General introduction
and thesis outline
Fanconi anemia: clinical hallmarks

Fanconi anemia, a rare and complex disorder

Nearly 90 years ago, in 1927, the Swiss pediatrician Guido Fanconi described a family with 3 brothers who suffered from a fatal disorder characterized by physical abnormalities (microcephaly, skin hyperpigmentation and hypoplasia of the testes) and anemia at the age of 5-7 years.1,2 Since 1931 this hereditary syndrome is called Fanconi anemia (FA).2 Progress in understanding the molecular basis and clinical features of FA has been remarkable since the first description of this disease.

FA is a very rare disease affecting less than 100 patients in the Netherlands.3 It is a complex, life-threatening disorder which has a major impact on patients and their families. The median lifespan of FA patients is currently 33 years.4,5 The clinical symptoms of this heterogeneous, multi-system disease can be divided into 4 categories: congenital anomalies, bone marrow failure, endocrine disorders and cancer susceptibility.

Congenital anomalies

Congenital anomalies are present in approximately two-thirds of the FA patients and may involve any of the major organ systems.6,7 Short stature, abnormal skin pigmentation (hyperpigmentation, hypopigmentation and café au lait spots) and thumb malformations are the most prevalent physical anomalies in FA.7 Other symptoms include intrauterine growth retardation, developmental delay, microcephaly, malformation of the radial bone and other skeletal deformities. Furthermore, abnormalities of the ears, eyes, heart, kidneys, urinary tract, gastrointestinal tract, central nervous system and reproductive system are frequently present.6,8 Some FA patients present with severe physical anomalies indicative of VACTERL association, a well-known congenital malformation association including vertebral, anal, cardiac, trachea-esophageal, renal and limb anomalies.10 Physical abnormalities are often subtle or even absent. It is important to realize that the absence of congenital malformations does not rule out FA.

Bone marrow failure

Bone marrow failure (BMF) is the hallmark of FA and often the first adverse event in FA patients. BMF commonly starts with thrombocytopenia, followed by neutropenia and anemia.11 Elevated levels of fetal hemoglobin and macrocytosis, both features of stress erythropoiesis, are frequently found in patients with inherited BMF syndromes such as FA.12 The majority of FA patients develop BMF during the first decade of life, at a median age of 7 years.6,13 The cumulative incidence of BMF by age 40 is 90%.14 The presence of many birth defects is associated with a high risk of developing early onset BMF.15
Transfusions can be given to treat severe anemia and thrombocytopenia. Granulocyte-colony stimulating factor can be considered to treat severe neutropenia. Furthermore, some patients may benefit from treatment with androgens. Nevertheless, the only curative treatment for BMF in FA is hematopoietic stem cell transplantation (SCT).

**Endocrine disorders**

Endocrine abnormalities are very common in FA. About 70-80% of the children and adults with FA have one or more endocrine disorders. These include short stature, hypothyroidism, impaired glucose tolerance or diabetes, obesity, dyslipidemia, metabolic syndrome and pituitary gland abnormalities. In addition, many FA patients have gonadal and pubertal disorders. Males are often infertile and females frequently have premature menopause and a significantly reduced window of fertility. Short stature, with or without growth hormone deficiency, is a well-known feature of FA. About 60% of FA patients are shorter than the reference population. Average height is around 150 cm in women and 161 cm in men. The etiology of endocrine disorders in FA is complex and not exactly understood. Also treatment for FA, such as androgens, blood transfusions, chemotherapy, irradiation and corticosteroids can affect the endocrine system. Baseline and annual endocrine evaluation should be performed in every FA patient. Early treatment of endocrine disorders may lead to reduced morbidity and improved quality of life.

**Cancer susceptibility**

FA patients have a very high risk of developing cancer and they develop cancer at a much younger age than the general population. Many patients develop more than one tumor during their lifetime. The median age for surviving free of any malignancy is 29 years, substantially lower than expected in the general population. Patients are particularly susceptible for hematological malignancies, such as myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML), which can be preceded by or associated with clonal cytogenetic abnormalities in the bone marrow, such as aberrations of chromosome 1, 3 and 7. The incidence of MDS is approximately 40% by age 50. The median age of AML is 11.3 years with a cumulative incidence of 15-20% by age 40. Because MDS and AML in FA patients are difficult to treat, it is advised to perform annual or biannual bone marrow aspirations to identify early signs of MDS or AML in order to proceed to SCT in time. Furthermore, FA patients are at very high risk of developing squamous cell carcinoma (SCC), mainly head and neck SCC (HNSCC), SCC of the esophagus and anogenital SCC. Other, non-SCC solid tumors include tumors of the liver, brain, kidney, skin,
breast and embryonal tumors.\textsuperscript{5,7} The cumulative incidence for solid tumors is 28\% by the age of 40 years with a ratio of observed to expected of 26.\textsuperscript{15}

\textit{Diagnosing Fanconi anemia}

The absence of characteristic physical findings, the low prevalence and the heterogeneous presentation of FA frequently causes a delay in accurate diagnosis. Sometimes FA is suspected shortly after birth because of FA-associated congenital anomalies. FA is mostly diagnosed in the first decade of life when children develop BMF. The median age at diagnosis is 6.5 years, but ranges from birth to adulthood.\textsuperscript{7} In rare cases FA is diagnosed in adulthood when patients present with unusual tumors at relatively young age or show unexpected toxicity when treated with chemotherapy or irradiation.

All siblings of a patient diagnosed with FA should be screened. Furthermore, FA should be tested for in all patients with FA-associated congenital abnormalities, unexplained pancytopenia at young age, MDS with FA-associated cytogenetic abnormalities (e.g. clonal aberrations of chromosome 3q) or early onset SCC of the upper aerodigestive tract or anogenital region.\textsuperscript{8} Also familial predisposition for AML and/or MDS should lead to evaluation of the potential diagnosis of FA. It is crucial to test for FA before proceeding to SCT or start treatment for cancer, since standard chemotherapy and irradiation is, in general, toxic to FA patients.

\textbf{Fanconi anemia: cellular characteristics}

\textit{Fanconi anemia, a DNA repair disorder}

To date, nearly 90 years after Guido Fanconi's first description of the disease, FA is known to be caused by mutations in one of the 20 currently known FA genes (see Table 1). These genes encode for proteins that form the FA pathway, which is specialized in repair of DNA damage.\textsuperscript{25} Some of the FA genes are also breast cancer (BRCA) susceptibility genes and therefore the pathway is also known as the FA/BRCA pathway.\textsuperscript{26}

Human cells are continuously challenged by DNA damage caused by exogenous and endogenous triggers. Cells have various methods to repair DNA damage and maintain genome integrity.\textsuperscript{26} The FA/BRCA pathway is essential to repair interstrand crosslinks (ICLs), a severe form of DNA damage. Unrepaired ICLs lead to stalled replication forks, DNA breakage and chromosomal rearrangements and, consequently, to the development of cancer. Cells with biallelic mutations in one of the FA genes are deficient in ICL repair. As a consequence, FA patients develop their clinical symptoms, have an increased risk of developing cancer and are, at the same time, hypersensitive to treatment of cancer with chemotherapy with crosslinking agents.
FA is also considered a stem cell disease. The unrepaired DNA damage causes a depletion of the hematopoietic stem cells and accumulation of mutations in other stem cells, most particularly squamous stem cells.

### Table 1. FA genes

<table>
<thead>
<tr>
<th>Gene</th>
<th>Alternative name</th>
<th>Locus</th>
<th>Patient frequency</th>
<th>Inheritance</th>
<th>Reference</th>
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Data extracted from the Rockefeller University - Fanconi Anemia Mutation Database at [www.rockefeller.edu/fanconi](http://www.rockefeller.edu/fanconi) and adapted from Wang AT, Smogorzewska A. SnapShot: Fanconi anemia and associated proteins. Cell 2015; 160 (1-2).25

AR = autosomal recessive, XLR = X-linked, AD = autosomal dominant, *dominant negative de novo mutation

### Endogenous crosslinkers

Chemotherapy, particularly alkylating drugs, are well-known exogenous causes of ICLs. However, the FA pathway developed in evolution to repair DNA damage that is caused in a physiological setting. In this context, reactive aldehydes, a by-product of several metabolic pathways, were recently identified as an important source of endogenous DNA damage.
Healthy cells catabolize these reactive metabolites with aldehyde dehydrogenases such as ALDH2 and simultaneously activate the FA pathway to maintain genome stability. Of interest, patients with FA who are also deficient in ALDH2 develop severe BMF at a very young age.27 The FA pathway seems to be essential to counteract the toxic, crosslinking effect of reactive aldehydes.27-29

Chromosomal breakage test and mutation analysis

The inability of FA cells to repair ICLs is used to diagnose FA by means of a chromosomal breakage test. In this test peripheral blood lymphocytes are treated with crosslinking agents such as Mitomycine-C (MMC) or Diepoxybutane (DEB).6,30 Normal, FA proficient cells can correct most of the chromosomal damage caused by MMC or DEB, however, cells of an FA patient show multiple chromosomal breaks and rearrangements. Other assays that assist the diagnosis of FA are growth inhibition tests and cell cycle studies using flow cytometric methods. FA deficient cells arrest in the G2/M phase of the cell cycle after treatment with crosslinking agents (see Figure 1).31

When FA is diagnosed with a chromosomal breakage test, mutation analysis should be performed to identify the underlying genetic mutations by Sanger sequencing or next generation sequencing methods.32 Identified mutations can be used to predict some aspects of the course of disease and tailor treatment (see also genotype-phenotype correlation). Furthermore, it enables testing of family members, prenatal testing and pre-implantation genetic diagnosis.

Mosaicism

Diagnosing FA with a chromosomal breakage test can be complicated by the presence of somatic mosaicism. A substantial part of FA patients develops somatic mosaicism during life.7 In mosaic FA patients one mutated allele in a hematopoietic stem cell (HSC) has spontaneously corrected and lost the FA hypersensitive phenotype. The restoration to (close to) normal function of a gene is called reversion. The coexistence of reverted, phenotypically wild type cells and mutated FA cells is referred to as reverse mosaicism. Molecular reversion mechanisms include intragenic mitotic recombination (crossing over and gene conversion), back mutation and compensatory second site mutation.33 MMC chromosomal breakage tests in mosaic FA patients will show a mixture of MMC-sensitive and MMC-resistant peripheral blood lymphocytes and could therefore give a false negative result. For these cases skin fibroblasts can be used to demonstrate sensitivity to crosslinking agents, since fibroblasts remain MMC-sensitive.30 Molecular analysis will reveal the presence of biallelic mutations in non-reverted, MMC-sensitive fibroblasts, while reverted, MMC-resistant peripheral blood cells show only one defective allele.33
Figure 1. Hypersensitivity of Fanconi anemia cells
This figure is reprinted with the courtesy of C. Stoepker.103 FA patient derived cells are hypersensitive for DNA interstrand cross-linking (ICL) agents, such as mitomycin C (MMC) and diepoxylbutane (DEB). After treatment with MMC or DEB, FA-deficient cells show (A) growth inhibition, (B) G2/M cell cycle arrest and (C) chromosomal aberrations, such as breaks and gaps.

Reverted HSCs have a proliferative advantage resulting in outgrowth of non-reverted cells. This could lead to improved or even normal blood counts and, consequently, some patients with mosaicism never develop BMF.33,34 However, the development of clonal cytogenetic abnormalities in the remaining fraction of non-reverted HSCs of mosaic FA patients cannot be excluded.33

Fanconi anemia: inheritance and genotype-phenotype correlations

Inheritance and carrier frequency
Worldwide, mutations in FANCA, FANCC and FANCG are most common. Approximately 85% of all patients in the International FA Registry (IFAR) have mutations in one of these three genetic subtypes.6 FA is primarily inherited as a recessive disorder. If both parents carry a mutation in the same FA gene, each of their children has a 25% chance of inheriting
the defective gene from both parents and subsequently will develop FA. FA occurs equally in males and females, except for subtype FA-B which exclusively affects males since it is inherited in an X-linked manner. Recently a new FA gene was found which was inherited in a dominant way. The mutation caused a dominant-negative effect and led to functional inactivation of the gene product of the wild type allele.

FA is found in all ethnic groups. The estimated FA carrier frequency in the United States is 1:181. Carrier frequencies of 1:100 and higher are found in certain ethnic groups, such as Ashkenazi Jews, the Afrikaner population in South Africa and Spanish Gypsies. The high frequency of defined mutations in these populations is caused by a founder effect and frequent marital relationships within the population. A founder effect is the loss of genetic variation that occurs when a new population is established by a very small number of individuals from a larger population.

**Genotype-phenotype correlations**

FA is a heterogeneous disorder. Presence and severity of congenital abnormalities, the age of onset of BMF and the type and risk of malignancies differ between and within FA subgroups. Generally, the genotype-phenotype correlations are not very strong. However, mutations in some FA genes can cause a distinct clinical phenotype and are, in these cases, important in the clinical management and counseling of FA patients. A strong genotype-phenotype correlation is seen in patients with mutations in FANCD1/BRCA2. These patients have a high frequency of severe birth defects and an extraordinary risk of developing early onset malignancies, mainly AML, Wilms’ tumor and midline brain tumors, with a cumulative probability of 97% by the age of 6 years. Different mutations in single genes can lead to distinct phenotypes. For example, intron 4 and exon 14 mutations in the FANCC gene are associated with a more severe phenotype and poorer survival compared to exon 1 mutations in the same gene. This could be explained by the fact that some mutations lead to some degree of protein function causing a milder phenotype than null-mutations, with no active protein at all.

Of interest, the phenotype may also vary between affected siblings sharing the same mutations. Additional modifying factors, such as environmental factors, modifier genes and chance, seem to influence the FA phenotype as well. Remarkably, also the genetic background of a specific patient can affect the phenotype: the IVS4+4A>T mutation in Ashkenazi Jews is associated with a very severe phenotype, whereas the same mutation in Japanese patients is not.
The Dutch mutation

The most prevalent mutation in Dutch FA patients is the c.67delG mutation in exon 1 of the FANCC gene, previously known as 322delG.48 The high frequency of this mutation is caused by a founder effect. Genealogical studies revealed that this founder mutation probably originated in the Netherlands.49 The c.67delG mutation is also found in Canadian Mennonites who share the same haplotype adjacent to this mutation, indicative of a common founder.49 The Dutch mutation is associated with a milder phenotype due to expression of a FANCC isoform with partial function.46 Patients with homozygous c.67delG mutations are considered to be less affected in terms of congenital abnormalities when compared to the majority of other FA patients.43,45 However, data on BMF, cancer risk and survival in this specific patient group are scarce.

Fanconi anemia: stem cell transplantation

Hematologic abnormalities in Fanconi anemia

Blood counts in FA patients are, in general, normal at birth and start to decrease in the first decade of life. Eventually all blood cell populations become deficient indicating a hematopoietic stem cell (HSC) dysfunction. Studies in humans and mice have shown that the HSC pool in FA already appears to be impaired in utero. It then further decreases during childhood, finally resulting in BMF early in life.11,50,51 The cause of this impaired HSC pool and subsequent BMF in FA is not exactly known, but it is thought to be the result of unresolved DNA damage caused by endogenous aldehyde-induced toxicity and the subsequent DNA damage-induced p53 activation resulting in the attrition of HSCs.29,51 Another mechanism that may contribute to BMF is the hypersensitivity of FA cells to certain inflammatory cytokines.52 The genetic instability caused by unresolved DNA damage leads to loss of HSCs resulting in BMF, but could also explain the genesis of neoplastic clones resulting in MDS and AML.11

Stem cell transplantation

The only curative treatment for the hematologic abnormalities in FA is allogeneic SCT. The aim of SCT is to replace the affected HSCs from a patient by stem cells from a healthy donor. These donor HSCs are derived from bone marrow, peripheral blood or umbilical cord blood. To prevent rejection of the donor stem cells, it is necessary to find a human leukocyte antigen (HLA) compatible related or unrelated donor. Chemotherapy and/or irradiation, given directly prior to a transplant, is called the conditioning regimen and is necessary to eradicate the patient’s stem cells before the infusion of donor stem cells. This treatment also has an immunosuppressive effect that prevents rejection of the donor stem cells by the
recipient's immune system. Major complications of SCT are mucositis, infections, graft-versus-host-disease (GVHD) and the development of new malignancies.

**Outcome of stem cell transplantation for Fanconi anemia**

First international results of SCT for FA are reported in the seventies.\(^\text{53-55}\) Initially, survival was very poor due to treatment-related toxicity.\(^\text{56}\) FA patients appeared to be hypersensitive to cyclophosphamide and irradiation as used in conventional conditioning regimens. The two major steps that resulted in increased survival were the introduction of reduced intensity conditioning regimens in the eighties-nineties and the introduction of fludarabine in 1997.\(^\text{57,58}\)

A recent multicenter, retrospective study of nearly 800 transplanted FA patients shows a better outcome in FA patients transplanted before the age of 10 years and before the development of MDS or AML. HLA matched related donor SCT and a fludarabine based conditioning regimen without irradiation are also associated with improved outcome.\(^\text{59}\)

Ideally, SCT should be performed at a young age, prior to complications of BMF or the development of MDS or AML. However, not all FA patients will progress to severe BMF or AML and it is therefore difficult to decide when to proceed to early, preemptive SCT, knowing that SCT is still a dangerous procedure with many possible complications.

Next steps in SCT for FA are further optimization of conditioning regimens and thereby reduction of possible long-term side effects. There is an ongoing debate on how to optimize these regimens without increasing the risk of graft failure. Elimination of irradiation would be especially attractive for FA patients given their clinical hypersensitivity for irradiation. This is somewhat unexpected as the cellular sensitivity is restricted to ICL agents. In the Netherlands a non-irradiation and busulfan-free conditioning regimen is currently used.

It is important to realize that SCT does not correct the non-hematological manifestations of FA. After transplant patients are still at risk to develop endocrine disorders and solid tumors. Moreover, SCT-related complications such as chronic GVHD can even further increase the risk of developing HSNCC.\(^\text{59,60}\)

**Fanconi anemia: head and neck squamous cell carcinoma**

**Sporadic head and neck squamous cell carcinoma**

HNSCC is one of the more prevalent types of cancer in the world. Exposure to carcinogens, such as smoking and the use of alcohol, is the most important risk factor. Furthermore, human papillomavirus (HPV) infection causes a subgroup of HNSCC. Besides exogenous risk factors, also specific genetic disorders, such as FA, are associated with a high risk of developing HNSCC.\(^\text{61}\)
HNSCCs are known to have a high morbidity and mortality. Tumor site, histology and stage at initial presentation, defined by tumor size, lymph node metastases and distant metastases, determine the prognosis of patients with HNSCC. Early stage HNSCC is treated with surgery or irradiation and has a favorable prognosis compared to advanced HNSCC which is treated with surgery combined with postoperative radiotherapy or cisplatin-based chemoradiation. Cisplatin-based chemoradiation is also applied as first line treatment, particularly for more advanced oropharyngeal cancer. Despite this aggressive, multimodality treatment, survival outcomes are still suboptimal. Targeted molecular therapy with cetuximab, an epidermal growth factor receptor-specific antibody, is a promising approach to improve treatment for HNSCC.

Head and neck squamous cell carcinoma in Fanconi anemia patients

Patients with FA are at extraordinary high risk of HNSCC. They have a 500- to 1,000-fold higher incidence of HNSCC than the general population with a cumulative incidence of 14% by the age of 40 years. In addition, FA patients develop HNSCC at a much younger age. Median age at HNSCC diagnosis in FA patients is 30 years compared to 63 years in the general population.

HNSCC in FA are most often located in the oral cavity. As already mentioned, SCT is an important additional risk factor for HNSCC in FA. Transplanted patients develop HNSCC more frequently (relative risk of 4.4) and earlier than non-transplanted FA patients. Since HPV is associated with a subgroup of sporadic HNSCC, it was a logical step to explore the potential role of HPV in FA HNSCC. Results of these investigations are controversial, but most studies did not find an increased attributable fraction of HPV in FA HNSCC. However, HPV was often involved in anogenital carcinomas in FA patients. Therefore, preventive HPV vaccination is advised for FA patients to avoid any potential, additional risk of HPV in these high risk patients.

Treatment options of FA patients with HNSCC are limited because of the sensitivity of FA patients to irradiation and chemotherapy. Early identification of HNSCC and prompt surgical intervention is of great importance and may lead to improved survival. Frequent 3-monthly screening is therefore recommended from the age of 10 years onwards.

Noninvasive genetic screening for head and neck squamous cell carcinoma

Field cancerization, the presence of large epithelial fields with premalignant genetic changes, plays an important role in the development of sporadic HNSCCs. Generally, these fields are not visible, but they can present as leukoplakia or erythroplakia. Most fields can be recognized under the microscope as dysplasias, graded as mild, moderate or severe. Genetic alterations in tumors and dysplasias were studied using microsatellite PCR. Based
on loss of heterozygosity (LOH) a genetic progression model was proposed (see Figure 2). With losses at chromosome 9p, 3p and 17p as early events, LOH of these chromosome arms is an important and reliable prognostic biomarker to predict malignant transformation of preneoplastic changes to oral cancer.\textsuperscript{71-74} In subsequent years the cancer genes playing a role in HNSCC were identified which encompass TP53 at 17p, CDKN2A (p16) at 9p, CCND1 (cyclinD1) at 11q13, EGFR at 7p, PIK3CA at 3q26 and many others that are less frequently changed.\textsuperscript{61,62}

**Figure 2.** Molecular carcinogenesis of head and neck squamous cell carcinoma.

This figure is reprinted with the courtesy of R.H. Brakenhoff and shows a proposed model of the development of head and neck squamous cell carcinoma (HNSCC) including the involved genes and molecular pathways.\textsuperscript{61} One or more genetic aberrations occur in a precursor or adult stem cell, including a TP53 mutation. Successively, a patch is formed of daughter cells containing these genetic aberrations. Due to growth advantage and/or escaping growth control, the clonal patch develops into a large field and replaces the surrounding, normal mucosal epithelium. Finally, a subclone transforms into an invasive tumor and then progresses to metastasis.

Three genetic HNSCC subtypes are presented: human papilloma virus (HPV) positive tumors, HPV negative tumors with high chromosome instability (high CIN) and HPV negative tumors with low CIN. No detailed molecular data are available for HPV negative low CIN tumors.

Yellow boxes: genetic and chromosome alterations; orange boxes: tumor-suppressive pathways; blue box: oncogenic pathways.

\(\uparrow\) = overexpression or gain; \(\uparrow\uparrow\) = high-level amplification; \(\downarrow\) = loss; \(\downarrow\downarrow\) = homozygous loss.
In recent years, a noninvasive approach to detect LOH in exfoliated oral epithelial cells has been developed. This approach is based on LOH analysis using 12 microsatellite markers. These markers, located on chromosomes 3p, 9p, 11q and 17p, involve areas close to genes that are associated with HNSCC development.\textsuperscript{75-77} Noninvasive LOH screening is an attractive method for detecting and monitoring oncogenetic changes in the oral epithelium of patients with a high HNSCC susceptibility, such as FA patients. LOH patterns in sporadic HNSCC are comparable to those identified in FA HNSCC and may be used for noninvasive genetic screening in these high risk patients.\textsuperscript{68}

**Fanconi anemia: care and social impact**

*Care for Fanconi anemia in the Netherlands*

Given the fact that FA is a rare and complex disease that affects many organ systems and carries a high psychological burden, ideally care for FA should be centralized in multidisciplinary expert teams. The majority of Dutch FA patients are currently treated at university medical centers. In 2007, the Dutch Childhood Oncology Group (DCOG-SKION) implemented a guideline for diagnosis, treatment and follow-up of FA patients. Together with this Dutch FA guideline also an FA patient registry was started to gather data on the genotype, phenotype and course of the disease of Dutch FA patients. This patient registry will enable better prediction of outcome and aid clinicians with decision making regarding therapy. Furthermore, it will enable Dutch participation in international research projects.

*Dutch Fanconi anemia patient organization*

The FA working group, part of the Dutch childhood cancer parent organization (VOKK), is an active organization that supports and represents the interests of Dutch FA patients. The working group provides information about FA to patients, their families and healthcare providers, and is a patient advocate for optimization of FA care in the Netherlands.

*International Fanconi anemia patient organizations*

Several countries have an active FA patient organization. In 1989, Lynn and Dave Frohnmayer, parents of 5 children of whom 3 were diagnosed with FA, started the Fanconi Anemia Research Fund (FARF). By raising more than 32 million dollar the FARF has given an impressive boost to scientific research on this rare genetic disorder.\textsuperscript{4} The mission of the FARF is to find effective treatments and a cure for FA and to provide education and support to affected families worldwide.\textsuperscript{4} In addition, the FARF organizes an annual international
scientific symposium and has developed an FA handbook providing guidelines for diagnosis and management of FA.⁸

New horizons, new challenges

Life expectancy of FA patients has increased significantly due to improved medical care and improved outcome after SCT. Median survival has increased from 21 years prior to 2000 to currently 33 years.⁴,⁵,⁷ To date, more than 80% of the FA patients reach adulthood.⁷ As a consequence, more patients will be confronted with other FA related problems, such as the development of solid tumors and endocrine disorders. Patients are increasingly being referred to physicians involved in adult healthcare since FA is no longer only a disease of childhood. However, these physicians have, understandably, little experience with this rare, complex disorder. Optimization of screening and treatment of solid tumors and endocrine disorders, and efficient organization of the transition process from pediatric to adult healthcare are the most important challenges for the years to come.

Aim and outline of this thesis

Aim

The studies presented in this thesis aim to increase knowledge on FA in the Dutch setting and thereby improve care for FA patients and their families. Genotype-phenotype studies are needed to gain insight into the natural history of this heterogeneous disorder and thereby improve clinical management and counseling of FA patients. The next question in SCT for FA is how to further optimize conditioning regimens and thereby reduce late effects. The increased lifespan raises new questions that need to be addressed. How to improve early detection and treatment of HNSCC and how to optimally organize care for FA, including transition from pediatric to adult healthcare, are the main questions that have to be answered in the coming years.

Outline

In Chapter 2 we report the clinical findings and molecular analysis of a pedigree with FANCD2 mutations. In Chapter 3 we describe the epidemiology and genotype of the Dutch FA cohort. Furthermore, we explore the phenotype and course of disease in patients homozygous for the Dutch FA mutation, which is considered to be a mild mutation. In Chapter 4 we demonstrate the characteristics and results of all consecutive SCTs that were performed in FA patients in the Netherlands over the past 4 decades. In addition, we report the results of the current Dutch non-irradiation and busulfan-free conditioning regimen
for FA patients transplanted with stem cells from matched related as well as alternative donors. In Chapter 5 we evaluate the potential of a novel, noninvasive assay to identify precancerous lesions in the oral epithelium of FA patients. We determine the prevalence of LOH in brushed cells of the oral epithelium of FA patients and analyze the association of LOH with clinical characteristics and HNSCC. In Chapter 6 we focus on healthcare for FA in the Netherlands. We show the results of a survey conducted in collaboration with the Dutch FA patient organization to evaluate current healthcare for FA in the Netherlands. Furthermore, we use the example of the rare disease FA to describe the challenges and opportunities in organizing healthcare for rare diseases. The results described in this thesis are discussed in Chapter 7. Finally, Chapter 8 provides the summary of this thesis.
References


3. DCOG. Dutch Childhood Oncology Registry. 2016.

4. website FARF.


103. Stoepker C. The role of the Fanconi anemia pathway in sporadic head and neck cancer. Enschede: VU University Medical Center; 2015.