profiling of FA-specific gains in the area of common amplification, 3q26-3q29, implicated *EVI1* in the FA leukemogenesis.[85] Cryptic *RUNX1* lesions including translocations, deletions, or

mutations were found in 21% of FA specimens with advanced MDS.[89] The presence of a clonal aberration seems to confer a poor prognosis in FA patients. Patients with clonal cytogenetic abnormalities are at >10-fold higher risk for developing MDS/AML (35% vs. 3%).

7. Management of hematologic complications

The management of the hematologic manifestations of FA includes close monitoring for evidence of BMF or progression to MDS/AML and initiation of supportive care (e.g. transfusions or androgen/cytokine therapy) or more definitive treatment (e.g. HSCT) when appropriate. There are benefits and risks of each approach. In addition, management is individualized based on stability of PB counts, presence of morphologic or cytogenetic abnormalities, and/or presence of high-risk genotypes (e.g. *FANCC IVS5*, *FANCD1/BRCA2*, or *FANCN* mutations).

7.1. Supportive care

Synthetic androgens can improve the peripheral blood counts in a variety of inherited BMF syndromes. The most dramatic responses have been observed in patients with dyskeratosis congenita, however, the majority of FA patients (70%) also have increased blood counts to treatment with androgens.[95] The red blood cells are often the most responsive cells. Depending on the dosage, increases in reticulocytes and hemoglobin generally occur within the first month or two of treatment.[96] Responses in the platelet count and the white cell count seem to be more variable. Platelet counts usually do not rise to normal levels and may take several months of therapy.[97] Responses may be transient and resistance to androgens may develop over the course of years.[96] However, some patients, from various complementation groups and usually with milder clinical manifestations of their FA, will respond well to low daily or even weekly doses of androgens. These patients, in the absence of malignant transformation in hematopoietic stem cells, may never require HSCT.[97]

The only FDA approved synthetic androgen for the treatment of BMF is oxymetholone at a starting dose of 2 mg/kg/day given orally (dose range 2-5 mg/kg/day).[98] Common side effects of oxymetholone administration include virilization, priapism, acne, growth spurt followed by premature closure of growth plates, behavioral changes, liver toxicities such as elevated liver enzymes,

cholestasis, peliosis hepatis (vascular lesion with multiple blood-filled cysts), hepatic tumors, hypertension, and blood lipid changes. Due to the risk of severe side effects, oxymethalone (and also the other androgens) should be slowly tapered as soon as possible after initial response (unless unacceptable side effects mandate a more rapid wean). Over time, the dose should be reduced to the minimal effective dose with acceptable side effects. Oxymetholone is highly androgenic and is especially troublesome for female patients. Moreover, given the toxic effects on the liver by oxymethalone (and other 17-alpha-alkylated androgens), regular liver function tests (LFTs; every 3-6 months) and liver ultrasounds (every 6-12 months) are important.[97-101] Oxymetholone therapy should be tapered if LFTs become abnormal and especially if adenomas develop, however, sudden withdrawal of androgen treatment might result in a drastic decline of the BM output within the next two months and needs to be monitored carefully. In most cases, cessation of androgens leads to regression of the adenomas, however, some adenomas may persist for many years even after discontinuation of androgen therapy.[101] In general, the presence of adenomas is not a contraindication for HSCT. Biopsies are important if there is any suspicion of malignant transformation to adenocarinoma. If no hematological response is achieved within 3-6 months of treatment, oxymetholone (and other androgens) should be discontinued. Some studies of HSCT in FA patients suggest that prior treatment with androgens can negatively impact transplant outcomes.[102-104] However, unpublished data from the German FA patient population does not indicate any negative effect of exposure to androgens prior to transplantation (W. Ebell, Berlin, personal communication).

Newer publications suggests that danazol, another synthetic androgen, which is widely used in women with endometriosis and in children and adolescents with hereditary angioedema at a starting dose of 5 mg/kg/day, might be an effective and well-tolerated treatment option for progressive BMF in FA.[97] Danazol treatment was not associated with a high degree of virilization or liver toxicities and therefore seems highly attractive particularly for female patients. On average in 4 patients, who were treated for 3 years with decreasing doses of danazol, the hemoglobin values rose by more than 4 g/dl and the PB platelets counts reached 68,000/µL.[97]

Granulocyte colony-stimulating factor (G-CSF) can improve the neutrophil count in some individuals and can be employed in the setting of recurrent or serious infections and low (<500/mm^3) absolute neutrophil counts (ANC). Treatment with G-CSF does not improve the output of the hematopoietic system in the BM but rather shifts hematopoietic production to the myeloid/granulocytic lineage.[107] Therefore, the use of G-CSF needs to be evaluated critically if the platelet or the hemoglobin values are already low. A bone marrow aspirate and biopsy is usually performed prior to the initiation of hematopoietic growth factor therapy. The starting dose of G-CSF is 5 µg/kg/day given subcutaneously and may be tapered to the lowest effective dose. Treatment may be discontinued if there is no response after 8 weeks of treatment.[96]

Many FA patients will receive transfusions for low hemoglobin and/or platelet levels. Because frequent transfusions can adversely influence HSCT outcomes, it should be approached with care.[108] RBC transfusion parameters include hemoglobin < 7g/dl or symptomatic anemia. Directed donations from family members are discouraged due to the risk of alloimmunization and possible graft rejection from matched related donor HSCT. Iron overload is a potential risk of frequent RBC transfusions and should be monitored and treated accordingly. Platelet transfusions are indicated in patients with bleeding or undergoing invasive procedures. Single donor units are recommended in order to decrease exposure to multiple donors. Anti-fibrinolytic agents such as Amicar may be useful for mucosal bleeding. Amicar is contraindicated for hematuria. Bleeding precautions such as the avoidance of non-steroidal anti-inflammatory drugs, constipation, at-risk sport activities, and the use of soft toothbrush are good common sense approaches for thrombocytopenia.

Practical surveillance recommendations are given in the appendix.

8. FA and viral infections

Recent *in vitro* studies clearly demonstrated that some human papilloma virus (HPV) strains replicate better in primary cells and cell lines which are deficient in the FA pathways.[110] There are also circumstantial evidences that both the primary manifestation of the BMF as well as further declines in peripheral blood counts and BM cellularity can be unequivocally linked to infections with human cytomegaly virus (CMV), varicella, human herpes virus 6 (HHV6), HPV (see below **10.1**), and

adenoviruses in FA patients.[111-115] Hypotheses for the acute decline in counts include a direct toxic effect of the viruses on the bone marrow stroma, the exquisite hypersensitivity of FA cells against TNFα and IFNγ which are strongly upregulated in viral infections,[116-119] and defects in the immunological responses against virally infected cells in FA patients. Therefore, an important supportive care management seems to be prophylactic vaccinations where available. However, formal proof for this has not been published yet.

9. Hematopoietic stem cell transplantation (HSCT)

At the current time, HSCT is the only curative therapy for the hematologic manifestations of FA. Initial transplants in FA patients utilizing high-dose cyclophosphamide with and without radiation had limited success due excessive organ toxicity, severe graft-versus-host disease (GVHD), and early post HSCT deaths. Gluckman *et al*. showed that extreme hypersensitivity to high-dose alkylators and radiation therapy was an inherent aspect of FA and that the preparative regimen for FA transplants must be modified.[123] Moreover, GVHD after allogeneic HSCT induced severe tissue damage with delayed or absent tissue repair in FA patients.[124] Subsequent lower doses of cyclophosphamide and radiation resulted in improved outcomes in patients with matched sibling donors (MSD).[125] However, this reduced intensity regimen was unsuccessful in sustaining engraftment, particularly in recipients of unrelated HSCT,[125] possibly related to the presence of crosslinker resistant T-cell mosaicism.[78]

Incremental changes to FA HSCT preparative regimens have significantly improved the overall survival and decreased the incidence of GVHD and graft failure. The addition of fludarabine to the preparative regimen clearly enhanced the immunosuppression (especially in mosaic FA patients) without added toxicity, thus improving engraftment.[126-128] *In* or *ex vivo* T-cell depletion of the graft dramatically decreased the incidence of both forms of GVHD.[83] In some regimens, radiation was omitted from the preparative regimen with still excellent outcomes. Today, the survival rate of FA patients undergoing MSD is 70-100% and for alternative donor (AD) transplantations, the overall survival rate is 50-90%.[83]

For FA patients whose hematologic manifestations have been successfully treated with HSCT, there is an increased risk for solid tumors. Long-term follow-up of FA patients post HSCT from the pre-fludarabine era indicate that more than half (53%) of patients develop head and neck carcinomas 15 years later.[124] The major risk factor here seems to be the combination of exposure to radiation and development of GVHD. Current HSCT therapies in FA patients aim to further decrease or eliminate these risk factors to improve short-term and long-term outcomes.[83]

9.1. HLA identical sibling transplants

At the current time, HSCT is the only curative therapy for the hematologic manifestations of FA. Initial transplants in FA patients utilizing high-dose cyclophosphamide with and without radiation had limited success due excessive organ toxicity, severe graft-versus-host disease (GVHD), and early post HSCT deaths. Gluckman *et al*. showed that extreme hypersensitivity to high-dose alkylators and radiation therapy was an inherent aspect of FA and that the preparative regimen for FA transplants must be modified.[123] Moreover, GVHD after allogeneic HSCT induced severe tissue damage with delayed or absent tissue repair in FA patients.[124] Subsequent lower doses of cyclophosphamide and radiation resulted in improved outcomes in patients with matched sibling donors (MSD).[125] However, this reduced intensity regimen was unsuccessful in sustaining engraftment, particularly in recipients of unrelated HSCT,[125] possibly related to the presence of crosslinker resistant T-cell mosaicism.[78]

Incremental changes to FA HSCT preparative regimens have significantly improved the overall survival and decreased the incidence of GVHD and graft failure. The addition of fludarabine to the preparative regimen clearly enhanced the immunosuppression (especially in mosaic FA patients) without added toxicity, thus improving engraftment.[126-128] *In* or *ex vivo* T-cell depletion of the graft dramatically decreased the incidence of both forms of GVHD.[83] In some regimens, radiation was omitted from the preparative regimen with still excellent outcomes. Today, the survival rate of FA patients undergoing MSD is 70-100% and for alternative donor (AD) transplantations, the overall survival rate is 50-90%.[83]

For FA patients whose hematologic manifestations have been successfully treated with HSCT, there is an increased risk for solid tumors. Long-term follow-up of FA patients post HSCT from the pre-fludarabine era indicate that more than half (53%) of patients develop head and neck carcinomas 15 years later.[124] The major risk factor here seems to be the combination of exposure to radiation and development of GVHD. Current HSCT therapies in FA patients aim to further decrease or eliminate these risk factors to improve short-term and long-term outcomes.[83]

9.2. Alternative donor (AD) transplants

Approximately two-thirds of FA patients requiring HSCT will not have a suitable related donor.[130] ADs defined as mismatched related or unrelated donors and umbilical cord blood have become viable options for these patients. Although this type of transplant in FA patients was previously associated with severe complications and low survival rates, there have been significant improvements in outcomes. Initial reports of HSCT in FA patients using matched unrelated donors (MUD) showed a 3-year survival of only 33%,[102] however, newer results approach 50-90%.

Recent abstracts reported at the American Society of Hematology (ASH) 54th Annual Meeting in 2012 represent the most current data from the largest single FA transplant center in the western hemisphere.[109] Between 1994 and 2006, the Minneapolis group treated 127 FA patients with unrelated donor bone marrow or umbilical cord blood (UCB) HSCT using cyclophosphamide, ATG, fludarabine (starting in 1999), and single fraction total body irradiation (TBI) with thymic shielding (TS) (starting in 2003) conditioning regimen. All patients received T-cell depleted grafts after 1994. Neutrophil recovery was twice as likely after fludarabine containing regimens than non-fludarabine containing regimens. Grade II-IV aGVHD was significantly reduced by the use of T-cell depletion (18%) compared to T-cell replete BM or UCB (50% and 38%, respectively: $p<0.01$). Similarly, cGVHD was significantly lower with T-cell deplete BM (9%) versus T-cell replete BM or UCB (25% and 15%, respectively; $p=0.04$). The probability of survival was 61% at 1 year and 54% at 5 years with a median follow-up of 9 years. In a subset of patients (n=10), who had no prior history of transfusions or opportunistic infections and treated with cyclophosphamide, fludarabine, ATG, and total body irradiation with TS, engraftment was 100%, acute and chronic GVHD 17% and 0%, and probability of survival was 92%.[109]

Non-radiation based HSCT for FA using AD from a multicenter trial in North America was reported by Boulad *et al*. at the 2012 ASH meeting.[133] Using the fludarabine, cyclophosphamide, ATG cytoreduction backbone, and substituting busulfan for radiation with T-cell depletion of peripheral blood stem cell graft, 27 patients were enrolled and transplanted with related mismatch or unrelated matched or mismatched donor from June 2009 to July 2012. All 27 evaluable patients engrafted, however, one patient suffered a secondary graft failure. Two patients developed grade I, and one patient developed grade II GVHD. Four of the 27 patients died. With a median follow-up of 7.9 months (range 0.5-37.8 months) 19 of 23 (83%) evaluable patients are alive and well (4 pts too early for outcome analysis).[133]

In a retrospective analysis of risk factors associated with improved outcomes in AD HSCT for FA, Wagner et al.[132] published that the use of fludarabine in the preparative regimen, younger recipient age at HSCT (<10 years), exposure to <20 blood product transfusions, and recipient CMV-negative serostatus correlated with improved survival. In addition, MacMillan reported that any transfusions before HSCT and also development opportunistic infections before HSCT were associated with inferior outcomes.[109] Based on these results, current practices include use of fludarabine containing conditioning regimens in the conjunction with T-cell depletion and earlier referral for transplantation prior to excessive transfusions or development of infections.

9.3. Umbilical cord blood transplantation (UCBT)

UCBT is increasingly becoming a treatment option for patients with both malignant and nonmalignant disorders. In recent years, transplantations utilizing UCB or BM from an unrelated donor have comparable survival results. However, reports comparing UCB and unrelated donor transplants demonstrated a reduction in the frequency and severity of acute and chronic GVHD after UCBT. The largest published report regarding unrelated UCBT in FA patients is a retrospective Eurocord analysis of 93 FA patients by Gluckman *et al*.[108] The overall survival was only 40±5% and the incidences of acute and chronic GVHD were 32% and 16%, respectively.[108] Neutrophil engraftment was 60 days was 60±5%. In multivariate analysis, factors associated with favorable outcomes were the use of fludarabine preparative regimens, high number of cells infused, and negative recipient CMV serology.[108] At

this moment, UCBT is indicated in those FA patients for whom an HLA-A, B, C, and DRB1 allele-matched unrelated volunteer donor cannot be identified, however, more experience is clearly needed.[136]

9.4. Advanced myelodysplastic syndrome and acute myeloid leukemia (MDS and AML)

Treatment of advanced MDS and AML in FA is not well established owing to the small numbers of patients and also the heterogeneity in the underlying FA defect. Whether patients are given pre-transplantation cytoreductive chemotherapy or taken directly to HSCT is dependent on the practices of the treating institution. There was a published report of four FA patients with advanced MDS/AML who received mini-FLAG (fludarabine and cytarabine, Ara-C) by Mehta *et al*.[137] Three of the four patients achieved clearance of the blast cells, and the final patient received additional chemotherapy with subsequent response. All patients tolerated the chemotherapy, however, post transplantation outcomes were not available to determine overall survival outcomes.[137] Other institutions proceed to HSCT directly using a cyclophosphamide, fludarabine, ATG, and 300 cGY TAI preparative regimen.[128] The rationale for employing radiation (or busulphan) being that the cyclophosphamide and fludarabine combination is insufficient to eliminate all malignant cells and thusly there is a high risk for relapse.[128]

9.5. Graft-versus-host disease (GVHD)

GVHD represents a particularly difficult complication for FA patients undergoing HSCT. Due to inherent DNA repair defect and the hypersensitivity to TNF and IFN,[116-119] there is an increased tendency of cells to undergo apoptosis after injury.[138] The historical incidence and severity of aGVHD (45-50%) in FA patients was higher when compared to non-FA patients with aplastic anemia who underwent matched sibling HSCT using similar preparative regimens.[139] Despite being younger at the time of transplant, the relative risk of FA patients for developing grade II-IV aGVHD was 2-fold higher when compared with non-FA counterparts. FA patients more often developed refractory GVHD which required second line therapy and corticosteroid doses >2 mg/kg.[124] In addition, FA patients are also likely to experience toxicities associated with GVHD medications, such as steroid-induced hyperglycemia and nephrotoxicity related to calcineurin inhibitors. The diagnosis of FA also appears to be

a risk factor for development of cGVHD.[124] Remarkably, in the pre-fludarabine area, the incidence of cGVHD in FA patients had approached almost 50%.[139]

GVHD prophylaxis regimens in FA HSCT vary from institution to institution (cyclosporine alone or in combination with methotrexate, steroids and other immunosuppressive agents), however, regimens consisting of cyclosporine alone had the highest incidence of GVHD while the combination of cyclosporine and methotrexate had the lowest GVHD rates.'[139,]

Finally, the development of acute and chronic GVHD was identified as a major risk factor for the development of SCCs of the head and neck region (HNSCCs).[124] The cumulative incidence of HNSCCs in FA patients with GVHD was 20% at 8.3 years post transplantation and 53% by 10 to 15 years.[124] Rosenberg et al. calculated a 4-fold higher incidence of carcinomas in FA patients after HSCT when compared to FA patients who had never received HSCT.[129] In addition, cGVHD was associated with post transplant mortality. Modifications of GVHD prophylaxis regimens and *in vivo* and/or *ex vivo* T-cell depletion have been utilized successfully in order to reduce the incidence and severity of GVHD with good results. Since the introduction of fludarabine into the conditioning regimens, the rates of aGVHD and especially cGVHD have dropped dramatically to less <20 and <10%, respectively. However, methods to reduce the incidence of GVHD, by necessity, resulted in poor and prolonged immune reconstitution and increased risk of infections.[83] The recent development of CD3+ and TCRαβ/CD19-negative depletion strategies of mobilized peripheral stem cell grafts confers a faster immune reconstitution i.e. NK cells recovery[145] that may be beneficial in FA HSCT to reduce opportunistic infections post transplantation. Graft engineering to maximize engraftment and immune reconstitution while minimizing GVHD are currently being developed for FA HSCT. [83]

9.6. Berlin transplant experience

Between October 1999 and October 2012, 36 FA patients have been transplanted at Charité Hospital in Berlin (W. Ebell, personal communication). Protocols GEFA02 (German Fanconi Anemia 02) and later GEFA03 (German Fanconi Anemia 03) were employed. The GEFA02 protocol included fludarabine, busulfan, ATG, and muromonab-CD3 (OKT3). Eighteen patients, ages 2-25 years, average age 11 years,

participated. Fifty-six percent of patients had MDS/AML, 61% had cytogenetic abnormalities, 83% had prior history of androgen administration, and 61% were multiply transfused (i.e. > 20 transfusions). Donors included matched related (MRD) 6%, mismatched related (MMRD) 6%, matched unrelated (MUD) 82%, and mismatched unrelated (MMUD) 6%. Both BM and peripheral stem cells (PBSC) were used and *ex vivo* T-cell depletion was completed for 6% of patients (n=2). All patients engrafted, however 11% had secondary graft failure and required retransplantation. Seventeen percent experienced grade II aGVHD and no patient developed grade III-IV aGVHD; 33% developed limited cGVHD and no patient had extensive cGVHD. The 2-year survival was 61%, and the overall survival 56% at a median of 123 months (range 77-160 months) follow-up with death occurring due to viral infections (n=6), progress of AML (n=1), and late-occurring SCC (n=1) in a high-risk complementation group being the causes of death.

The GEFA03 protocol employed fludarabine, busulfan, cyclophosphamide, and MabCampath (Campath) as alternative immunosuppressive agent. Eighteen patients completed the transplant. The average age of the patients was 11 years (3-19 years). Approximately 33% of patients had MDS/AML, 28% with cytogenetic abnormalities, 67% had received prior androgen therapy, and 61% were multiply transfused. Similar to GEFA02, donors included MRD (11%), MMRD (11%), MUD (50%), and MMUD (28%). Two-thirds (67%) of patient received bone marrow, while 33% received peripheral blood stem cells with *ex vivo* T-cell depletion for mismatched transplants. All patients engrafted and no graft failure occurred. Approximately 22% of patients developed grade I aGVHD and no one developed grade II-IV aGVHD. Only 6 % developed limited cGVHD and no patient experienced extensive cGVHD. So far, no SCC has been observed in this patient group. The 2-year survival is 94% the overall survival is thus far 89% at a mean of 22 months (range 4-82 months) follow-up. Two patients died on this protocol: one from viral infection and one from progression of the underlying clonal disease.

The GEFA03 results reflect the world-wide trend that results for AD transplantation in FA are improving. Despite high-risk patient and transplant characteristics such as the presence of MDS/AML, clonal cytogenetic aberrations, prior androgen use, history of multiple transfusion, and donor-recipient HLA mismatches, outcomes thus far have comparable to the international data. The overall survival is slowly approaching that

of MSD transplants. Nevertheless, the strong immunosuppression resulting in excellent engraftment rates and very low rates of GVHD ≤ grade II is associated with an increased risk for infections that may require additional prophylactic measures and therapies.[83] Long-term data follow-up data of the German transplant patients and the current prospective North American multicenter FA trial will help to shed light on the question whether radiation may also be omitted in AD FA HSCT, given the excellent results acchieved with the use of busulfan instead of irradiation.

10. Solid tumors

The cumulative incidence of solid tumors in FA patients by age 45 years is approximately 30%, and continues to rise with the patient's age. Tumors involving the brain, breasts, lungs, gastrum, colon, kidneys, and bone have been reported, however, SCCs particularly of the head and neck, esophageal, and gynecologic regions are the most frequent.[147] The tumors occur in FA patients at a young age compared to the normal population; therefore the diagnosis of FA should be considered in any unusually young patient with oropharyngeal or anogenital SCC and especially in the absence of risk factors. FA patients who have received HSCT are at higher risk of developing SCCs,[129] particularly SCCs of the tongue which correlated with the presence and severity of GVHD.[148] Liver adenomas (and carcinomas) can also occur and predominantly affect those who have received androgen treatment.[149] In an analysis of German FA Registry, Rosenberg *et al.* evaluated a cohort of 181 patients with FA and reported solid tumors in 10 patients.[150] The ratio of observed to expected cancers was 26 for all solid tumors. Significantly elevated ratios of observed to expected cancers were observed for esophageal (6281), vulvar (2411), head and neck (240), breast (34) and brain (23) tumors.[150]

All cells of FA patients are exquisitely sensitive to DNA cross-linking agents. Chemotherapy agents widely used in sporadic SCCs, such as cisplatin or carboplatin, therefore cannot be used in this patient population due to extremely high toxicities. Organ failure induced even at reduced cisplatin dosing lead to fatal outcomes. Radiotherapy used for local treatment of SCC,[36] is also associated with severe complications in FA patients.[154-156] A recent study reported that one-third of FA patients (four out of 12) died during the course of radiation therapy. Pancytopenia was observed in half of the patients and most also suffered from severe mucositis

and dysphagia.[154] Likewise, in 2002, the outcome for 14 FA patients with SCCs who received radiotherapy was summarized.[157] Cancers in this cohort included 10 head and neck, 3 esophageal, and 1 vaginal SSC. Although the numbers are small, radiation at doses >34 Gray was associated with severe toxicities including mucositis, edema, ulceration, and/or local bleeding in all patients, and only two patients were alive 3 and 10 months after the initial diagnosis of the cancer.[157]

Based on these experiences, the most important therapeutic option in FA patients with SCC is the complete surgical resection of the cancer at an early stage. Surgery can either be performed conventionally or in certain cases by laser resection. Cervical lymph node dissections for patients with head and neck SCC should be added if any lymph node metastasis appears possible, as loco-regional tumor control by other means is more difficult to achieve.[154]

10.1. HPV/P53

Infection with HPV16 virus activates the FA/BRCA pathway in normal cells and increases the genomic instability in FA cells. A genetic cross showed that *Fancd2* deficient mice transgenic for the HPV16 E7 protein have a higher incidence of chemically induced head and neck SCCs compared to the *Fancd2* deficient control animals.[160] In FA patients, however, there is conflicting data regarding the impact of HPV in SCC oncogenesis. Kutler *et al.* detected HPV DNA in 84% of SCC samples from 25 FA patients, compared to only 36% in their non-FA control tumor group.[161] Fifteen of the 18 head and neck SCCs (83%) and six of the seven of the anogential SCCs (86%) were positive for HPV in the FA patient group.[161] Since the HPV E6 protein inhibits p53, they also sequenced *TP53* in the tumors from both groups. No mutations in *TP53* were detected in samples from FA patients compared to 36% of tumors in the control group.[162] These findings in mice[160] and the observations in FA patients support the hypothesis that defects in the FA/BRCA pathway are associated with increased susceptibility to HPV infections and a higher propensity for HPV associated SCCs.[162]

In sharp contrast, van Zeeburg *et al.* did not detect HPV DNA in four head and neck SCC cell lines from FA patients or in seven head and neck SCC cell lines from non-FA patients with sporadic tumors.[163] In addition, *TP53* mutations were detected in all FA head and neck SCC cell lines and in four of the seven cell lines from sporadic

cases. In a follow-up publication, van Zeeburg *et al.* again did not detect HPV DNA in 16 head and neck SCC specimens from FA patients and indirect HPV analysis by p16 immunostaining only showed positive staining in tumors from two of the thirteen analyzed FA patients.[165] *TP53* mutations were detected in the majority (eight out of thirteen) of analyzed patients.[165]

These conflicting findings hinder definitive conclusions regarding the role of HPV infections in SSCs in FA patients. However, it is worth mentioning that the incidence of HPV infection and *TP53* mutations in nonFA patients reported by Kutler *et al.* in their control group of sporadic SCCs[161] is similar to what has been reported by others. In addition, there are other reports of high percentages of HPV infection in FA patients.

Although the impact of HPV in SCC oncogenesis in FA patients has not been fully clarified, standard HPV vaccination for all FA patients may be beneficial. As of September 2011, over 40 million doses of vaccines have been distributed in the US and it is considered safe.[168] HPV vaccination appears to have the greatest effect when administered between 11 to 12 years of age, before any exposure to HPV through sexual contacts and when the immune response is superior.[168] There are two different vaccines available, the quadrivalent Gardasil® manufactured by Merck[169] and the bivalent Cervarix® from GlaxoSmithKline.[168] Both contain recombinant virus-like particles without any viral DNA. While Gardasil® is effective against high-risk oncogenic HPV16 and 18 types as well as low-risk condylomata acuminata (genital warts) HPV6 and 11 strains, Cervarix® includes only HPV16 and 18, but may induce higher antibody titers after standard vaccination. HPV vaccination recommendations were first established for female patients (routine vaccination for 11 or 12 years olds and up to 26 years for those who were not previously vaccinated), however, in 2009, the quadrivalent HPV vaccine was approved by the FDA for use in boys and men to prevent genital warts and anal cancers in which >80% of cases are associated with HPV infection.[168] In October 2011, the Advisory Committee on Immunization Practices (ACIP) in the US recommended routine vaccination with the quadrivalent vaccine in all 11 or 12 years old males and for those who have note been previously vaccinated up to the age of 21 years.[168] Although vaccination of males is associated with significant costs, it is an important opportunity to reduce the spread of HPV from

males to females and to decrease the burden of HPV related diseases in both genders.[168] Based on the exquisite susceptibility of FA cells to HPV infections, the excellent safety profiles of the available vaccines, and the general recommendation to vaccinate 11 or 12 years old children in the US, we consider it important to vaccinate FA patients with the quadrivalent HPV vaccine.

Practical surveillance recommendations are given in the appendix.

10.2 Screening and Prevention for ENT SCCs

The high incidence of head and neck SCCs, combined with the limited therapeutic options for FA patients, underscore the importance of regular and rigorous surveillance and early surgical interventions in order to achieve cure. As two thirds of head and neck SCCs in FA patients are located in the oral cavity,[1] surveillance should ideally be performed by a specialist (e.g. ENT or oral surgeon) and should also include naso-, oro-, and hypopharynx as well as larynx and possibly esophagus, especially in older patients and if there are any signs of reflux or dysphagia. Semiannual examinations of the upper aerodigestive tract and not only the oral cavity, may be indicated as early as 10 to 12 years of age and particularly, if the patient had undergone HSCT. Without prior transplantation, screening may start later at the age of 15 years. Extensive examinations that include endoscopy of the upper aerodigestive tract every 6 to 8 weeks appears necessary in FA patients with leukoplakia and recurrent oral lesions.[161]

Practical surveillance recommendations are given in the appendix.

11. Gynecological treatment/cancers

Female FA patients face a variety of gynecological problems such as structural abnormalities, delayed puberty, decreased fertility, early menopause, and a high risk of SCC of the cervix, vagina, vulva, and anus. Gynecologists caring for FA patients need to have an excellent understanding of the unique health issues in this area associated with FA. In general, the pregnancy rates in untransplanted FA women are in the range of 10%-20%, depending on other manifestations of FA and especially on the hematological parameters. Pregnancies after transplantations have been described however seem to be even less frequently. Pregnancy in a FA woman is

classified as high-risk and menopausal symptoms may present at a young age.[176-181]

Some centers recommend early yearly gynecological evaluation by 12 years of age and annual cervical examinations by age 18 years. Biopsy is encouraged for any suspicious appearing lesions. The role of HPV in FA associated SCCs of the lower genital tract is unclear, however, as outlined above, HPV vaccination in all FA patients seems appropriate given the vaccines' strong safety profiles.

Breast cancer in FA patients with defects in early FA genes usually develops later in life. Without additional damage inflicted by radiation in the conditioning for HSCT, there is not a high propensity of occurrence as in *BRCA1* or *BRCA2* mutation carriers.[1] There is no published/systematic data available on FA patients with defects in the late cancer-associated genes *BRIP1* or *SLX4*.

Practical surveillance recommendations are given in the appendix.

12. Endocrinology

Endocrine abnormalities are present in 73%-80% of FA patients.[182-186] The endocrinopathies may be constitutional, related to comorbid medical conditions, or be influenced treatments for FA such as chronic RBC transfusions, androgen therapy, and/or HSCT. The most commonly observed features are short stature/growth hormone (GH) deficiency (51%), hypothyroidism (37%), abnormal glucose/insulin metabolism including diabetes mellitus (39%), obesity (27%) and dyslipidemia (55%), and osteopenia/osteoprosis (92%).[184] In some pubertal or postpubertal patients, hypogonadism, gonadal dysfunction, and infertility are encountered. FA women may also experience premature menopause.[184] Because of these complex endocrinopathies that may occur in FA patients, a thorough baseline testing and annual endocrine evaluations should be performed.[183]

The average height for a person with FA is 2.1 standard deviations (SD) below normal, translating to an average height of 150 cm for females and 160 cm for males. Yet unidentified genetic factors, hormonal deficiencies, and therapies related to FA contribute to short stature in FA patients. For example, patients with the IVS4 *FANCC* mutations are -4.3 SD below normal in height and the majority of FA patients who are

small for gestational age remain with small stature.[183] Patients with GH and/or thyroid hormone deficiencies also have shorter heights. Androgen therapy may also accelerate growth plate closure leading to shorter adult height. However, it must be noted that almost half of FA patients are of normal height and approximately 10% of FA patients are above average height.

Testing for thyroid hormone and thyroid stimulating hormone (TSH) levels demonstrate borderline or mild hypothyroidism in many FA patients.[182-186] FA children supplemented with thyroid hormone demonstrated improved growth rates. GH testing also shows hypothalamic hypoactivity resulting in partial GH deficiency. Magnetic resonance imaging (MRI) of the brain of FA patients may show midline defects such as absence of the corpus callosum or the septum pellucidum. Pituitary stalk interruption syndrome has also been reported suggesting GH deficiency.[184] Treatment with exogenous GH can increase height, however, there is a paucity of information on published information regarding the long-term safety of exogenous GH replacement in FA.

Glucose metabolism may be perturbed in FA patients and may be related to androgen or corticosteroid therapy, hemosiderosis, and overweight/obesity.[184] It is estimated that approximately 10% of FA patients had subclinical diabetes mellitus.[184] A significant proportion of FA patients also have abnormal lipid profiles. Although the majority of FA patients are underweight, a significant proportion of patients in North America are overweight: Metabolic syndrome described as overweight, dyslipidemia, and insulin resistance was present in 21% of FA patients.[184]

Both premature and delayed puberty have been reported in FA. Hypogonadism, gonadal failure, and infertility also occur. There can be developmental abnormalities of the reproductive organs in FA patients. Both primary gonadal defect and hypothalamic/pituitary dysfunction are thought to be the basis for reduced fertility.[184] In addition, hypogonadism and possibly chronic anemia may be related to FA associated osteopenia/osteoporosis.

Practical surveillance recommendations are given in the appendix.

13. Surgery/correction of congenital abnormalities

Following skin anomalies and short stature, skeletal anomalies constitute the third most frequently encountered anomaly in FA patients (Table 1). The most common problem involves the upper limbs particularly the thumbs and radial border of the forearm. The malformations may be subtle such as mild hypoplasia or dramatic such as complete absence, floating digit, triphalangeal thumb, or duplication. The presence any upper limb anomaly should instigate early referral to an experience upper extremity/hand surgeon for possible surgical treatment. Both surgical and nonsurgical interventions maximize functional outcomes especially when administered early. Indeed, early intervention (i.e. 6-24 months of age) exploits brain plasticity to adapt to new situations, facilitates normal development of gross and fine motor skills, and avoids development of compensatory mechanisms. Importantly, the presence of thumb and/or radial border anomalies warrants an evaluation for FA.

Similar recommendations including early referral to subspecialists, early intervention/treatment, and consideration of a possible diagnosis FA can be made for other congenital anomalies associated with FA. FA patients with hearing, eye, cardiac, intestinal, renal, genital, and other skeletal anomalies benefit from prompt evaluation and management by subspecialists.

14. Registry design

This is a prospective and retrospective natural history registry. We anticipate enrollment of around 30 patients per year. In addition, we will try to also enroll patients already diagnosed with FA in (at least) the last 15 years. Patients who are identified as being eligible according to the inclusion criteria will enter the registry.

15. Participating centers

The pediatric hematology and oncology of Hannover Medical School, Hannover, Germany, is the Coordinating Center for the Fanconi Anemia Registry 01 (FAR01) study. Centers from centers outside of Germany may participate.

16. Registry population

Written informed consent is required for participation. Patients ≥18 years of age will give consent or their legal guardian in case of mental retardation, and for patients ≤17 years of age, their parent(s) or legal guardian(s) must give consent, the patients if adequate.

17. Inclusion criteria

Patients with FA enrolled in this registry are to meet the following inclusion criteria:

- Adult patients: Written informed consent by the patient or legal guardian in case of mental retardation
- Children and adolescents: Written informed consent by the caretakers and whenever possible assent by the patient
- Confirmed diagnosis of FA (no age restriction)

18. Enrollment and patient registration

Enrollment is to start January 1, 2014. Follow-up of the patients is planned for at least 10 years.

19. Objectives

- To describe the clinical course of FA
- To define genetic and environmental modifiers

20. Endpoints

- Development of bone marrow failure
- Development of clonal genetic changes
- Development of cancer or leukemia or myelodysplastic syndromes
- Development of endocrine abnormalities
- Physical anomalies
- Death

21. Documentation of the diagnostic procedures

Prior to registration of a patient, the diagnosis needs to be confirmed (Table 2).

Table 2. Test for patients with suspected FA

Test	**Material**	**Send to established FA lab, e.g.**
G2-arrest/Chrom. Breakage *If positive:* Complementation group and mutation testing	10 ml hep. PB	Dr. Reinhard Kalb Institut für Humangenetik, Biozentrum Am Hubland 97074 Würzburg Tel.: +49 931 31 84361

21.1. Initial procedures in patients with confirmed FA:

Reasons for FA testing and Date of Diagnosis

- **History of FA patient**

  Pregnancy complications, birth mode, birth complication, gestational age at birth, birth weight, birth height, birth head circumference, APGAR, severe previous illnesses (e.g. endocrine abnormalities), cancer (e.g., AML, MDS, head and neck SCC, vulvar/vaginal SCC, cervical SCC, esophageal SCC, brain tumor, nephroblastoma), menses, puberty, surgeries, transfusions, medications, history of gastrointestinal dysplasia, esophageal atresia, duodenal atresia/stenosis, annular pancreas, intestinal atresia, dysplasia of other small or large intestine, rectal atresia, anal atresia, esophagotracheal fistula
- **Family history**

  Ethnicity, Consanguinity, Cancer
- **Vaccination history**

  HPV (type of vaccine, how often?)

  DTPa, Hib, IPV, HepA, HepB, MMR, Pneumococci V, Meningococci V, VZV, FSME, Rotavirus vaccine

Physical exam, including current height, weight, head circumference. Specific anomalies should be ruled out or diagnosed (Table 3**)**

Table 3. Anomalies to be diagnosed or ruled out during initial physical exam

Abnormalities of the head Head dysplasia, microcephaly, macrocephaly, cleft lip or palate, microretrog nathia, macroglossia, high palate, flat nose, low-set ears, low hairline
Abnormalities of the eyes Eye dysplasia, hypertelorism, epicanthus, ptosis, strabismus, blue sclerae, microphthalmia
Abnormalities of the ears Outer ear dysplasia (left and right), middle ear dysplasia (left and right), Inte rior ear dysplasia (left and right)
Abnormalities of the neck Neck dyplasia, short neck, torticollis, pterygium colli
Abnormalities of the skin and nails Skin dysplasia, café-au-lait spots, hyperpigmentation, hypopigmentation, nail dystrophia
Abnormalities of the chest and lungs Mammary dysplasia
Abnormalities of the urogenital system hypogonadism, microgenitosomia
Abnormalities of the extremities Extremities (incl hand/feet) dysplasia, triphalangeal thumb, polydactylia, cleft hand, hypoplasia thumb, thenar hypoplasia, radial aplasia/hypoplasia, toes/foot dysplasia
Abnormalities of the skeletal system Skeletal dysplasia, scoliosis, hypoplasia/aplasia of radius, scapula abduction, hip dysplasia

- **Abdominal ultrasound** (splenic dysplasia, asplenia, accessory spleen, splenic atrophy, renal agenesis, dystopic kidney, dysplastic kidney, horseshoe kidney, double kidney, ureteral dysplasia)
- **Echocardiogram, ECG** (cardiovascular dysplasia, VSD, ASD, tetralogy of Fallot, stenosis/insufficiency of the mitral valve, pulmonary V. stenosis/ insufficiency, aortic isthmus stenosis, pulmonary hypertension)
- **Ophthalmology** (cataract, glaucoma, visual acuity)
- **ENT:** surveillance to include naso-, oro-, and hypopharynx as well as larynx and possibly esophagus, especially in older patients and if there are any signs of reflux or dysphagia. Semiannual examinations of the upper aerodigestive tract and not only the oral cavity, may be indicated as early as 10 to 12 years of age and particularly, if the patient had undergone HSCT. Without prior

transplantation, screening may start later at the age of 15 years.

- **Hearing test**
- **Gynecology:** Female FA patients face a variety of gynecological problems such as structural abnormalities, delayed puberty, decreased fertility, early menopause, and a high risk of SCC of the cervix, vagina, vulva, and anus. Yearly gynecological evaluation by 12 years of age and annual cervical examinations by age 18 years. Biopsy is encouraged for any suspicious appearing lesions.
- **Head MRI** (may be postponed in young children to avoid anesthesia. Use local protocols to screen for brain anomalies)
- **X-ray left hand (**bone age)
- **Other scans performed for clinical indications**

Laboratory tests performed locally:

- **Hematology**
  CBC, Reticulocytes, Differential count, HbF, Leukocyte subsets
- **Endocrinology**
  8:00 am TSH and FT4, oral glucose tolerance test, HbA1c, 25OH vitamin D.
  If growth rate slow: IGF-I, IGFBP3
  If delayed puberty: LH, FSH, Estradiol or Testosterone, DXA (bone density scan)
- **Chemistry**
  GPT, GOT, total bilirubin, AP, albumin, BUN (Blood urea nitrogen), creatinine, cholesterol, HDL, LDL, triglycerides (fasting)
- **Serology**
  CMV, EBV, Hep B, Hep C, HIV, HSV, HHV6, Parvovirus, immunoglobulins G, M, A
- **Urine analysis**

Laboratory tests performed in reference laboratories (Table 4)

Table 4: Laboratory tests performed in reference laboratories (Results can be exchanged with related studies EWOG-SAA, -MDS, AML-BFM)

Test	Material	Send to
BM Pathology	BM biopsy (buffered formalin 4%) PB and BM slides, 1 each	PD Dr. med. Irith Baumann Pathology Böblingen Medical Center Bunsenstr. 120 71032 Böblingen, Germany Tel.: +49 7031 668 2259
PB & BM Morphology	Unstained PB and BM slides, 5 each	Prof. Dr. med. C. Kratz Fanconi Anemia Registry Pediatric Hematology and Oncology Hannover Medical School Carl-Neuberg-Str. 1 30625 Hannover, Germany Tel.: +49 511 532 6711
Oncogenetic studies*	5(-10) ml hep. BM 5 ml EDTA BM	
Research	5 ml hep. PB 5 ml EDTA PB 5-10 ml hep. BM (optional: Skin biopsy)	

*Material will be forwarded to Prof. Brigitte Schlegelberger.

21.2 Documentation of the regular (annual) follow-up

- **History**
- **Family history**
- **Vaccination history**
- **Physical Exam, including height, weight, head circumference**
- **ENT:** surveillance to include naso-, oro-, and hypopharynx as well as larynx and possibly esophagus, especially in older patients and if there are any signs of reflux or dysphagia. Semiannual examinations of the upper aerodigestive tract and not only the oral cavity, may be indicated as early as 10 to 12 years of age and particularly, if the patient had undergone HSCT. Without prior transplantation, screening may start later at the age of 15 years.
- **Gynecology:** Female FA patients face a variety of gynecological problems such as structural abnormalities, delayed puberty, decreased fertility, early menopause, and a high risk of SCC of the cervix, vagina, vulva, and anus. Yearly gynecological evaluation by 12 years of age and annual cervical examinations by age 18 years. Biopsy is encouraged for any suspicious appearing lesions.
- **Laboratory tests performed in reference laboratories** (Table 4)

- **Hematology**
  CBC, Reticulocytes, Differential count, HbF
- **Endocrinology**
  8:00 am TSH and FT4, oral glucose tolerance test, HbA1c, 25OH vitamin D.
  If the growth rate is too slow: AM FT4, TSH, GH, IGF-I, IGFBP3, bone age,
  If GH deficiency (developed de novo): MRI head
  If delayed puberty: LH, FSH, Estradiol or Testosterone, DXA, bone age
- **Chemistry**
  GPT, GOT, total bilirubin, AP, albumin, BUN, creatinine, cholesterol, HDL, LDL, triglycerides (fasting)

21.3. Patients undergoing HSCT

For patients undergoing HSCT, the recorded data corresponds to the HSCT dataset of the GPOH-working group for data management with FA- specific extensions.

22. Data handling and reporting

Data will be entered by participating institutions employing a remote data entry data base. Alternatively, for clinics with low recruitment rate or who do not participate in other studies/registries of the GPOH, paper forms are to be filled which are then entered by the registry center.

22.1. Reporting and recording of data

Follow-up information is required on a 12-monthly basis.

23. Statistical analysis

Overall survival (OS) is defined as the time from birth until last follow-up or event (death from any cause). Event-free survival (EFS) is defined as the time from birth to last follow-up or first event (disease progression, Graft rejection or failure, secondary malignancy, death of any cause). Survival times will be calculated according to the Kaplan-Meier method and comparisons between different patient groups will be performed using the log-rank test. The incidences of transplant-related mortality (TRM), (relapse/disease progression (what would be the definition?), secondary

malignancies and incidence of the development of bone marrow failure, clonal genetic changes and endocrine abnormalities will be calculated according to Kalbfleisch and Prentice. Subgroups will be compared with Gray's test. For multivariate analyses, the Cox proportional hazard regression model will be used.
The analysis of the distribution of qualitative and quantitative variables will be done using suitable descriptive univariate and multivariate methods. Two-sided 95% confidence intervals will be calculated for all estimates.

Statistical analyses will employ the statistical software SPSS (Statistical Package for Social Sciences) and SAS (Statistical Analysis System). All analyses will be documented and saved. The transfer of the data from the study database will be performed after checking the data for plausibility.

24. Changes in protocol

Any change or addition to this protocol requires a written protocol amendment. If an amendment significantly affects the safety of the patients, the scope of the investigation or the scientific quality of the registry, it should be formally approved by the Ethics Committee, and communicated to the regulatory authority, as required by law. After approval, an amendment becomes an integral part of the protocol. The Principal Investigators are authorized to decide the discontinuation of the registry due to relevant medical or administrative reasons.

The above-mentioned requirements do not preclude any immediate action taken by the investigators in the interests of the patients' safety. In the case where such an immediate change to the protocol is implemented and the principal investigators should be notified immediately.

25. Ethical and legal considerations

The study will be conducted in accordance with the Declaration of Helsinki (Appendix 2), the current revision of ICH Topic E6 (Appendix 3), Guideline for GCP: "Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95), and the legal requirements of each participating country in its valid version. It is mandatory that all considerations

regarding the protection of the patients be carried out in accordance with the Declaration of Helsinki. The data protection will be granted according to the local law.

To ensure compliance the investigators agree, by written consent to this protocol, to fully cooperate with compliance checks by allowing access to all documentation by authorized individuals.

26. Patient information and informed consent

In sections 25 and 26, “patient” refers to adult patients and parent(s)/legal guardian(s) of patients who are minors. All patients must sign and personally date an approved informed consent form after receiving detailed written and verbal information about the reason, the nature, and the methods of the registry. The informed consent complies with regulatory requirements. The written informed consent must be obtained before the entry of the patient into the registry.
Furthermore, the patient must be notified that participation is voluntary and that he/she may withdraw from the registry at any time and that withdrawal of consent will not affect his/her right to the most appropriate medical treatment or affect the doctor/patient relationship. A written patient information leaflet will be handed to the patient, whose contents have to be discussed with the patient by the investigator. The investigator will provide the patient ample time and opportunity to inquire about details of the registry and to decide whether or not to participate in the registry. All questions about the registry will be answered to the satisfaction of the patient. The patient should be given sufficient time to read and understand the statement him/herself before signing his/her consent and dating the document. Neither the investigator nor the registry staff will coerce or unduly influence a patient to participate or to continue to participate in the registry. Personal information will be treated as strictly confidential and will not be publicly available. The patient will receive a copy of the written informed consent once signed, and the original version of the informed consent has to be kept in the investigator file.

27. Patient withdrawal

A patient may withdraw from the registry at any time, at his or her own request, for any reason, specified or unspecified, and without penalty or loss of benefits to which the patient is otherwise entitled.

28. Disclosure and confidentiality

Throughout this registry, all data will be treated confidentially. For data recording and analysis, patients will be identified only by a patient identification number and never by their full name, and/or initials. The legal provisions by the respective Laws will be heeded.

The investigators are responsible for keeping sufficient information for every patient (name, date of birth, internal clinic number, patient identification number, gender, informed consent), in order to identify the patient. According to the ICH-GCP-guidelines these documents (Patient Identification List) have to be archived for at least 15 years.

By conducting this registry, the investigators agree that they and their staff will maintain all information in strict confidence. The investigators are requested to insist on similar confidentiality for this information from other bodies such as the Hospital Scientific Committees and Ethic Committees/Institutional Review Boards that have been consulted by the investigator. Registry documents will be stored appropriately to further ensure their confidentiality. It is understood that the confidential information provided to the investigators will not be disclosed to others without direct written authorization from the patient and/or his/her family. Such information will not be communicated by telephone to potential or enrolled patients or to any other individual.

29. Independent Ethics Committee (IEC) / Institutional Review Board (IRB)

Prior to implementation of this registry, the protocol, patient information forms and the proposed informed consent must be reviewed and approved by the Independent Ethics Committee (IEC)/Institutional Review Board (IRB). Signed and dated approval by the IEC/IRB must be obtained by prior to registry initiation and patient enrollment. The investigators are committed in accordance with local requirements to inform the IEC/IRB of any emergent problem and/or protocol amendments.

30. Insurance

The aim of this registry is the collection of epidemiological data based on a standardized diagnostic approach and not the investigation of clinical or pharmacological properties of drugs. The registry is therefore exempt from clinical trials insurance coverage according to law. Patients are covered by the public liability insurance of their hospitals.

31. References

1 Kutler, D. I. *et al.* A 20-year perspective on the International Fanconi Anemia Registry (IFAR). *Blood* **101**, 1249-1256, doi:10.1182/blood-2002-07-2170 (2003).

2 Tischkowitz, M. & Dokal, I. Fanconi anaemia and leukaemia - clinical and molecular aspects. *British journal of haematology* **126**, 176-191, doi:10.1111/j.1365-2141.2004.05023.x (2004).

3 Bagby, G. C. & Alter, B. P. Fanconi anemia. *Seminars in hematology* **43**, 147-156, doi:10.1053/j.seminhematol.2006.04.005 (2006).

4 Green, A. M. & Kupfer, G. M. Fanconi anemia. *Hematology/oncology clinics of North America* **23**, 193-214, doi:10.1016/j.hoc.2009.01.008 (2009).

5 Shimamura, A. & Alter, B. P. Pathophysiology and management of inherited bone marrow failure syndromes. *Blood reviews* **24**, 101-122, doi:10.1016/j.blre.2010.03.002 (2010).

6 Parikh, S. & Bessler, M. Recent insights into inherited bone marrow failure syndromes. *Current opinion in pediatrics* **24**, 23-32, doi:10.1097/MOP.0b013e32834eca77 (2012).

7 Fanconi, G. Familial constitutional panmyelocytopathy, Fanconi's anemia (F.A.). I. Clinical aspects. *Seminars in hematology* **4**, 233-240 (1967).

8 Rosenberg, P. S., Tamary, H. & Alter, B. P. How high are carrier frequencies of rare recessive syndromes? Contemporary estimates for Fanconi Anemia in the United States and Israel. *American journal of medical genetics. Part A* **155A**, 1877-1883, doi:10.1002/ajmg.a.34087 (2011).

9 Tipping, A. J. *et al.* Molecular and genealogical evidence for a founder effect in Fanconi anemia families of the Afrikaner population of South Africa. *Proceedings of the National Academy of Sciences of the United States of America* **98**, 5734-5739, doi:10.1073/pnas.091402398 (2001).

10 Fares, F. *et al.* Carrier frequency of autosomal-recessive disorders in the Ashkenazi Jewish population: should the rationale for mutation choice for screening be reevaluated? *Prenatal diagnosis* **28**, 236-241, doi:10.1002/pd.1943 (2008).

11 Callen, E. *et al.* A common founder mutation in FANCA underlies the world's highest prevalence of Fanconi anemia in Gypsy families from Spain. *Blood* **105**, 1946-1949, doi:10.1182/blood-2004-07-2588 (2005).

12 Alter, B. P., Joenje, H., Oostra, A. B. & Pals, G. Fanconi anemia: adult head and neck cancer and hematopoietic mosaicism. *Archives of otolaryngology--head & neck surgery* **131**, 635-639, doi:10.1001/archotol.131.7.635 (2005).

13 Waisfisz, Q. *et al.* Spontaneous functional correction of homozygous fanconi anaemia alleles reveals novel mechanistic basis for reverse mosaicism. *Nature genetics* **22**, 379-383, doi:10.1038/11956 (1999).
14 Gross, M. *et al.* Reverse mosaicism in Fanconi anemia: natural gene therapy via molecular self-correction. *Cytogenetic and genome research* **98**, 126-135, doi:69805 (2002).
15 Auerbach, A. D. & Wolman, S. R. Susceptibility of Fanconi's anaemia fibroblasts to chromosome damage by carcinogens. *Nature* **261**, 494-496 (1976).
16 Knipscheer, P. *et al.* The Fanconi anemia pathway promotes replication-dependent DNA interstrand cross-link repair. *Science* **326**, 1698-1701, doi:10.1126/science.1182372 (2009).
17 Qiao, F. *et al.* Phosphorylation of fanconi anemia (FA) complementation group G protein, FANCG, at serine 7 is important for function of the FA pathway. *The Journal of biological chemistry* **279**, 46035-46045, doi:10.1074/jbc.M408323200 (2004).
18 Ishiai, M. *et al.* FANCI phosphorylation functions as a molecular switch to turn on the Fanconi anemia pathway. *Nature structural & molecular biology* **15**, 1138-1146, doi:10.1038/nsmb.1504 (2008).
19 Wilson, J. B. *et al.* FANCG promotes formation of a newly identified protein complex containing BRCA2, FANCD2 and XRCC3. *Oncogene* **27**, 3641-3652, doi:10.1038/sj.onc.1211034 (2008).
20 Collins, N. B. *et al.* ATR-dependent phosphorylation of FANCA on serine 1449 after DNA damage is important for FA pathway function. *Blood* **113**, 2181-2190, doi:10.1182/blood-2008-05-154294 (2009).
21 Garcia-Higuera, I. *et al.* Interaction of the Fanconi anemia proteins and BRCA1 in a common pathway. *Molecular cell* **7**, 249-262 (2001).
22 Smogorzewska, A. *et al.* Identification of the FANCI protein, a monoubiquitinated FANCD2 paralog required for DNA repair. *Cell* **129**, 289-301, doi:10.1016/j.cell.2007.03.009 (2007).
23 Timmers, C. *et al.* Positional cloning of a novel Fanconi anemia gene, FANCD2. *Molecular cell* **7**, 241-248 (2001).
24 Shimamura, A. *et al.* A novel diagnostic screen for defects in the Fanconi anemia pathway. *Blood* **100**, 4649-4654, doi:10.1182/blood-2002-05-1399 (2002).
25 Hays, L. E. Multifunctionality of the FA pathway. *Blood* **121**, 3-4, doi:10.1182/blood-2012-11-464636 (2013).
26 Liu, T., Ghosal, G., Yuan, J., Chen, J. & Huang, J. FAN1 acts with FANCI-FANCD2 to promote DNA interstrand cross-link repair. *Science* **329**, 693-696, doi:10.1126/science.1192656 (2010).
27 MacKay, C. *et al.* Identification of KIAA1018/FAN1, a DNA repair nuclease recruited to DNA damage by monoubiquitinated FANCD2. *Cell* **142**, 65-76, doi:10.1016/j.cell.2010.06.021 (2010).
28 Smogorzewska, A. *et al.* A genetic screen identifies FAN1, a Fanconi anemia-associated nuclease necessary for DNA interstrand crosslink repair. *Molecular cell* **39**, 36-47, doi:10.1016/j.molcel.2010.06.023 (2010).
29 Rahman, N. & Scott, R. H. Cancer genes associated with phenotypes in monoallelic and biallelic mutation carriers: new lessons from old players. *Human molecular genetics* **16 Spec No 1**, R60-66, doi:10.1093/hmg/ddm026 (2007).

30 Meindl, A. *et al.* Germline mutations in breast and ovarian cancer pedigrees establish RAD51C as a human cancer susceptibility gene. *Nature genetics* **42**, 410-414, doi:10.1038/ng.569 (2010).
31 Auerbach, A. D. Fanconi anemia and its diagnosis. *Mutation research* **668**, 4-10, doi:10.1016/j.mrfmmm.2009.01.013 (2009).
32 Tischkowitz, M. D. & Hodgson, S. V. Fanconi anaemia. *Journal of medical genetics* **40**, 1-10 (2003).
33 Glanz, A. & Fraser, F. C. Spectrum of anomalies in Fanconi anaemia. *Journal of medical genetics* **19**, 412-416 (1982).
34 Pinto, F. O. *et al.* Diagnosis of Fanconi anemia in patients with bone marrow failure. *Haematologica* **94**, 487-495, doi:10.3324/haematol.13592 (2009).
35 Mankad, A. *et al.* Natural gene therapy in monozygotic twins with Fanconi anemia. *Blood* **107**, 3084-3090, doi:10.1182/blood-2005-07-2638 (2006).
36 Huck, K. *et al.* Delayed diagnosis and complications of Fanconi anaemia at advanced age--a paradigm. *British journal of haematology* **133**, 188-197, doi:10.1111/j.1365-2141.2006.05998.x (2006).
37 Kalb, R. *et al.* Hypomorphic mutations in the gene encoding a key Fanconi anemia protein, FANCD2, sustain a significant group of FA-D2 patients with severe phenotype. *American journal of human genetics* **80**, 895-910, doi:10.1086/517616 (2007).
38 Futaki, M. *et al.* The IVS4 + 4 A to T mutation of the fanconi anemia gene FANCC is not associated with a severe phenotype in Japanese patients. *Blood* **95**, 1493-1498 (2000).
39 Neveling, K., Endt, D., Hoehn, H. & Schindler, D. Genotype-phenotype correlations in Fanconi anemia. *Mutation research* **668**, 73-91, doi:10.1016/j.mrfmmm.2009.05.006 (2009).
40 Shimamura, A. Inherited bone marrow failure syndromes: molecular features. *Hematology / the Education Program of the American Society of Hematology. American Society of Hematology. Education Program*, 63-71, doi:10.1182/asheducation-2006.1.63 (2006).
41 Alter, B. P. Bone marrow failure: a child is not just a small adult (but an adult can have a childhood disease). *Hematology / the Education Program of the American Society of Hematology. American Society of Hematology. Education Program*, 96-103, doi:10.1182/asheducation-2005.1.96 (2005).
42 Faivre, L. *et al.* Association of complementation group and mutation type with clinical outcome in fanconi anemia. European Fanconi Anemia Research Group. *Blood* **96**, 4064-4070 (2000).
43 Lo Ten Foe, J. R. *et al.* Expression cloning of a cDNA for the major Fanconi anaemia gene, FAA. *Nature genetics* **14**, 320-323, doi:10.1038/ng1196-320 (1996).
44 Morgan, N. V., Tipping, A. J., Joenje, H. & Mathew, C. G. High frequency of large intragenic deletions in the Fanconi anemia group A gene. *American journal of human genetics* **65**, 1330-1341, doi:10.1086/302627 (1999).
45 Tachibana, A. *et al.* The FANCA gene in Japanese Fanconi anemia: reports of eight novel mutations and analysis of sequence variability. *Human mutation* **13**, 237-244, doi:10.1002/(SICI)1098-1004(1999)13:3<237::AID-HUMU8>3.0.CO;2-F (1999).
46 Tamary, H. *et al.* Fanconi anaemia group A (FANCA) mutations in Israeli non-Ashkenazi Jewish patients. *British journal of haematology* **111**, 338-343 (2000).

47 Bouchlaka, C. *et al.* Fanconi anemia in Tunisia: high prevalence of group A and identification of new FANCA mutations. *Journal of human genetics* **48**, 352-361, doi:10.1007/s10038-003-0037-z (2003).
48 Levran, O. *et al.* Spectrum of sequence variations in the FANCA gene: an International Fanconi Anemia Registry (IFAR) study. *Human mutation* **25**, 142-149, doi:10.1002/humu.20125 (2005).
49 Gillio, A. P., Verlander, P. C., Batish, S. D., Giampietro, P. F. & Auerbach, A. D. Phenotypic consequences of mutations in the Fanconi anemia FAC gene: an International Fanconi Anemia Registry study. *Blood* **90**, 105-110 (1997).
50 Verlander, P. C. *et al.* Carrier frequency of the IVS4 + 4 A-->T mutation of the Fanconi anemia gene FAC in the Ashkenazi Jewish population. *Blood* **86**, 4034-4038 (1995).
51 Kupfer, G. M. & D'Andrea, A. D. The effect of the Fanconi anemia polypeptide, FAC, upon p53 induction and G2 checkpoint regulation. *Blood* **88**, 1019-1025 (1996).
52 Meetei, A. R. *et al.* X-linked inheritance of Fanconi anemia complementation group B. *Nature genetics* **36**, 1219-1224, doi:10.1038/ng1458 (2004).
53 McCauley, J. *et al.* X-linked VACTERL with hydrocephalus syndrome: further delineation of the phenotype caused by FANCB mutations. *American journal of medical genetics. Part A* **155A**, 2370-2380, doi:10.1002/ajmg.a.33913 (2011).
54 Umana, L. A., Magoulas, P., Bi, W. & Bacino, C. A. A male newborn with VACTERL association and Fanconi anemia with a FANCB deletion detected by array comparative genomic hybridization (aCGH). *American journal of medical genetics. Part A* **155A**, 3071-3074, doi:10.1002/ajmg.a.34296 (2011).
55 Sims, A. E. *et al.* FANCI is a second monoubiquitinated member of the Fanconi anemia pathway. *Nature structural & molecular biology* **14**, 564-567, doi:10.1038/nsmb1252 (2007).
56 Colnaghi, L. *et al.* Patient-derived C-terminal mutation of FANCI causes protein mislocalization and reveals putative EDGE motif function in DNA repair. *Blood* **117**, 2247-2256, doi:10.1182/blood-2010-07-295758 (2011).
57 Howlett, N. G. *et al.* Biallelic inactivation of BRCA2 in Fanconi anemia. *Science* **297**, 606-609, doi:10.1126/science.1073834 (2002).
58 Offit, K. *et al.* Shared genetic susceptibility to breast cancer, brain tumors, and Fanconi anemia. *Journal of the National Cancer Institute* **95**, 1548-1551 (2003).
59 Wagner, J. E. *et al.* Germline mutations in BRCA2: shared genetic susceptibility to breast cancer, early onset leukemia, and Fanconi anemia. *Blood* **103**, 3226-3229, doi:10.1182/blood-2003-09-3138 (2004).
60 Hirsch, B. *et al.* Association of biallelic BRCA2/FANCD1 mutations with spontaneous chromosomal instability and solid tumors of childhood. *Blood* **103**, 2554-2559, doi:10.1182/blood-2003-06-1970 (2004).
61 Alter, B. P., Rosenberg, P. S. & Brody, L. C. Clinical and molecular features associated with biallelic mutations in FANCD1/BRCA2. *Journal of medical genetics* **44**, 1-9, doi:10.1136/jmg.2006.043257 (2007).
62 Kopic, S. *et al.* Hepatoblastoma in a 4-year-old girl with Fanconi anaemia. *Acta Paediatr* **100**, 780-783, doi:10.1111/j.1651-2227.2010.02116.x (2011).
63 Myers, K. *et al.* The clinical phenotype of children with Fanconi anemia caused by biallelic FANCD1/BRCA2 mutations. *Pediatric blood & cancer* **58**, 462-465, doi:10.1002/pbc.23168 (2012).

64 Reid, S. *et al.* Biallelic mutations in PALB2 cause Fanconi anemia subtype FA-N and predispose to childhood cancer. *Nature genetics* **39**, 162-164, doi:10.1038/ng1947 (2007).
65 Levitus, M. *et al.* The DNA helicase BRIP1 is defective in Fanconi anemia complementation group J. *Nature genetics* **37**, 934-935, doi:10.1038/ng1625 (2005).
66 Meetei, A. R. *et al.* A human ortholog of archaeal DNA repair protein Hef is defective in Fanconi anemia complementation group M. *Nature genetics* **37**, 958-963, doi:10.1038/ng1626 (2005).
67 Kim, Y. *et al.* Mutations of the SLX4 gene in Fanconi anemia. *Nature genetics* **43**, 142-146, doi:10.1038/ng.750 (2011).
68 Vaz, F. *et al.* Mutation of the RAD51C gene in a Fanconi anemia-like disorder. *Nature genetics* **42**, 406-409, doi:10.1038/ng.570 (2010).
69 Schuster, B. *et al.* Whole exome sequencing reveals uncommon mutations in the recently identified Fanconi anemia gene SLX4/FANCP. *Human mutation* **34**, 93-96, doi:10.1002/humu.22221 (2013).
70 Cumming, R. C. *et al.* Fanconi anemia group C protein prevents apoptosis in hematopoietic cells through redox regulation of GSTP1. *Nature medicine* **7**, 814-820, doi:10.1038/89937 (2001).
71 Davies, S. M. *et al.* GST genotype may modify clinical phenotype in patients with Fanconi anaemia. *British journal of haematology* **131**, 118-122, doi:10.1111/j.1365-2141.2005.05721.x (2005).
72 Langevin, F., Crossan, G. P., Rosado, I. V., Arends, M. J. & Patel, K. J. Fancd2 counteracts the toxic effects of naturally produced aldehydes in mice. *Nature* **475**, 53-58, doi:10.1038/nature10192 (2011).
73 Rosado, I. V., Langevin, F., Crossan, G. P., Takata, M. & Patel, K. J. Formaldehyde catabolism is essential in cells deficient for the Fanconi anemia DNA-repair pathway. *Nature structural & molecular biology* **18**, 1432-1434, doi:10.1038/nsmb.2173 (2011).
74 Garaycoechea, J. I. *et al.* Genotoxic consequences of endogenous aldehydes on mouse haematopoietic stem cell function. *Nature* **489**, 571-575, doi:10.1038/nature11368 (2012).
75 Soulier, J. Fanconi anemia. *Hematology / the Education Program of the American Society of Hematology. American Society of Hematology. Education Program* **2011**, 492-497, doi:10.1182/asheducation-2011.1.492 (2011).
76 Alter, B. P. The association between FANCD1/BRCA2 mutations and leukaemia. *British journal of haematology* **133**, 446-448; author reply 448, doi:10.1111/j.1365-2141.2006.06049.x (2006).
77 Lo Ten Foe, J. R. *et al.* Somatic mosaicism in Fanconi anemia: molecular basis and clinical significance. *European journal of human genetics : EJHG* **5**, 137-148 (1997).
78 MacMillan, M. L. *et al.* Haematopoietic cell transplantation in patients with Fanconi anaemia using alternate donors: results of a total body irradiation dose escalation trial. *British journal of haematology* **109**, 121-129 (2000).
79 Gregory, J. J., Jr. *et al.* Somatic mosaicism in Fanconi anemia: evidence of genotypic reversion in lymphohematopoietic stem cells. *Proceedings of the National Academy of Sciences of the United States of America* **98**, 2532-2537, doi:10.1073/pnas.051609898 (2001).
80 Soulier, J. *et al.* Detection of somatic mosaicism and classification of Fanconi anemia patients by analysis of the FA/BRCA pathway. *Blood* **105**, 1329-1336, doi:10.1182/blood-2004-05-1852 (2005).

81 Hoehn, H. in *Monogr Hum Genet* Vol. 15 149-172 (Karger, 2007).
82 May, M. Mutations to the rescue. *Nature medicine* **17**, 405-407, doi:10.1038/nm0411-405 (2011).
83 MacMillan, M. L., Hughes, M. R., Agarwal, S. & Daley, G. Q. Cellular therapy for fanconi anemia: the past, present, and future. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation* **17**, S109-114, doi:10.1016/j.bbmt.2010.11.027 (2011).
84 Butturini, A. *et al.* Hematologic abnormalities in Fanconi anemia: an International Fanconi Anemia Registry study. *Blood* **84**, 1650-1655 (1994).
85 Cioc, A. M., Wagner, J. E., MacMillan, M. L., DeFor, T. & Hirsch, B. Diagnosis of myelodysplastic syndrome among a cohort of 119 patients with fanconi anemia: morphologic and cytogenetic characteristics. *American journal of clinical pathology* **133**, 92-100, doi:10.1309/AJCP7W9VMJENZOVG (2010).
86 Alter, B. P. *et al.* Fanconi anemia: myelodysplasia as a predictor of outcome. *Cancer genetics and cytogenetics* **117**, 125-131 (2000).
87 Alter, B. P., Scalise, A., McCombs, J. & Najfeld, V. Clonal chromosomal abnormalities in Fanconi's anaemia: what do they really mean? *British journal of haematology* **85**, 627-630 (1993).
88 Meyer, S., Neitzel, H. & Tonnies, H. Chromosomal aberrations associated with clonal evolution and leukemic transformation in fanconi anemia: clinical and biological implications. *Anemia* **2012**, 349837, doi:10.1155/2012/349837 (2012).
89 Quentin, S. *et al.* Myelodysplasia and leukemia of Fanconi anemia are associated with a specific pattern of genomic abnormalities that includes cryptic RUNX1/AML1 lesions. *Blood* **117**, e161-170, doi:10.1182/blood-2010-09-308726 (2011).
90 Tonnies, H. *et al.* Clonal chromosomal aberrations in bone marrow cells of Fanconi anemia patients: gains of the chromosomal segment 3q26q29 as an adverse risk factor. *Blood* **101**, 3872-3874, doi:10.1182/blood-2002-10-3243 (2003).
91 Alter, B. P. Diagnosis, genetics, and management of inherited bone marrow failure syndromes. *Hematology / the Education Program of the American Society of Hematology. American Society of Hematology. Education Program*, 29-39, doi:10.1182/asheducation-2007.1.29 (2007).
92 Smith, A. R. & Wagner, J. E. Current clinical management of Fanconi anemia. *Expert review of hematology* **5**, 513-522, doi:10.1586/ehm.12.48 (2012).
93 Dokal, I. Inherited aplastic anaemia. *The hematology journal : the official journal of the European Haematology Association / EHA* **4**, 3-9, doi:10.1038/sj.thj.6200215 (2003).
94 Dokal, I. & Vulliamy, T. Inherited aplastic anaemias/bone marrow failure syndromes. *Blood reviews* **22**, 141-153, doi:10.1016/j.blre.2007.11.003 (2008).
95 Shukla, P., Ghosh, K. & Vundinti, B. R. Current and emerging therapeutic strategies for Fanconi anemia. *The HUGO Journal* **6** (2012).
96 Alter, B. P. & Kupfer, G. in *GeneReviews* (eds R. A. Pagon *et al.*) (1993).
97 Scheckenbach, K. *et al.* Treatment of the bone marrow failure in Fanconi anemia patients with danazol. *Blood cells, molecules & diseases* **48**, 128-131, doi:10.1016/j.bcmd.2011.11.006 (2012).
98 Velazquez, I. & Alter, B. P. Androgens and liver tumors: Fanconi's anemia and non-Fanconi's conditions. *American journal of hematology* **77**, 257-267, doi:10.1002/ajh.20183 (2004).

99 Shahidi, N. T. & Diamond, L. K. Testosterone-induced remission in aplastic anemia of both acquired and congenital types. Further observations in 24 cases. *The New England journal of medicine* **264**, 953-967 (1961).

100 Shahidi, N. T. Aplastic anaemia: aetiological and therapeutic dilemmas. *British journal of haematology* **43**, 163-165 (1979).

101 Pavlatos, A. M., Fultz, O., Monberg, M. J., Vootkur, A. & Pharmd. Review of oxymetholone: a 17alpha-alkylated anabolic-androgenic steroid. *Clinical therapeutics* **23**, 789-801; discussion 771 (2001).

102 Guardiola, P. *et al.* Outcome of 69 allogeneic stem cell transplantations for Fanconi anemia using HLA-matched unrelated donors: a study on behalf of the European Group for Blood and Marrow Transplantation. *Blood* **95**, 422-429 (2000).

103 Pasquini, R. *et al.* HLA-matched sibling hematopoietic stem cell transplantation for fanconi anemia: comparison of irradiation and nonirradiation containing conditioning regimens. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation* **14**, 1141-1147, doi:10.1016/j.bbmt.2008.06.020 (2008).

104 Chaudhury, S. *et al.* Fludarabine-based cytoreductive regimen and T-cell-depleted grafts from alternative donors for the treatment of high-risk patients with Fanconi anaemia. *British journal of haematology* **140**, 644-655, doi:10.1111/j.1365-2141.2007.06975.x (2008).

105 Zeidler, C. & Welte, K. Hematopoietic growth factors for the treatment of inherited cytopenias. *Seminars in hematology* **44**, 133-137, doi:10.1053/j.seminhematol.2007.04.003 (2007).

106 Dufour, C. & Svahn, J. Fanconi anaemia: new strategies. *Bone marrow transplantation* **41 Suppl 2**, S90-95, doi:10.1038/bmt.2008.63 (2008).

107 Croop, J. M. *et al.* Mobilization and collection of peripheral blood CD34+ cells from patients with Fanconi anemia. *Blood* **98**, 2917-2921 (2001).

108 Gluckman, E. *et al.* Results of unrelated cord blood transplant in fanconi anemia patients: risk factor analysis for engraftment and survival. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation* **13**, 1073-1082, doi:10.1016/j.bbmt.2007.05.015 (2007).

109 MacMillan, M. *et al.* Alternative Donor Hematopoietic Cell Transplantation for Patients with Fanconi Anemia. *Blood* (2012).

110 Hoskins, E. E. *et al.* The fanconi anemia pathway limits human papillomavirus replication. *Journal of virology* **86**, 8131-8138, doi:10.1128/JVI.00408-12 (2012).

111 Shapiro, P. *et al.* Multiple hepatic tumors and peliosis hepatis in Fanconi's anemia treated with androgens. *Am J Dis Child* **131**, 1104-1106 (1977).

112 Johansson, E., Niemi, K. M., Siimes, M. & Pyrhonen, S. Fanconi's anemia. Tumor-like warts, hyperpigmentation associated with deranged keratinocytes, and depressed cell-mediated immunity. *Archives of dermatology* **118**, 249-252 (1982).

113 Socie, G. *et al.* Squamous cell carcinomas after allogeneic bone marrow transplantation for aplastic anemia: further evidence of a multistep process. *Transplantation* **66**, 667-670 (1998).

114 Nowzari, H., Jorgensen, M. G., Ta, T. T., Contreras, A. & Slots, J. Aggressive periodontitis associated with Fanconi's anemia. A case report. *Journal of periodontology* **72**, 1601-1606, doi:10.1902/jop.2001.72.11.1601 (2001).

115 Cherubini, G. *et al.* The FANC pathway is activated by adenovirus infection and promotes viral replication-dependent recombination. *Nucleic acids research* **39**, 5459-5473, doi:10.1093/nar/gkr084 (2011).
116 Haneline, L. S. *et al.* Multiple inhibitory cytokines induce deregulated progenitor growth and apoptosis in hematopoietic cells from Fac-/- mice. *Blood* **91**, 4092-4098 (1998).
117 Koh, P. S., Hughes, G. C., Faulkner, G. R., Keeble, W. W. & Bagby, G. C. The Fanconi anemia group C gene product modulates apoptotic responses to tumor necrosis factor-alpha and Fas ligand but does not suppress expression of receptors of the tumor necrosis factor receptor superfamily. *Experimental hematology* **27**, 1-8 (1999).
118 Otsuki, T. *et al.* Tumor necrosis factor-alpha and CD95 ligation suppress erythropoiesis in Fanconi anemia C gene knockout mice. *Journal of cellular physiology* **179**, 79-86, doi:10.1002/(SICI)1097-4652(199904)179:1<79::AID-JCP10>3.0.CO;2-O (1999).
119 Dufour, C. *et al.* TNF-alpha and IFN-gamma are overexpressed in the bone marrow of Fanconi anemia patients and TNF-alpha suppresses erythropoiesis in vitro. *Blood* **102**, 2053-2059, doi:10.1182/blood-2003-01-0114 (2003).
120 Fagerlie, S. R., Koretsky, T., Torok-Storb, B. & Bagby, G. C. Impaired type I IFN-induced Jak/STAT signaling in FA-C cells and abnormal CD4+ Th cell subsets in Fancc-/- mice. *J Immunol* **173**, 3863-3870 (2004).
121 Fagerlie, S. R. & Bagby, G. C. Immune defects in Fanconi anemia. *Critical reviews in immunology* **26**, 81-96 (2006).
122 Gluckman, E. *et al.* Bone marrow transplantation in Fanconi anaemia. *British journal of haematology* **45**, 557-564 (1980).
123 Gluckman, E., Devergie, A. & Dutreix, J. Radiosensitivity in Fanconi anaemia: application to the conditioning regimen for bone marrow transplantation. *British journal of haematology* **54**, 431-440 (1983).
124 Guardiola, P. *et al.* Acute graft-versus-host disease in patients with Fanconi anemia or acquired aplastic anemia undergoing bone marrow transplantation from HLA-identical sibling donors: risk factors and influence on outcome. *Blood* **103**, 73-77, doi:10.1182/blood-2003-06-2146 (2004).
125 Socie, G. *et al.* Transplantation for Fanconi's anaemia: long-term follow-up of fifty patients transplanted from a sibling donor after low-dose cyclophosphamide and thoraco-abdominal irradiation for conditioning. *British journal of haematology* **103**, 249-255 (1998).
126 Kapelushnik, J., Or, R., Slavin, S. & Nagler, A. A fludarabine-based protocol for bone marrow transplantation in Fanconi's anemia. *Bone marrow transplantation* **20**, 1109-1110, doi:10.1038/sj.bmt.1701016 (1997).
127 Aker, M., Varadi, G., Slavin, S. & Nagler, A. Fludarabine-based protocol for human umbilical cord blood transplantation in children with Fanconi anemia. *Journal of pediatric hematology/oncology* **21**, 237-239 (1999).
128 MacMillan, M. L. & Wagner, J. E. Haematopoeitic cell transplantation for Fanconi anaemia - when and how? *British journal of haematology* **149**, 14-21, doi:10.1111/j.1365-2141.2010.08078.x (2010).
129 Rosenberg, P. S., Socie, G., Alter, B. P. & Gluckman, E. Risk of head and neck squamous cell cancer and death in patients with Fanconi anemia who did and did not receive transplants. *Blood* **105**, 67-73, doi:10.1182/blood-2004-04-1652 (2005).

130 Smith, A. R. & Wagner, J. E. Alternative haematopoietic stem cell sources for transplantation: place of umbilical cord blood. *British journal of haematology* **147**, 246-261, doi:10.1111/j.1365-2141.2009.07828.x (2009).
131 Gluckman, E. *et al.* Bone marrow transplantation for Fanconi anemia. *Blood* **86**, 2856-2862 (1995).
132 Wagner, J. E. *et al.* Unrelated donor bone marrow transplantation for the treatment of Fanconi anemia. *Blood* **109**, 2256-2262, doi:10.1182/blood-2006-07-036657 (2007).
133 Boulad, F. *et al.* Chemotherapy-Only Preparative Regimen for T-Cell Depleted Hematopoietic Stem Cell Transplantation of Patients with Fanconi Anemia From Alternative Donors. *Blood* (2012).
134 Gluckman, E. & Rocha, V. History of the clinical use of umbilical cord blood hematopoietic cells. *Cytotherapy* **7**, 219-227, doi:10.1080/14653240510027136 (2005).
135 Bizzetto, R. *et al.* Outcomes after related and unrelated umbilical cord blood transplantation for hereditary bone marrow failure syndromes other than Fanconi anemia. *Haematologica* **96**, 134-141, doi:10.3324/haematol.2010.027839 (2011).
136 http://www.eurocord-ed.org/download_docs_embed/_PARTNERS_FOLDER_/ch208_gluckman_hbmfs_final.pdf.
137 Mehta, P. A. *et al.* Chemotherapy for myeloid malignancy in children with Fanconi anemia. *Pediatric blood & cancer* **48**, 668-672, doi:10.1002/pbc.20843 (2007).
138 Wang, L. *et al.* Increased apoptosis is linked to severe acute GVHD in patients with Fanconi anemia. *Bone marrow transplantation*, doi:10.1038/bmt.2012.237 (2012).
139 Boulad, F. *et al.* Stem cell transplantation for the treatment of Fanconi anaemia using a fludarabine-based cytoreductive regimen and T-cell-depleted related HLA-mismatched peripheral blood stem cell grafts. *British journal of haematology* **111**, 1153-1157 (2000).
140 Tan, P. L. *et al.* Successful engraftment without radiation after fludarabine-based regimen in Fanconi anemia patients undergoing genotypically identical donor hematopoietic cell transplantation. *Pediatric blood & cancer* **46**, 630-636, doi:10.1002/pbc.20538 (2006).
141 Torjemane, L. *et al.* Bone marrow transplantation from matched related donors for patients with Fanconi anemia using low-dose busulfan and cyclophosphamide as conditioning. *Pediatric blood & cancer* **46**, 496-500, doi:10.1002/pbc.20286 (2006).
142 Ayas, M. *et al.* Matched-related allogeneic stem cell transplantation in Saudi patients with Fanconi anemia: 10 year's experience. *Bone marrow transplantation* **42 Suppl 1**, S45-S48, doi:10.1038/bmt.2008.114 (2008).
143 Boyer, M. W. *et al.* Low risk of graft-versus-host disease with transplantation of CD34 selected peripheral blood progenitor cells from alternative donors for Fanconi anemia. *Journal of pediatric hematology/oncology* **25**, 890-895 (2003).
144 Huck, K. *et al.* Favourable long-term outcome after matched sibling transplantation for Fanconi-anemia (FA) and in vivo T-cell depletion. *Klinische Padiatrie* **220**, 147-152, doi:10.1055/s-2008-1065326 (2008).
145 Handgretinger, R. New approaches to graft engineering for haploidentical bone marrow transplantation. *Seminars in oncology* **39**, 664-673, doi:10.1053/j.seminoncol.2012.09.007 (2012).

146 Rosenberg, P. S., Greene, M. H. & Alter, B. P. Cancer incidence in persons with Fanconi anemia. *Blood* **101**, 822-826, doi:10.1182/blood-2002-05-1498 (2003).
147 Alter, B. P. *et al.* Malignancies and survival patterns in the National Cancer Institute inherited bone marrow failure syndromes cohort study. *British journal of haematology* **150**, 179-188, doi:10.1111/j.1365-2141.2010.08212.x (2010).
148 Oksuzoglu, B. & Yalcin, S. Squamous cell carcinoma of the tongue in a patient with Fanconi's anemia: a case report and review of the literature. *Annals of hematology* **81**, 294-298, doi:10.1007/s00277-002-0455-6 (2002).
149 Masserot-Lureau, C. *et al.* Incidence of liver abnormalities in Fanconi anemia patients. *American journal of hematology* **87**, 547-549, doi:10.1002/ajh.23153 (2012).
150 Rosenberg, P. S., Alter, B. P. & Ebell, W. Cancer risks in Fanconi anemia: findings from the German Fanconi Anemia Registry. *Haematologica* **93**, 511-517, doi:10.3324/haematol.12234 (2008).
151 Kitao, H. & Takata, M. Fanconi anemia: a disorder defective in the DNA damage response. *International journal of hematology* **93**, 417-424, doi:10.1007/s12185-011-0777-z (2011).
152 Deans, A. J. & West, S. C. DNA interstrand crosslink repair and cancer. *Nature reviews. Cancer* **11**, 467-480, doi:10.1038/nrc3088 (2011).
153 Spanier, G. *et al.* Fatal course of tonsillar squamous cell carcinoma associated with Fanconi anaemia: a mini review. *Journal of cranio-maxillo-facial surgery : official publication of the European Association for Cranio-Maxillo-Facial Surgery* **40**, 510-515, doi:10.1016/j.jcms.2011.08.013 (2012).
154 Birkeland, A. C. *et al.* Postoperative clinical radiosensitivity in patients with fanconi anemia and head and neck squamous cell carcinoma. *Archives of otolaryngology--head & neck surgery* **137**, 930-934, doi:10.1001/archoto.2011.154 (2011).
155 Marcou, Y., D'Andrea, A., Jeggo, P. A. & Plowman, P. N. Normal cellular radiosensitivity in an adult Fanconi anaemia patient with marked clinical radiosensitivity. *Radiotherapy and oncology : journal of the European Society for Therapeutic Radiology and Oncology* **60**, 75-79 (2001).
156 Tan, I. B. *et al.* Fanconi's anemia in adulthood: chemoradiation-induced bone marrow failure and a novel FANCA mutation identified by targeted deep sequencing. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **29**, e591-594, doi:10.1200/JCO.2011.35.1064 (2011).
157 Alter, B. P. Radiosensitivity in Fanconi's anemia patients. *Radiotherapy and oncology : journal of the European Society for Therapeutic Radiology and Oncology* **62**, 345-347 (2002).
158 Spardy, N. *et al.* The human papillomavirus type 16 E7 oncoprotein activates the Fanconi anemia (FA) pathway and causes accelerated chromosomal instability in FA cells. *Journal of virology* **81**, 13265-13270, doi:10.1128/JVI.01121-07 (2007).
159 Hoskins, E. E. *et al.* Fanconi anemia deficiency stimulates HPV-associated hyperplastic growth in organotypic epithelial raft culture. *Oncogene* **28**, 674-685, doi:10.1038/onc.2008.416 (2009).
160 Park, J. W. *et al.* Deficiencies in the Fanconi anemia DNA damage response pathway increase sensitivity to HPV-associated head and neck cancer. *Cancer research* **70**, 9959-9968, doi:10.1158/0008-5472.CAN-10-1291 (2010).

161 Kutler, D. I. *et al.* High incidence of head and neck squamous cell carcinoma in patients with Fanconi anemia. *Archives of otolaryngology--head & neck surgery* **129**, 106-112 (2003).
162 Kutler, D. I. *et al.* Human papillomavirus DNA and p53 polymorphisms in squamous cell carcinomas from Fanconi anemia patients. *Journal of the National Cancer Institute* **95**, 1718-1721 (2003).
163 van Zeeburg, H. J. *et al.* Generation and molecular characterization of head and neck squamous cell lines of fanconi anemia patients. *Cancer research* **65**, 1271-1276, doi:10.1158/0008-5472.CAN-04-3665 (2005).
164 van Zeeburg, H. J., Snijders, P. J., Joenje, H. & Brakenhoff, R. H. Re: Human papillomavirus DNA and p53 polymorphisms in squamous cell carcinomas from Fanconi anemia patients. *Journal of the National Cancer Institute* **96**, 968; author reply 968-969 (2004).
165 van Zeeburg, H. J. *et al.* Clinical and molecular characteristics of squamous cell carcinomas from Fanconi anemia patients. *Journal of the National Cancer Institute* **100**, 1649-1653, doi:10.1093/jnci/djn366 (2008).
166 Han, T. J. *et al.* Synchronous multifocal HPV-related neoplasm involving both the genital tract and the head-and-neck area: a case report of Fanconi anemia. *Radiotherapy and oncology : journal of the European Society for Therapeutic Radiology and Oncology* **92**, 138-141, doi:10.1016/j.radonc.2009.03.009 (2009).
167 de Araujo, M. R. *et al.* High prevalence of oral human papillomavirus infection in Fanconi's anemia patients. *Oral diseases* **17**, 572-576, doi:10.1111/j.1601-0825.2011.01803.x (2011).
168 http://www.cdc.gov/media/releases/2011/t1025_hpv_12yroldvaccine.html.
169 http://www.ema.europa.eu/ema/.
170 Reinhard, H., Peters, I., Gottschling, S., Ebell, W. & Graf, N. Squamous cell carcinoma of the tongue in a 13-year-old girl with Fanconi anemia. *Journal of pediatric hematology/oncology* **29**, 488-491, doi:10.1097/MPH.0b013e318063ef14 (2007).
171 Masserot, C. *et al.* Head and neck squamous cell carcinoma in 13 patients with Fanconi anemia after hematopoietic stem cell transplantation. *Cancer* **113**, 3315-3322, doi:10.1002/cncr.23954 (2008).
172 Scheckenbach, K., Wagenmann, M., Freund, M., Schipper, J. & Hanenberg, H. Squamous cell carcinomas of the head and neck in Fanconi anemia: risk, prevention, therapy, and the need for guidelines. *Klinische Padiatrie* **224**, 132-138, doi:10.1055/s-0032-1308989 (2012).
173 http://www.fanconi.org/images/uploads/other/FN49.pdf.
174 Kujan, O., Glenny, A. M., Oliver, R. J., Thakker, N. & Sloan, P. Screening programmes for the early detection and prevention of oral cancer. *Cochrane Database Syst Rev*, CD004150, doi:10.1002/14651858.CD004150.pub2 (2006).
175 http://www.awmf.org/leitlinien/aktuelle-leitlinien/ll-liste/deutsche-krebsgesellschaft-dkg.html.
176 Seaward, P. G., Setzen, R. & Guidozzi, F. Fanconi's anaemia in pregnancy. A case report. *South African medical journal = Suid-Afrikaanse tydskrif vir geneeskunde* **78**, 691-692 (1990).
177 Alter, B. P. *et al.* Fanconi's anaemia and pregnancy. *British journal of haematology* **77**, 410-418 (1991).
178 Alter, B. P., Kumar, M., Lockhart, L. L., Sprinz, P. G. & Rowe, T. F. Pregnancy in bone marrow failure syndromes: Diamond-Blackfan anaemia and

Shwachman-Diamond syndrome. *British journal of haematology* **107**, 49-54 (1999).
179 Dalle, J. H., Champagne, M. A. & Duval, M. Pregnancy after bone marrow transplantation in Fanconi anaemia. *British journal of haematology* **137**, 76; author reply 76, doi:10.1111/j.1365-2141.2007.06506.x (2007).
180 Oshima, K. *et al.* Fanconi anemia in infancy: report of hematopoietic stem cell transplantation to a 13-month-old patient. *International journal of hematology* **89**, 722-723, doi:10.1007/s12185-009-0329-y (2009).
181 Nabhan, S. K. *et al.* Fertility recovery and pregnancy after allogeneic hematopoietic stem cell transplantation in Fanconi anemia patients. *Haematologica* **95**, 1783-1787, doi:10.3324/haematol.2010.023929 (2010).
182 Aynsley-Green, A., Zachmann, M., Werder, E. A., Illig, R. & Prader, A. Endocrine studies in Fanconi's anaemia. Report of 4 cases. *Archives of disease in childhood* **53**, 126-131 (1978).
183 Wajnrajch, M. P. *et al.* Evaluation of growth and hormonal status in patients referred to the International Fanconi Anemia Registry. *Pediatrics* **107**, 744-754 (2001).
184 Giri, N., Batista, D. L., Alter, B. P. & Stratakis, C. A. Endocrine abnormalities in patients with Fanconi anemia. *The Journal of clinical endocrinology and metabolism* **92**, 2624-2631, doi:10.1210/jc.2007-0135 (2007).
185 Sherafat-Kazemzadeh, R. *et al.* Small pituitary size in children with Fanconi anemia. *Pediatric blood & cancer* **49**, 166-170, doi:10.1002/pbc.21148 (2007).
186 Rose, S. R. *et al.* Endocrine phenotype of children and adults with Fanconi anemia. *Pediatric blood & cancer* **59**, 690-696, doi:10.1002/pbc.24095 (2012).