Fanconi Anemia RESEARCH FUND, INC.

Deutsche Fanconi-Anämie-Hilfe e.V.

Study Title

Reducing the burden of squamous cell carcinoma in Fanconi anemia

PREAMBLE

Fanconi anemia (FA), named for Swiss pediatrician, Guido Fanconi, is a rare genetic disease that leads to bone marrow failure. Though considered primarily a blood disease, FA may affect all systems of the body. It is a complex and chronic disorder that is psychologically demanding. FA is also a cancer-prone disease, affecting individuals decades earlier than the general population. FA individuals have an especially high risk of developing malignant lesions, particularly solid tumors of the head and neck region and oral cavity (mouth and esophagus). The aggressive nature of these tumors combined with delayed diagnosis mandate substantial surgical intervention and radiotherapy.

A non-invasive brush biopsy approach followed by conventional cytology and/or DNA cytometry and LOH assay to detect pre-cancerous lesions has proven successful in identifying early malignant lesions. Based on these results we are proposing a longitudinal screen of an international FA cohort for the early detection and monitoring of oral cancer and pre-cancer, employing soft brush biopsy combined with cytology, DNA cytometry and LOH analysis. Included in these studies will be a questionnaire to correlate presence and progression of cancer and pre-cancer with exposure to environmental risk factors such as alcohol and tobacco exposure. In cases where visible lesions are observed, we will provide the cytology and cytometry analysis results to the individual, parent of children participant and designated treating physician. The de-identified research data will be aggregated in a dataset accessible for research purposes through research databases such as the Synapse repository and analysis platform (WIRB # 20112068).

The aim of the proposed study is improvement of early detection, timely diagnosis, and individualized treatment of SCC arising in the context of FA individuals, with the ultimate goal to improve treatment outcome and reduce disease burden. Secondary aims are to identify FA individuals with cancer and pre-cancer and to foster tissue sampling for the FA research community.

We are seeking WIRB's opinion regarding the feasibility of this study.

Table of Content

Chapter 1 **Abbreviations:**

AML: acute myeloid leukemia
FA: Fanconi anemia
GvHD: graft versus host disease
HNSCC: head and neck squamous cell carcinoma
HSCT: hematopoietic stem cell transplantation
LOH: loss of heterozygosity
SCC: squamous cell carcinoma
WHO: World Health Organization

Chapter 2 **Background**

Fanconi anemia (FA) is an inherited disease that leads to bone marrow failure (aplastic anemia). It is primarily a recessive disorder: if both parents carry a mutation in the same FA gene their child has a 25% risk of inheriting the mutation and having the FA condition. About 5% of FA individuals do not carry a mutation on the known FA genes, suggesting that more FA genes will be discovered in the future. FA affects both males and females from all ethnic origins. The current median lifespan for a person with FA is 29 years although some individuals live into their 50s. While FA is primarily a blood disorder, it affects many other body systems, leading to a number of complications. Many individuals develop a variety of cancers such as acute myeloid leukemia (AML) or squamous cell carcinoma (SCC) at an unexpectedly early age. Individuals who have had a successful bone marrow transplant and are cured of the blood disorder still must have regular examinations to watch for signs of cancer.

FA individuals have a 500-700 fold increased risk over the general population for developing SCC of the head and neck regions, most notably in the oral cavity and esophagus[1,2]. The causes for such elevated risk are varied and not all known. When diagnosed early these tumors can be treated successfully and relatively easily. However, when detected late, prognosis of these aggressive tumors remains poor, despite extensive surgical intervention and radiotherapy. Regular surveillance for these complications is therefore essential and FA individuals are advised to be examined regularly for early signs of abnormal lesions. Despite these guidelines for regular surveillance, not all healthcare providers are aware of the elevated risks of cancer in FA and few FA individuals seek regular medical attention for these complications.

The most current method for detecting malignant lesions is through visual inspection by experienced oncologists and head/neck surgeons. One problem with this approach is the fact that FA individuals often present with visible lesions of the oral cavity from graft-versus-host disease, particularly following HSCT, and consequently the majority of visible lesions might not be pre-cancerous. Furthermore, precursor lesions are not always visible to the naked eye or they are asymptomatic and missed.

In 2007, Dr. Brakenhoff piloted a non-invasive molecular assay to detect precursor lesions in the oral cavity. This novel method using LOH analysis has been adapted to test soft swabs of the oral mucosa. FA individuals accept and tolerate these soft-brush biopsies (including soft swabs for cytological evaluation and DNA cytometry) much better than the traditional scalpel biopsies and are more likely to comply with regular screening recommendations.

Based on these observations we propose to conduct a longitudinal study to (1) raise awareness about the high cancer risk in FA, (2) collect soft-brush biopsy samples to detect, differentiate and follow-up benign vs. malignant oral lesions as determined by cytology and/or DNA Cytometry and/or LOH analysis, (3) correlate presence and progression of cancer with exposure to environmental risk factors, (4) collect material and stimulate research collaborations, and (5) provide critical educational material and data to individuals, parents and researchers to improve treatment outcome and reduce the burden of SCC in FA.

Chapter 3 **Study sponsors and Principal Investigators**

The Fanconi Anemia Research Fund, Inc. in the United States and the Deutsche Fanconi-Anaemie-Hilfe e.V. (German Parent and Patient Organization for Fanconi Anemia), are the sponsors of this project. The Board of Director and scientific advisors for FARF and for FANCONI.de are listed in **Appendix A**

The Fanconi Anemia Research Fund is a non-profit organization created to promote discovery of effective methods to treat and someday cure problems associated with Fanconi anemia. In addition to funding relevant research projects, the foundation provides educational and support services to the FA community worldwide.

The Deutsche Fanconi-Anaemie-Hilfe e.V. (FANCONI.de) is the active equivalent to FARF. Founded in 1990 by German Families with FA children FANCONI.de also supports international research projects for Fanconi anemia and provides support to family and patients worldwide.

The Co-Principal Investigators for this study in the U.S. are Laura Hays, PhD., Executive Director of FARF and Amy Frohnmayer, M.A., a member of the Board of Directors of the Fanconi Anemia Research Fund. Ralf Dietrich, Executive Director of FANCONI.de and Eunike Velleuer, MD will help coordinate the study.

Chapter 4 **Purpose of the Study**

This study aims to inform FA individuals and their families of the risks of oral and oropharyngeal SCC; to collect, test and analyze samples from FA participants with follow-

up over time; to provide materials and stimulate research collaborations and innovation and ultimately to reduce the burden of SCC in FA individuals.

Chapter 5 **Who can participate?**

This study is designed for FA individuals, irrespective of age, gender, nationality or ethnicity. We will recommend that sample collection be conducted at a dental practice or hospital for individuals with increased risk of bleeding as indicated by visible bruises and petechiae in the prior 2 weeks, or platelets lower than 20,000 if platelet count is known.

Chapter 6 **Subject recruitment/sites**

Our goal is to reach as many FA individuals as possible. To that end we have prepared an announcement flyer that we propose to distribute widely to all FA-related support groups, associations, research meetings and other events, hospitals and academic institutions (**Appendix B)**. Specifically, we will post the announcement on the FARF website, and send it via letter mail, email, social media sites (through FARF) to FA individuals and family on the FARF mailing list or provide it to attendees at FA family meetings, FA adult meetings and/or Camp Sunshine.

Camp Sunshine offers an annual meeting for individuals with FA and their families. This event takes place each summer and incorporates scientific presentations about this disease as well as support group gatherings. This meeting represents a unique opportunity to reach large numbers of people with FA in one location within a relatively short period of time. A verbal announcement will be made at the beginning of the meeting informing families about the purpose and procedures of this study and inviting people to participate. The decision to participate (or abstain) will have no effect on regular camp activity. FA individuals will be given the opportunity to participate at other times, for instance, through home visitations depending on funding availability.

We will also provide information sheets, one for individuals over 14 years old (**Appendix C)** and one for children 7-14 years old (**Appendix D)**., that explain the details of the study. Interested participants will be able to review the study goals and procedure description at their leisure.

Participation is voluntary and does not impact standard of care. The decision to participate or not to participate will not affect current benefit or standing. There is no penalty of loss of benefit for declining to participate.

Interested participants will confirm their consent/assent to participate by signing the informed consent form. At least one designated study investigator will be present to explain the study procedures in person, respond to participant questions, and collect consent/assent.

Individuals who have consented will be given a unique and random code identifier to be used instead of their names on all material collected. The sponsors and study staff will keep the identity of the individual confidential.

The following measures will be taken to guarantee participant privacy. Nobody will be forced to do anything with which he or she feels uncomfortable. It will be made clear that participation is an invitation, not a recommendation. The intervention and interviews, including sensitive questions about substance use, will be conducted in a private setting in a separate room.

Chapter 7 **Experimental Components and Procedure**

The experimental components of this study are:

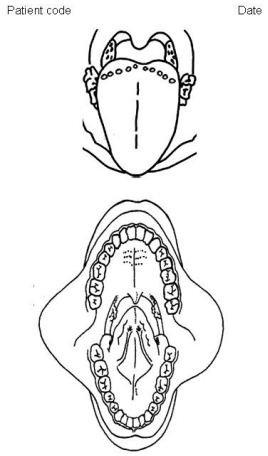
- A health questionnaire
- Non-invasive oral cavity screening: photographs and maps of oral lesions
- Sample collection with self-administered soft-brush biopsy procedure
- Cellular and molecular analysis of samples
- Report of cytological results of visible lesions to individuals and their treating physicians if applicable
- Optional additional sample collection for research and methods development Optional transfer of de-identified data onto a centralized data repository (such as Synapse) to be used in future research

The procedure will take place at FA family meetings, home visits, and local meetings in academic hospitals. The sequence of events for consented participants is as follows:

- **Step 1: Conduct interview and complete health questionnaire**

We will use structured interviews and a questionnaire to estimate the quality of surveillance of FA individuals by head and neck specialists according to the *Fanconi Anemia Guidelines for Diagnosis and Management* [3]. Specifically, we will ask contact information for the participant and his/her health care provider. We will also ask demographic information and medical history as related to FA. The questionnaire is included in **Appendix E**

- **Step 2: Inspect, photograph and map the lesions**



We will perform a careful and standardized inspection based on the WHO oral cancer diagnosis protocol and we will document any visible oral lesion in an individual “oral cavity map” (figure 1). In addition we will document the lesions using the Karl Storz Endoscopic Oral Documentation System and digital pictures [4]. Note that the pictures will focus exclusively on the lesion and will not contain any facial features that can reasonably re-identify the participant.

Figure 1: individual oral cavity map

- **Step 3: Collect samples via soft-brush biopsy**

Soft brush biopsies are much more accepted than traditional surgical biopsies. For the purpose of this study, the biopsies for the LOH analysis (described below) will always be performed and the cytological brush biopsy will be used in cases where there is a visible lesion. These minimally invasive procedures can be performed by the participant him- or herself with instructions or under the guidance of trained personnel. These procedures do not require standardized conditions, making them particularly well suited for data collection in FA family meetings or homes.

The diagnostic value of the brush biopsy procedure has been validated in numerous studies [5–10]. Figures 2 and 3 illustrate the soft brush biopsy procedures for the LOH and cytology analyses.

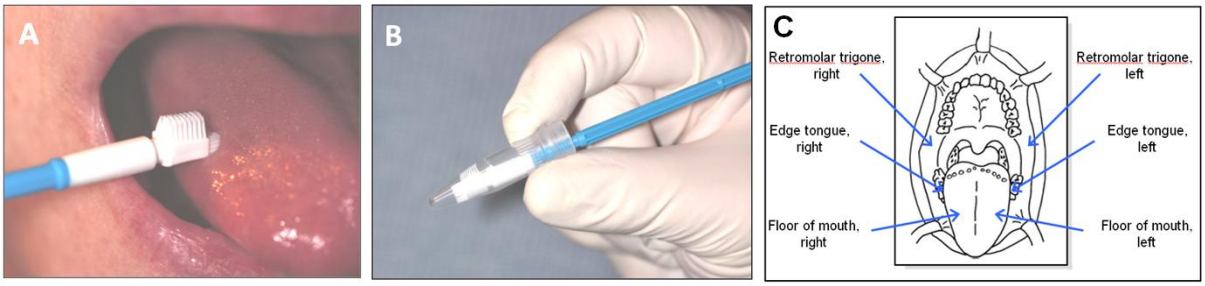


Figure 2: Procedure for brush sampling in the oral cavity for LOH analysis.
(A) The tissue is brushed superficially five times with a sterile Rovers Orcellex Brush (Rovers Medical Devices B.V., Oss, NL). (B) The brush containing the sample is placed in a 1.5 ml reaction tube filled with a fixative (CytoLyt from Hologic, Bedford, MA, USA). Each vial is labeled with a unique individual code. (C) Generally, samples from six separate high-risk areas of the oral cavity are collected from each participant. Each location is noted on the respective vial by the letters A-F. If visible lesions at different anatomical regions are present, they will be brushed as well (labeled with the letters beginning from G). Three separate brushes are used on each area and the steps above are repeated for each brush to provide controls.

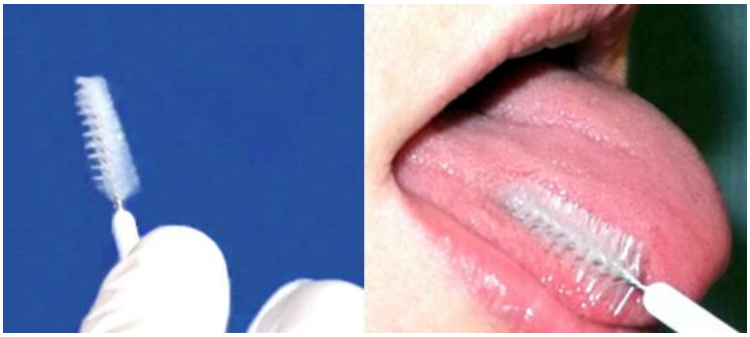


Figure 3: Procedure for cytological brush biopsy.
(A) If visible lesions, which have not been biopsied previously, are present, each is brushed ten times with three separate Cervi Brushes (CellPath Ltd, Newtown, UK) (B) All brushes of the same lesion are then twisted into a tube containing an alcohol-based fixing solution.

Each collection tube is labeled with the unique individual code.

- **Step 4: Optional saliva and tissue collection**

Participants will have the option to provide saliva and an additional soft brush biopsy for other research in FA. Saliva will be collected through a small absorbent saliva pad to evaluate the presence of biomarkers for oral cancer detection. Depending on how much saliva the participant has, the pad may remain in the mouth of the participant for 5-10 minutes. The collected saliva is pressed out of the pad and into a tube containing an alcohol-based fixing solution. The additional soft brush biopsy will be used to evaluate the specific bacteria (microbiome) present in the mouth.

- **Step 5: Data analysis**

Coded specimen will be sent to various laboratories for analysis. The laboratories will be blinded as to the identity of the subject.

Genetic changes in the oral cavity: The brush biopsies taken from six high-risk locations within the oral cavity will be sent to the laboratory of Prof. Brakenhoff, Netherlands. For participants who have not received a bone marrow transplant, an LOH analysis will be conducted. In a preliminary analysis it was shown that genetic changes detectable by loss of heterozygosity analysis (LOH) are a strong and significant predictor of cancer in the FA population. LOH analysis describes allelic loss at chromosome arms 3p, 9p, 11q and 17p. Samples from participants who have already had a bone marrow transplant will be stored until a technique has been found to distinguish between the individuals' and the bone marrow donor's genetic information.

Cytological analysis: Brush biopsy samples from participants with visible lesions will be sent to the laboratory of Prof. Biesterfeld at the University of Dusseldorf, Germany for cytological evaluation including DNA image cytometry to determine the proportion cells with DNA content suggestive of aneuploidy if suspicious lesions are detected. This test indicates chromosomal changes, which are known to be present in pre-cancerous and cancerous tissue[10].

Microbiome analysis: Brush biopsy samples from participants will be sent to the laboratory of Prof. Henrich at the University of Dusseldorf, Germany (Institute for Medical Microbiology and Hospital Hygiene) for microbiological evaluation. After DNA extraction and labeling, sequencing will be performed on the HiSeq2000 Illumina platform. Species-specific differences will be analyzed using TaqMan-PCR.

Biomarker of oral cancer in saliva samples: The goal of the study is to evaluate in FA a panel of salivary oral cancer genomic and proteomic biomarkers shown to correlate with oral cancer development in non-FA individuals. The saliva sample of FA participants will be assayed via. qPCR and conventional ELISA to determine the levels of 7 pre-validated salivary oral cancer transcriptomic (IL8, IL1b, DUSP1, HA3, OAZ1, S100P, SAT) and 7 proteomic (MRP14, CD59, Catalase, M2BP, Profilin, IL8 and IL1 b) biomarkers.

- **Step 6: Data reporting**

The cytology and cytometry results will be provided to the study investigators who will use the protected database containing unique identification codes to determine the identities of participants and send them their data reports. These reports will include: a diagram of the mouth indicating location of any visible lesion(s); high resolution photos of the lesion(s); results from the cytological analysis which indicates the morphology of the cells. In case suspicious cells were identified a DNA image cytometry report will be included and participants will be strongly encouraged to visit their local physician for further investigation.

Due to heightened susceptibility to cancer, all participants will be encouraged to visit their local physicians often as per recommendations outlined in the *Fanconi Anemia Guidelines for Diagnosis and Management*, and especially if they develop a visible lesion that does not disappear within 2-4 weeks.

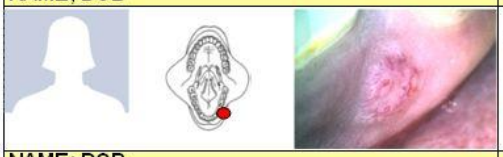
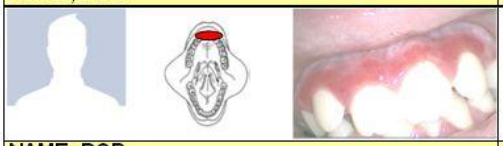
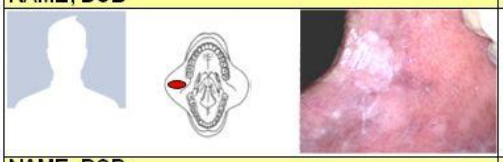
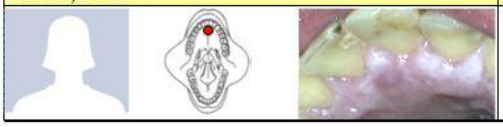
NAME; DOB	Cytology	DNA-Cytometry	Recommendation
	**negative;** hyperkeratotic oral mucosa; mild inflammation; no dysplasia.	no	clinical follow up
NAME; DOB	Cytology	DNA-Cytometry	Recommendation
	**suspicious;** abnormal regenerating epithelia with a hemorrhagic component	active regenerative epithelial of the oral mucosa	clinical follow up
NAME; DOB	Cytology	DNA-Cytometry	Recommendation
	**suspicious;** abnormal regenerating epithelia; hemorrhagic component	**aneuploid,** indicative for an early malignant transformation.	biopsy !
NAME; DOB	Cytology	DNA-Cytometry	Recommendation
	**suspicious;** acute erosive inflammation of the oral mucosa with bacterial overlay and progression into regenerating epithelia	The euploid-polyploid DNA-distribution is compatible with active regenerative epithelial of the oral mucosa	clinical follow up
NAME; DOB	Cytology	DNA-Cytometry	Recommendation
	**negative;** normal oral mucosa with mild inflammation; no dysplasia. signs for an infection with candida albicans.	no	treat against candida

Figure 4: Example of a cumulative report to a treating physician from five FA individuals of cytological smears. Included are the individual mouth maps as well as the pictures of the visible lesions.

- **Step 7: Follow up**

Follow up visits, including mouth inspection, repeated brushing and collection of an updated participant medical history, will be proposed to participants at least every second year. In addition, we will ask an update on the status of visible oral lesions twice a year.

Chapter 8 **Data Storage and Confidentiality**

We take data security, privacy and confidentiality very seriously. Each individual's contact information will be kept secure and confidential, and separated from the research data. To protect every participant's privacy we will not share identifiable information with researchers and laboratories performing the assays. Instead we will use a unique code identifier on the specimen and on the data. This means that no organization outside the participant, the sponsor and study staff, and regulatory agencies will be able to link the data/health information to the participant. Note that all these personnel will have completed mandatory training on protection of human subjects in research.

With each participant's permission, we will transfer electronically the de-identified, coded research data to research databases such as the Sage Bionetworks' data repository and analysis platform, Synapse. We will make it available to selected researchers with the goal to identify biomarkers that can be used as reliable predictors for the development of oral SCC or other FA Research. The research results will not include information that can link the data to the subject.

With participant's permission, the coded specimen leftovers will be frozen and stored by the laboratories for future analysis and research or methods development.

Chapter 9 **Potential Risks and Discomforts**

The sampling of cells from the oral cavity with a small brush is generally painless but it may give a tickling sensation. Some participants may show some redness of the brushed area, which usually disappears within a few hours or 1 to 2 days. In a European study, less than 10% of participants with visible lesions reported some slight pain and bleeding of the brushed lesions for a couple of minutes. Longer lasting bleedings, swelling or infections cannot be excluded but have not been observed so far. Only individuals who have known platelet counts above 20,000 or no bruising or unusual bleeding in the preceding 2 weeks will be accepted for local brushings. FA individuals with lower platelet counts may be included in the study provided that the brushing is done in a local dental practice or hospital.

Receiving results from the cytology and cytometry tests may cause emotional stress, especially if signs for pre-cancerous or already cancerous lesions are identified. The study coordinator will recommend that participants get in contact with their local physicians to exclude or confirm cancer with the help of a classical scalpel biopsy.
The participant will be informed that an ultimate cancer diagnosis can only be confirmed by a specialist based on the results of the local pathologist. In some cases, the pathology will not confirm an abnormality either because the test will show changes in cells earlier than a

pathologist can recognize or because of a false positive, in which case a tissue biopsy would not have been necessary.
The local specialist will be responsible for recommending further steps for treatment and to find local experts for the individual, with whom he/she can talk about emotional stress after the final diagnosis.

One risk to contemplate is the potential loss of privacy and confidentiality in case of public disclosure due to unintended data breaches, including hacking or other activities outside of the procedures authorized by this study. In such cases, the data may be misused or used for unauthorized purposes by someone sufficiently skilled. We are taking such risk very seriously and are using IT measures to reduce this risk such as coding, encryption and keeping identifiable data in locked cabinets.

Chapter 10 **Potential Benefits**

Improved information, photographic documentation of visible changes over time, a policy of targeted rather than routine biopsy guided by the results of cytological analyses, can lead to earlier detection of suspicious lesions. Benefits to the subjects include increased awareness of the intrinsically high risk of HNSCC in FA and of additional risk factors that may motivate individuals to participate in early lesion detection. These efforts should result in improved prevention and/or therapy of this devastating tumor variety. Ultimately, individuals may benefit from conventional and novel therapeutic strategies that may specifically target SCC [11–13].

Beyond improved patient care, the concept of a specialized network formed by patients, caregivers and their respective physicians will facilitate exchange of knowledge and stimulate cooperative research on the still elusive pathogenesis of SCC in FA.

Chapter 11 **Conclusion**

The aim of this study can be summarized by three primary points: The first is to inform individuals with FA about the highly elevated risk for squamous cell carcinoma in Fanconi Anemia, invite them to attend regular check ups with local caregivers and to invite them to participate in the study using a non-invasive test. The second is to test samples that may indicate whether pre-cancer lesions or cancer is present in the mouths of these individuals. The third is to identify biomarkers that can be used for early detection as reliable predictors for the development of oral SCC (or potentially for development of SCC in other high risk organs) in FA individuals.

A strength of this study comes from previous experience that has shown that contact with individuals within this population has met the needs of these people and led to increased surveillance.

Participants will be made aware that they should not rely solely on the observations made in this study. Investigators cannot exclude the possibility that potential lesions are missed during the oral exam.

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APPENDICES

Appendix A Sponsor's Board of Directors and Scientific Advisors

Fanconi Anemia Research Funds

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Appendix B Proposed announcement Flyer

Deutsche Fanconi-
Anämie-Hilfe e.V.

For people with Fanconi anemia (FA), mouth cancer remains a serious problem. People with FA get mouth cancer at much younger ages than in the general population and often without any known cause. When discovered early, mouth cancer can be treated relatively easily, but treatment becomes much more difficult when cancer is found at later stages.

New methods are being developed to detect very early signs of mouth cancer in simple, noninvasive ways. One of these methods involves brushing samples from the mouth with soft brushes and testing these samples for abnormal cells. We are currently conducting a research study to see if this test would be helpful for people with FA. We are also interested in learning more about molecules in the saliva that could indicate the presence of cancer and improving research collaborations in FA.

We are recruiting people with FA to help us with this research study through participating in one or more of the following: brushing certain places in the mouth with soft brushes under our guidance, donating saliva, completing a health questionnaire, and keeping in touch with us.

We would greatly appreciate your help with this project. If you think you might be interested in joining or want to learn more, please review the study description at <include links> or contact one of the project investigators, Laura Hays, PhD or Amy Frohnmayer, by email: study@fanconi.org or by calling the Fanconi Anemia Research Fund Office at 1-541-687-4658.

Appendix C Information sheet for adolescents over 14 and adults

Fanconi Anemia RESEARCH FUND, INC.

Deutsche Fanconi-Anämie-Hilfe e.V.

Information for people with Fanconi anemia (aged 14 and older)

Title of the study: "Reducing the burden of squamous cell carcinoma in Fanconi anemia"

Hello,

We would like to invite you to be part of a research study. You are being asked to participate because you have been diagnosed with Fanconi anemia (FA).

Why are we doing this project?

People with FA have a higher than average risk of developing mouth cancer at young ages. Because of this, it's important that you have your mouth checked regularly by an experienced head and neck doctor (ENT). Mouth cancer can be treated relatively easily when detected early, but when discovered in later stages, treatment becomes more difficult. This project will help us learn if we can use simple, noninvasive techniques to collect and test samples for early signs of oral cancer and pre-cancer.

What will you be asked to do?

- **Health questionnaire:** We will ask you to answer questions about yourself, your medical history, and current health. You may choose to leave any questions you do not wish to answer blank.

- **Photograph and Oral map:** We will examine the inside of your mouth carefully and document any spot, mark or visible lesion on a diagram ("oral cavity map") so we can remember where they are.

- **Brush samples:** We will ask you to give us small amount of cells from your mouth using a soft brush. First, we will ask you to gently brush six places in your mouth with a soft brush under our guidance for LOH analysis (LOH means Loss of Heterozygosity). You will not receive a report from this analysis. (*Note for FA participants who have received a bone marrow transplant: At this time, this test does not work well for people who have had a bone marrow transplant. Researchers would still like to have brushes from transplanted patients to try to improve this test. These samples will be frozen and stored until a better method is found.*)

  We will also ask you to brush any visible lesions under our guidance for further cytological analysis. This test looks at the shape and morphology of the cells for any pre-cancerous and cancerous changes. You and your doctor will receive a report of this test on the visible lesions if you wish.

- **Extra brush samples**: We would like to test for early signs of serious mouth problems in FA using other research procedures. If you agree to provide extra samples we will ask you to brush certain places in your

mouth once more under our guidance, as well as any visible spots, for another study looking at specific bacteria present in the mouth. You will not receive a report from this analysis.

- **Saliva sample:** If you agree to donate a saliva sample, you will be asked not to eat, drink, smoke, or use oral hygiene products (like toothpaste or mouthwash) for at least half an hour before. You will rinse your mouth with water, place a white pad (like a small sponge) in your mouth, and then, after about 2-5 minutes, put the pad into a cup we provide.

What do we hope to learn with the samples you donate?

We hope to use the samples you brush from your mouth to see whether it is possible to use a new method, called an "LOH" analysis, to find pre-cancerous cells very early in people with FA. When we find visible spots in the mouth, brush samples from these areas can be tested for suspicious cells using an existing test, called a "cytological analysis." Extra brush samples will be used to investigate whether there is a link between certain types of bacteria and the development of head and neck cancer in people with FA. Through collection of saliva, researchers hope to learn whether we can find molecules in saliva that would indicate the presence of early cancer.

You do not need to agree to participate in all of the above in order to be part of this project.

Possible risks Side effects of participating in this study

Brushing the mouth and collecting saliva are generally painless. The brushing might give you a tickling sensation and may cause irritation in places where the skin is already sensitive.

Analysis of brush samples may indicate an abnormality that is later found to be normal by a traditional tissue biopsy. This could happen because the brush test finds pre-cancer cells earlier than a pathologist can find them. However, it is also possible that the brush test was done too early or was inaccurate and a tissue biopsy was therefore not necessary.

Possible benefit of participating in this study

A possible advantage of participating is that if a suspicious spot is seen, you and your doctor will be alerted right away and you will be able to watch it carefully.

CAUTION: We could miss abnormal areas in our inspection. You should continue having regular checkups with your local doctor or dentist. If you find unusual spots in your mouth that do not vanish after a couple of weeks, talk to your doctor right away.

Time to think it over

Please take all the time you need to think about whether you would like to participate in this project. Please do not hesitate to talk with family, friends and/or the project staff if you have any questions before you decide.

Confidentiality

The information that we collect about you during this project will remain confidential. Only the research team will have access to your name and personal information. For minors, please note that your parents can request and receive your data including your responses to the questionnaire.

Participation is voluntary

You are completely free to decide whether you want to participate in this research project or not. If you decide to participate, you are free to change your mind and discontinue participation at any time.

Further information

We will ask to collect brush samples from FA patients who wish to participate at least every second year. If after reading this document you would like more information, or have new questions at any time before, during, or after the project, you can contact Laura Hays, PhD or Amy Frohnmayer by email: study@fanconi.org or call the Fanconi Anemia research Fund at 1- 541-687-4658.

Signing the consent form

If you decide to participate, we will ask you to confirm that you agree through signing an informed consent form. You may choose whether you want to participate in all parts of this project or only in some. If you change your mind about participation, you may contact us and indicate your desire to discontinue participation at any time. Parents must also sign the consent form to let their child participate in the study.

Appendix D Information sheet for Participants up to age 14

Fanconi Anemia RESEARCH FUND, INC.

Deutsche Fanconi-Anämie-Hilfe e.V.

Information for children with Fanconi anemia (up to age 14)

Title of the study: "Reducing the burden of squamous cell carcinoma in Fanconi anemia"

Hello,

We would like to invite you to be part of a research study. You are being asked to participate because you have been diagnosed with Fanconi anemia (FA).

Why are we doing this study?

Some people with FA can have serious problems in their mouth and throat when they become older. We are trying to see if we can find early signals for these problems by brushing small amounts of material from several places in your mouth and testing them for abnormalities. People without FA have used this test. We want to know if this test can be helpful for children and adult with FA.

1. What will happen?

We will ask you questions about yourself, your health and how you are doing. If you do not want to answer a question you do not have to.

We will look at the inside of your mouth carefully and take pictures of your mouth with a camera. If you have spots we will mark them on a diagram ("oral cavity map") so we can remember where they are.

We will ask you to gently brush 6 places in your mouth with a small soft brush twice. We will show you how to do it. (Note for FA individuals who have received a bone marrow transplant: At this time, this test does not work well for people who have had a bone marrow transplant. Researchers would still like to have brushes from transplanted patients to try to solve these problems. These samples will be frozen and stored until a better method is found).

If you have spots in the mouth we will ask you to brush them with another special brush for a different test ("DNA ploidy" test) to see if the spots are harmless or may become a problem. In our experience, most visible spots in the mouth of people with FA are harmless and disappear after a while. Later your parent and your doctor will receive the result of this test.

We will ask if you agree to give us extra samples to test for early signs of seroious mouth problems in FA with other research procedures. For that we will ask you to brush the inside of your mouth once more and to give us some of your saliva to see what bacteria you have in your mouth. To give saliva you cannot eat,

drink, smoke, or use oral hygiene products (like toothpaste or mouthwash) for at least half an hour before. You will rinse your mouth with water and place a white pad (like a small sponge) in your mouth for about 2-5 minutes, then put the pad into a cup we provide.

You do not need to agree to participate in all of the above in order to be part of this study.

2. Possible risks and side effects of participating in this study

Brushing the mouth and collecting saliva are generally painless. The brushing might give you a tickling sensation and sometimes it can cause irritation in places that are already sensitive.

The brush test could find an abnormal spot that is later found to be normal by a tissue biopsy. It could also be that the brush test was done too early or that it was inaccurate, and a tissue biopsy was therefore not necessary.

3. Possible benefit of participating in this study

If a suspicious spot is seen, you and your doctor will be alerted right away and you will be able to watch it carefully.

CAUTION: we could miss an abnormal area during our inspection. You should continue having regular check-ups with your local doctor or dentist. If you find unusual spots in your mouth that do not seem to vanish after a couple of weeks, talk to your doctor right away.

4. Time to think it over

Please take all the time you need to think about whether you would like to participate in this study. Talk with your family, friends and/or the study staff if you have questions before you decide.

5. Confidentiality

The information that we collect about you during this study will remain confidential. Only the research team will have access to your name and your personal information.

6. Participation is voluntary

You are completely free to decide whether you want to participate in this research study or not. If you decide to participate, you are free to change your mind and stop at any time.

7. Further information

We will ask to collect brush and saliva samples from FA individuals who wish to participate at least every second year. If after reading this document you want more information, or you have more questions at any time before, during and after the study, you can contact Laura Hays, PhD or Amy Frohnmayer by e-mail: study@fanconi.org or call the Fanconi Anemia Research Fund Office at : 1-541-556-4322

8. Signing the consent form

If you decide to participate, we will ask your parent(s) to confirm that you agree and to sign a consent form. You may choose whether you want to participate in all parts of this study or only in some.. If you change your mind at any time you and your parent(s) can contact us.

Appendix E Study Questionnaire

Deutsche Fanconi-
Anämie-Hilfe e.V.

Reducing the burden of squamous cell carcinoma in Fanconi Anemia
- Initial study questionnaire -

Today's Date (MM/DD/YYYY): _____/_____/_____

Please note: If you do not want to answer a question, leave it blank.
(*Note for minors: We are required by law to share your questionnaire with your parents or guardians if they ask for it.)*

1. Participant's Contact Information

Participant's First name _____ Last name _____

Gender: ☐ Male ☐ Female Date of birth (MM/DD/YYYY) _____/_____/_____

(If participant is a minor: contact information of parent or guardian):

First name _____ Last name _____

Street _____ City _____

State _____ Zip _____ Country _____

Adult participant or parent/guardian home phone _____

Adult participant or parent/guardian mobile phone _____

Adult participant or parent/guardian email _____

2. *Treating Physician's Information*

(Alternate name and address of the physician who could receive your data report.)

Physician's name _____

Institution/Hospital _____

Street _____

City _____ State _____ Zip _____

Telephone _____ Email _____

3. Diagnosis of Fanconi Anemia

Date of diagnosis (MM/YYYY) _____/_____

Complementation group (if known) _____

Gene mutation(s) (if known) Maternal _____ Paternal _____

4. Summary of Medical History

(*This information helps us understand the presentation of Fanconi anemia in your case.*)

Do you have anomalies due to FA? ☐ Yes ☐ No

(If yes, please check all that apply):

☐ Cardiac	☐ Neurological	☐ Ears/Hearing
☐ Reproductive/Gynecological	☐ Endocrine	☐ Eyes/Vision
☐ Respiratory	☐ Thumb/Radius	☐ Gastrointestinal
☐ Skeletal	☐ Growth	☐ Skin
☐ Kidney	☐ Genital	☐ Other

If other, please specify _____

In case we need more information about a specific anomaly may we contact your physician?

☐ Yes ☐ No

Do you have hematologic manifestations (low blood counts)? ☐ Yes ☐ No

Age of onset of decreased blood counts: ☐ 0 – 3y ☐ 4 – 10y ☐ older than 10y

5. Hematologic Manifestations

(*Blood counts can help us evaluate whether you have a higher risk of infection in your mouth or a higher risk for bleeding*)

What were your most recent blood counts?

Date (MM/DD/YYYY) ___/___/_____WBC ____ ANC _____ HGB _____ PLTS ____

Have you had a blood transfusion within the past 3 months? ☐ Yes ☐ No

Did you have unusual bleedings within the past 3 months? ☐ Yes ☐ No

If yes, please specify _____

Did you have recurrent infections within the past 3 months? ☐ Yes ☐ No

If yes, please specify _____

6. ***Visible Oral Lesions***

Have you had a visible oral lesion that has stayed for at least 6 weeks?

☐ Yes ☐ No (If No, skip to question 7)

Have you consulted a doctor/dentist about the lesion(s)? ☐ Yes ☐ No

Site(s) of oral lesion(s) _____

Was a biopsy performed? ☐ Yes ☐ No

Type of diagnosed oral lesion:

☐	Leukoplakia	☐	Yeast Infection	☐	Cancer
☐	Erythroplakia	☐	Bacterial Infection	☐	Unknown
☐	Graft versus Host Disease	☐	Viral Infection	☐	Other

If other, please specify _____

If you received any treatment for your visible lesion please indicate: _____

_____

7. ***Cancer History***

Have you ever been diagnosed with Squamous Cell Carcinoma (SCC)?

☐ Yes ☐ No (If No, skip to question 8)

Date(s) of cancer diagnosis (MM/YYYY) ____/_____ ____/_____ ____/_____

Location of cancer *(please check all that apply):*

☐	Mouth	☐	Esophagus	☐	Anus	☐	Cervix
☐	Pharynx	☐	Skin	☐	Vulva	☐	Other

If other, please specify _____

Did you develop metastasis of the cancers described above? ☐ Yes ☐ No

Did you receive surgery for these cancer(s)? ☐ Yes ☐ No

If yes, Date(s) (MM/YYYY) ____/_____ ____/_____ ____/_____

Did you receive chemotherapy or equivalent? ☐ Yes ☐ No

If yes, Date(s) (MM/YYYY) ____/_____ ____/_____ ____/_____

Chemotherapy agent(s) used _____

Frequency _____

Did you receive radiation? ☐ Yes ☐ No

If yes, year(s) (YYYY) _____ _____ _____

Total Dosages (Gy) _____ _____ _____

8. Smoking Habits

Have you ever smoked cigarettes regularly? ☐ Yes ☐ No (If No, skip to question 9)

Cigarettes smoked per week on average? _____
For how many years/months did you smoke regularly? Years _____ Months _____

If you stopped smoking, when did you stop (MM/YYYY)? ____/ _____

Do you (did you ever) consume regularly one ore more of the following:

☐	Pipe	☐	Water pipe	☐	Snuff	☐	Other
☐	Chewing tobacco	☐	Cigars	☐	Marijuana		

If yes to any of the above or other, please specify (kind, duration, amount, stopped, restarted):
_____

9. Drinking habits

Do you (did you ever) drink alcohol occasionally or regularly?

☐ Yes ☐ No (If No, skip to question 9)

How many drinks do (did) you usually consume on a drinking day?

☐	1 drink	☐	2 drinks	☐	3 drinks	☐	4 drinks	☐	5 or more drinks

How frequently do (did) you drink alcohol?

☐	Daily	☐	2 times a week	☐	Once a month
☐	Nearly every day	☐	Once a week	☐	6-11 times a year
☐	3-4 times a week	☐	2-3 ties a month	☐	1-5 times a year

What kind of drinks do/did you prefer?

☐	Beer	☐	Wine / Champagne	☐	Hard Liquor	☐	Cocktails	☐	Other

If other please specify _____

For how many years/months did you drink regularly? Years _____ Months _____

If you stopped drinking, when did you stop? (MM/YYYY) ____/ _____

Additional information about drinking habits (e.g. stopped, restarted) _____

10. ***Therapy***

(*We want to find out if special kinds of treatments people with FA receive have an impact on the development of squamous cell carcinoma.)*

Did you receive any of the following treatments?

Androgens ☐ Yes ☐ No Treatment duration (MM/YYYY) ____/ _____

Growth Hormones ☐ Yes ☐ No Treatment duration (MM/YYYY) ____/ _____

Bone marrow transplant (BMT) ☐ Yes ☐ No

Date of BMT (DD/MM/YYYY) _____/_____/_____

Radiation used? ☐ Yes ☐ No

Graft versus Host Disease (GvHD)? ☐ Yes Acute ☐ Yes Chronic ☐ No

Treatment agent(s) for GvHD used _____

Frequency _____

Other Therapy (please specify) _____

Treatment duration (MM/YYYY) ____/ _____

11. ***HPV***

(*We want to find out if HPV has an impact on the development of squamous cell carcinoma in FA.*)

Have you had the HPV vaccine? ☐ Yes ☐ No If yes age(s) at vaccination ___ ___ ___

Name of vaccine: ☐ Gardasil ☐ Cervarix ☐ other (please specify) _____

Has the participant ever had a HPV infection? ☐ Yes ☐ No ☐ Not known

12. ***Questions related to the Microbiome Study***

(Answering these questions will help us conduct the microbiome assay. Leave these questions blank if you do not want to answer.)

Did you receive one or more antibiotic treatments within the past 3 months? (Antibiotics may affect the microbiome in your mouth)

☐ Yes ☐ No If yes, please specify:

Name of antibiotic _____ Treatment duration (days) _____

Name of antibiotic _____ Treatment duration (days) _____

Name of antibiotic _____ Treatment duration (days) _____

13. ***Questions related to the Salivary Biomarkers Study***

(Answering these questions will help us conduct the Salivary Biomarkers study. Leave these questions blank if you do not want to answer.)

What is your ethnicity?

☐	Caucasian	☐	Asian	☐	I rather not say
☐	African American	☐	Hispanic	☐	Other

If other please specify _____

Do you have:

☐	Diabetes Type I	☐	Diabetes Type II (Insulin dependent)
☐	Hepatitis	☐	HIV

Do you have prior history of cancer (other than Squamous Cell Carcinoma)?

☐ Yes ☐ No If yes, please describe briefly (where and date only): _____
_____

Do you have a family history of cancer? ☐ Yes ☐ No

If yes, please indicate the kind of cancer (breast, rectal, lung, etc.): _____
_____