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Quercetin chemoprevention for Squamous cell carcinoma in patients with Fanconi anemia

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TABLE OF CONTENTS

I. PURPOSE OF STUDY

Primary Objectives:

1. To determine the efficacy of Quercetin in reducing buccal micronuclei (a surrogate marker of DNA damage and susceptibility to squamous cell carcinoma due to genomic instability) in post-HCT patients with fanconi anemia (FA).

Correlative studies:

Measure the impact of Quercetin therapy on additional potential surrogate markers:

1. Peripheral blood ROS and salivary ROS
2. Salivary total antioxidant capacity
3. Biomarkers measured via Exhaled Breath Condensate (EBC) - anti-oxidants, aldehydes etc.
4. Oral microbiome
5. Skin elasticity

II. SIGNIFICANCE OF STUDY IN RELATION TO HUMAN HEALTH

Fanconi anemia (FA) is an autosomal recessive disease characterized by progressive bone marrow failure, variable congenital abnormalities and a predisposition to malignancy, particularly acute myeloid leukemia (AML) and squamous cell carcinoma (SCC)[1]. The genes encoding for 22 subgroups have been identified so far[2-5] Cells from FA patients exhibit hypersensitivity to alkylating agents such as mitomycin C and diepoxybutane (DEB). Currently, FA is diagnosed by testing for chromosome breakage after lymphocyte stimulation and exposure to mitomycin C (MMC) or diepoxybutane (DEB)[6]. Chromosome fragility, defined by an increased percentage of chromosome breaks, is diagnostic for FA [7]. Although it is the most common form of constitutional aplastic anemia it is quite uncommon and the true incidence of FA is not known. A total of 754 patients from North America with the DEB confirmed diagnoses of FA were registered into the International Fanconi Anemia registry (IFAR) by 2001[8]. The major cause of morbidity and mortality for children with FA is progressive bone marrow failure[9] and development of myelodysplastic syndrome (MDS)/leukemia or squamous cell carcinoma (SCC) of head and neck (HNSCC)/genitourinary system [1].

Currently, the only curative treatment option for the hematological complications of FA include hematopoietic cell transplantation (HCT), with its associated toxicities from the transplant conditioning regimens, graft-versus-host disease and increased risk of post-transplant malignancy compared to patients without FA (i.e. adding to their baseline increased risk of malignancy).

Improved transplant outcomes[10-12] are modifying the natural history of Fanconi Anemia. Improved transplant survival, no radiation exposure, almost no GVHD, increases the importance of addressing later SCC even further. HNSCCs are the most common solid tumor in individuals with FA The incidence is 500- to 700-fold higher than in the general population. Individuals successfully treated with HCT are also at increased risk (4.4 fold higher risk) for solid tumors, in addition to the baseline increased risk related to FA[13]. The HNSCCs in FA show distinct differences compared to HNSCCs seen in the general population. They occur at an earlier age (20-40 years) than in the general population, are most commonly in the oral cavity (e.g., tongue), present at an advanced stage, and respond poorly to therapy (<20% overall survival). Moreover, individuals with FA are at increased risk for second primary

cancers in the skin and genitourinary tract. The pattern of second primaries resembles that observed in human papilloma virus (HPV)-associated HNSCC in the general population[14]. In addition, solid tumors may be the first manifestation of FA in individuals who have no birth defects and have not experienced bone marrow failure. These individuals commonly end up having significant and at times life threatening complication after treatment with chemotherapy and radiation (due to their baseline inability to repair DNA damage) before diagnosis of FA is made.

Based on studies in both animals and human subjects indicating that high levels of systemic ROS and increased sensitivity of hematopoietic progenitors to ROS plays a key role in pathogenesis of bone marrow failure in patients with FA (see section III) and these can be reversed by antioxidant Quercetin. We recently completed our pilot study of safety, feasibility (of long term administration) and pharmacokinetics (PK) of Quercetin in patients with FA, who have not undergone HSCT. Results of our own pilot study of quercetin, a natural anti-oxidant showed excellent tolerance, feasibility of long term supplementation and biologically relevant blood levels in patients with FA (NCT#01720147) (see section III).

An elevated oxidative status with concomitant depletion of antioxidants has been found in breast cancer and head and neck cancer, suggesting their contribution to carcinogenesis[15,16].

Recently, ROS were demonstrated to mediate signaling of c-met, the hepatocyte growth factor receptor, resulting in increased metastatic capacity[17,18]. The potential chemotherapeutic efficacy of quercetin has also been demonstrated in many types of cancers including SCC[19-24].

In fact Quercetin is known to affect various other pathways involved in carcinogenesis in addition to its effect on the Redox pathway to cause apoptosis and inhibit proliferation[19]. We propose to target these key pathways (also known to be involved in pathogenesis of SCC in FA) to prevent development and/or early progression of SCC in post-HCT patients with FA.

The results of the proposed project will use important preclinical data (see below) to support the development of a novel therapeutic approach for prevention and/or treatment of SCC in FA. We anticipate that quercetin will prevent or delay the development of SCC and associated complications, there by ameliorating or delaying the need for potentially lethal treatment with chemotherapy and/or radiation therapy for the same. This will be included in standard clinical care of patients with FA, potentially for many years.

III. PREVIOUS WORK DONE IN THIS AREA

Studies in both animals and human samples indicate that high levels of systemic reactive-oxygen species (ROS) and increased sensitivity of hematopoietic progenitors to ROS play a key role in the pathogenesis of marrow failure associated with FA and responds to treatment with anti-oxidants (Inflammatory reactive oxygen species-mediated hemopoietic suppression in Fancc-deficient mice[25]. Quercetin, a naturally occurring anti-oxidant is the best studied dietary flavonol that lacks major side effects[26]. It has a wide range of biological activities including but not limited to free radical scavenging, anti-inflammation, antioxidant and antineoplastic properties.

We recently reported results of our Phase 1 open label, single arm, pilot study of safety, tolerability and pharmacokinetics (PK) of Quercetin (3, 30, 40, 5, 7-pentahydroxyflavone) in patients with FA[27] .

Standard 3x3 Phase 1 design was used.

Initial treatment phase was 16 weeks followed by an optional continuation phase of 20 more months.

Dose limiting toxicity and Stopping rules were defined and an IND was obtained from FDA (**IND# 113343**).

In this prospective, pharmcokinetically guided, stepwise dose finding Phase I study of Quercetin in patients with FA, we have shown the following:

1) **Quercetin is safe and well tolerated in patients with FA.**
   Twelve patients with FA with a median age of 7 years (range: 3-21), were treated with oral quercetin twice a day for 16 weeks. Quercetin was well tolerated without attributable adverse events. Four patients were noted to have significant weight gain; 4-10 kg over 4 - 18 months. This is a desirable effect in this population as patients with FA usually have trouble gaining weight.

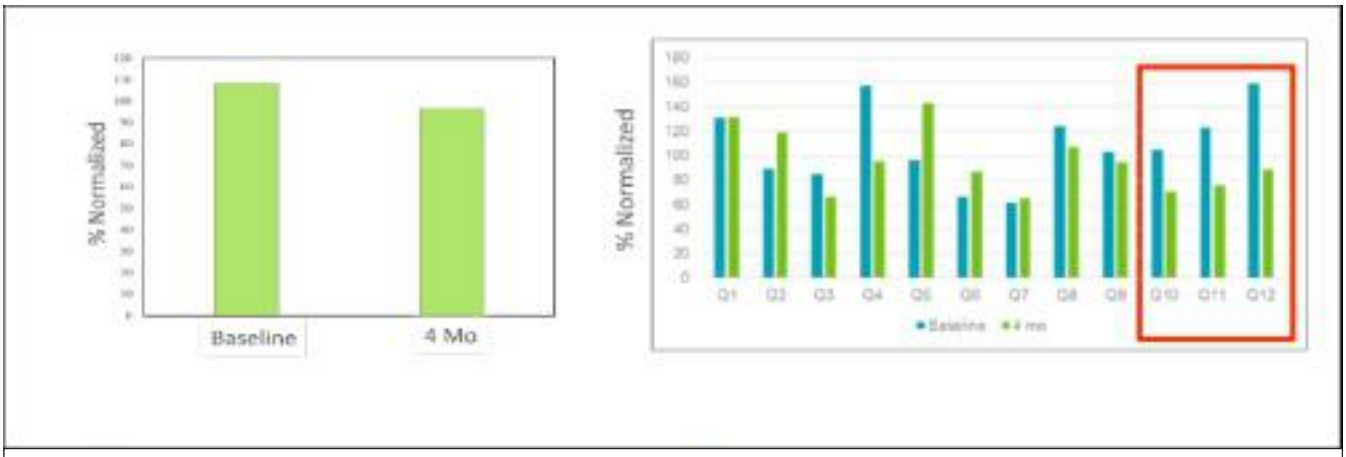
2) **Long term administration of oral Quercetin is feasible in patients with FA.**
   Patients received *up to two years* of quercetin without hardly missing any doses. Parental withdrawal related to inability to administer the study agent was considered to be the evidence of lack of feasibility. In addition, if ≥3 out of first 6 patients missed ≥2 weeks of therapy/month during first 4 months, we would have concluded that long term oral quercetin therapy is not feasible. However both of these stopping rules of lack of feasibility were not met.

3) **Goal levels of Quercetin were achieved in patients with FA.**
   Pharmacokinetic (PK) measurements of Quercetin were performed around 1st dose and again at 16 weeks. Quercetin concentration-time profiles were similar to previous reports in non-FA individuals (108).

4) **Impact of Quercetin on ROS (marker of DNA damage) reduction and preservation of stem cell reserve were used as surrogate markers for prevention of progressive marrow failure.**
   As shown in the **Table 1** below, Quercetin dose was adjusted as needed based on serial assessments of Quercetin levels (per PK) and change in peripheral blood ROS for each cohort of 3 patients. Peripheral Blood ROS levels (marker of DNA damage) were reduced after Quercetin therapy; highest reduction children taking the optimized dose **(Fig 1).**
   Peripheral Blood ROS (surrogate marker of DNA damage) levels were consistently reduced at the highest dose tested (last cohort of 3 patients). **(Fig 2).**

Dose level	Patient #	Weight adjusted maximum daily dose (mg/day)	Levels achieved AUC0-24 (ng.h/mL) First dose/ 4 months	Peripheral blood ROS level at 4 months	Overall assessment for each cohort (PK + effect on PB ROS)
1	1	750-1150-1500	5701/NA	Stable	Mixed response
	2		6680/11156	Increased	
	3		3640/6892	Decreased	
2	4	1500	1308/1601	Decreased	Unfavorable
	5		3500/2308	Increased	
	6		3602/3672	Increased	
3	7	3000	7553/8759	Stable	Reasonable
	8		4418/5938	Decreased	
	9		4934/5304	Decreased	
4	10	4000	5232/9952	Decreased	Optimal
	11		5930/4439	Decreased	
	12		7281/5186	Decreased	

Table 1. Fourth dose level achieved biologically relevant levels in all 3 patients



A. **B.**

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Total ROS normalized to control normal individuals respective to the time. Values at baseline compared to ones after 4 months of Quercetin therapy. A. Median total ROS in all 12 patients, baseline and 4 months. B. Total ROS levels in individual patients, baseline and 4 months.

Fig 1. Peripheral Blood ROS levels are reduced after Quercetin therapy; highest reduction in children taking the optimized dose

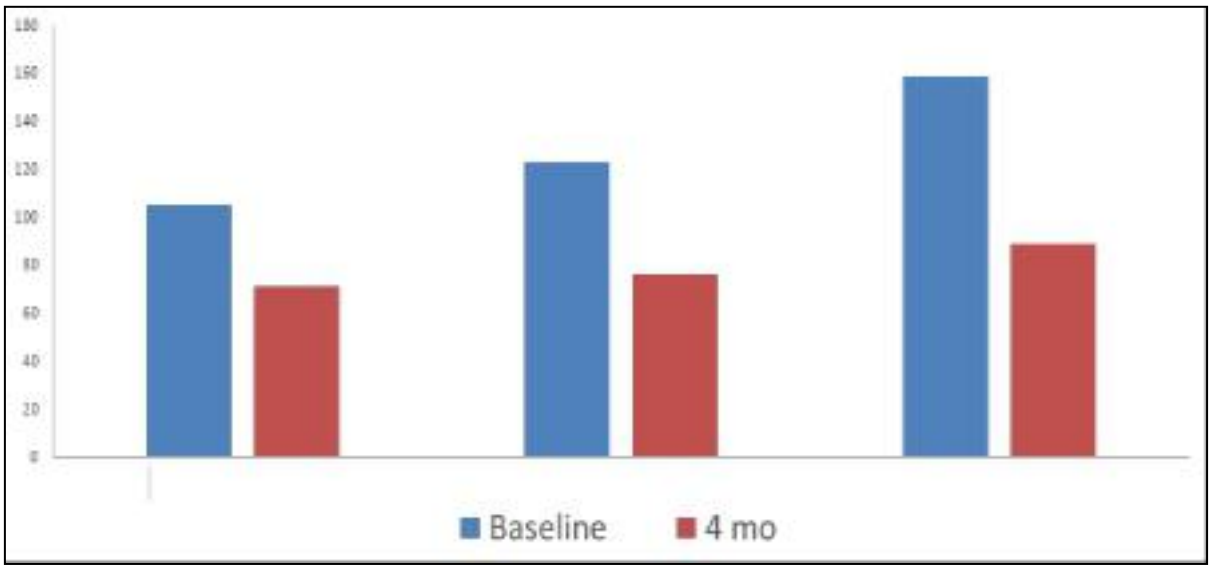


Fig 2. Peripheral Blood ROS (surrogate marker of DNA damage) levels are consistently reduced at the highest dose tested

Final weight adjusted maximum daily dose of 4000mg/day achieved the highest and most consistent ROS reduction. We concluded that long-term quercetin therapy is safe, and well tolerated in patients with FA and biologically relevant blood levels can be achieved at the currently recommended dosing.

Quercetin for chemoprevention of SCC:

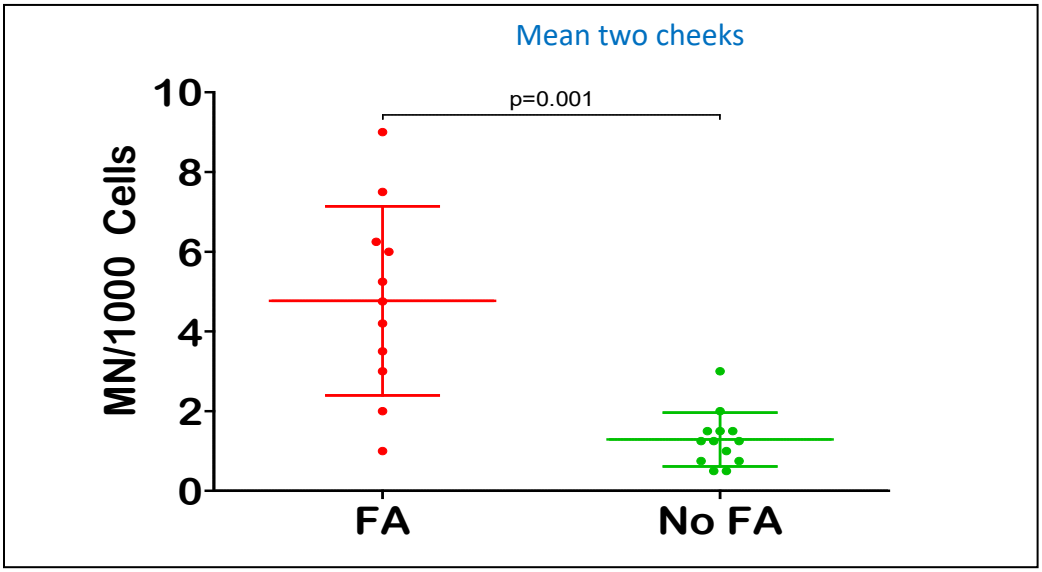
HCT can improve hematological complications of FA (i.e. marrow failure, MDS/AML), however these patients still remain at risk of SCC after HCT. Surgery remains the mainstay of treatment for SCC in patients with FA due to their chemotherapy and radiation sensitivity. Thus, there is

an **urgent need** to develop a new strategy for its treatment and prevention (See section II, SIGNIFICANCE OF STUDY).

- Quercetin inhibits proliferation and induces apoptosis and arrest many types of human cancer cells [28-34].

- Saliva has an important role in intraoral oxidative balance. It has been suggested that an increase in the amount of intraoral free radicals in saliva may promote the onset of oral SCC. Brazil fanconi group measured total oxidant status (TOS) and total antioxidant capacity (TAC) by calorimetric methods and showed that ration of TOS/TAC was 4.8 times higher in patients with leukoplakia (pre-SCC lesion) group compared to FA without leukoplakia and 3.2 when compared to healthy controls[35]. **<u>These data suggest that higher TOS/TAC ratio is associated with transformation and needs to be balanced to prevent development of leukoplakia and subsequent SCC.</u>**

- Micronuclei (MN) are chromosome fragments that are left behind during anaphase and appear in the cytoplasm of the daughter cells as small nuclei. Our collaborator Jordi Surrallés' lab (Barcelona, Spain) recently showed that **MN frequency** in the buccal mucosa is a useful **in vivo biomarker of chromosome fragility (ongoing DNA damage)** in the patients with FA; and patients with FA have a statistically significant increase in the MN frequency in buccal epithelial cells compared to the control group [36] **(Fig 3).**



Mean ± SD
FA patients n=11
Controls n=13

Fig 3. Patients with FA have a statistically significant increase in the MN frequency in buccal epithelial cells compared to the control group

In collaboration with Dr Surrallés' lab, we evaluated MN frequencies in 4 patients from above Quercetin study before and after Quercetin therapy. Results are shown in the **Table 2** below.

Patient	Time from start of Quercetin treatment (days)	Number of MN/1000cells-buccal brushings from both sides (Average) (%)
1	11	**4.75**
	109	**2.25 (52.6%)** ↓
2	31	**3.25**
	136	**2.25 (31%)** ↓

Table 2. Reduction of micronuclei (in vivo marker of chromosome fragility/ongoing DNA damage) in the buccal brushings from patients with FA after Quercetin therapy

Quercetin reduced MN frequencies in the buccal brushings from patients with FA, suggesting its role in potentially preventing development of SCC in patients with FA.

- In fact, a recent report showed that chemoprevention by Quercetin of oral SCC can be successfully achieved and is mediated by suppression of the NF-κB signaling pathway in non-FA DMBA-treated Hamsters [37].

  Hamsters fed quercetin developed no tumors or very small tumors compared to larger, more invasive tumors in those who did not receive Quercetin.

  These results are extremely encouraging especially in reference to FA, as the only potentially curative option for SCC in patients with FA is complete surgical excision (small tumors are more amenable to complete excision).

- Similarly, our own preliminary data in FA SCC cell lines confirm SCC tumor cell kill by Quercetin in a dose dependent manner.

  Using a combination of human cell lines deficient (and proficient) for FA, we were able to demonstrate that **quercetin induces reactive oxygen species (ROS) (Fig 4), proliferative arrest (Fig 5), and cell death in cancer cell lines (Fig 6)**, while non-cancerous keratinocytes remained unaffected with decreased ROS as expected.

  Quercetin has anti-oxidant effects on healthy cells but can cause oxidative stress in cancer cells (**Fig 4**); a well-known mechanism in tumor kill.

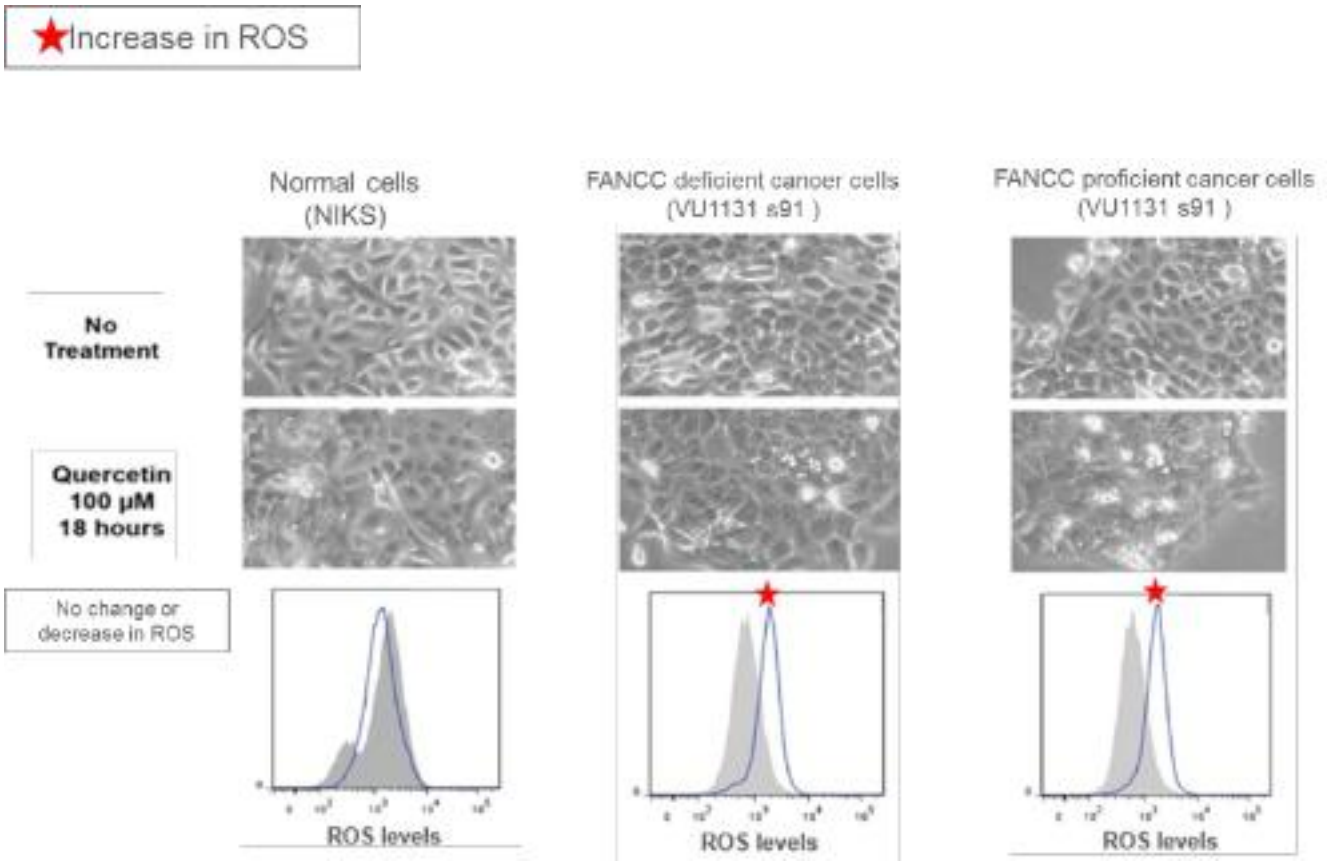


Fig 4. Quercetin has anti-oxidant effects on healthy cells but causes oxidative stress in cancer cells

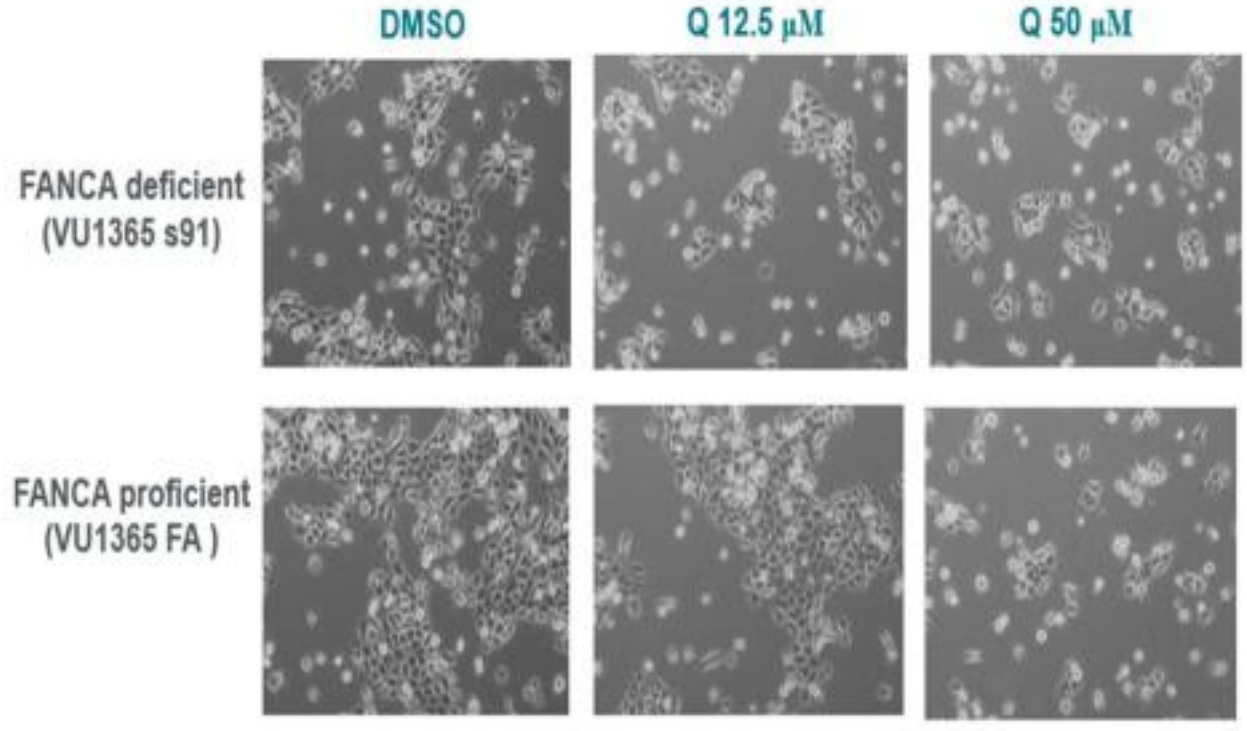


Fig 5. Quercetin diminishes cell number in FA deficient and control cancer cells

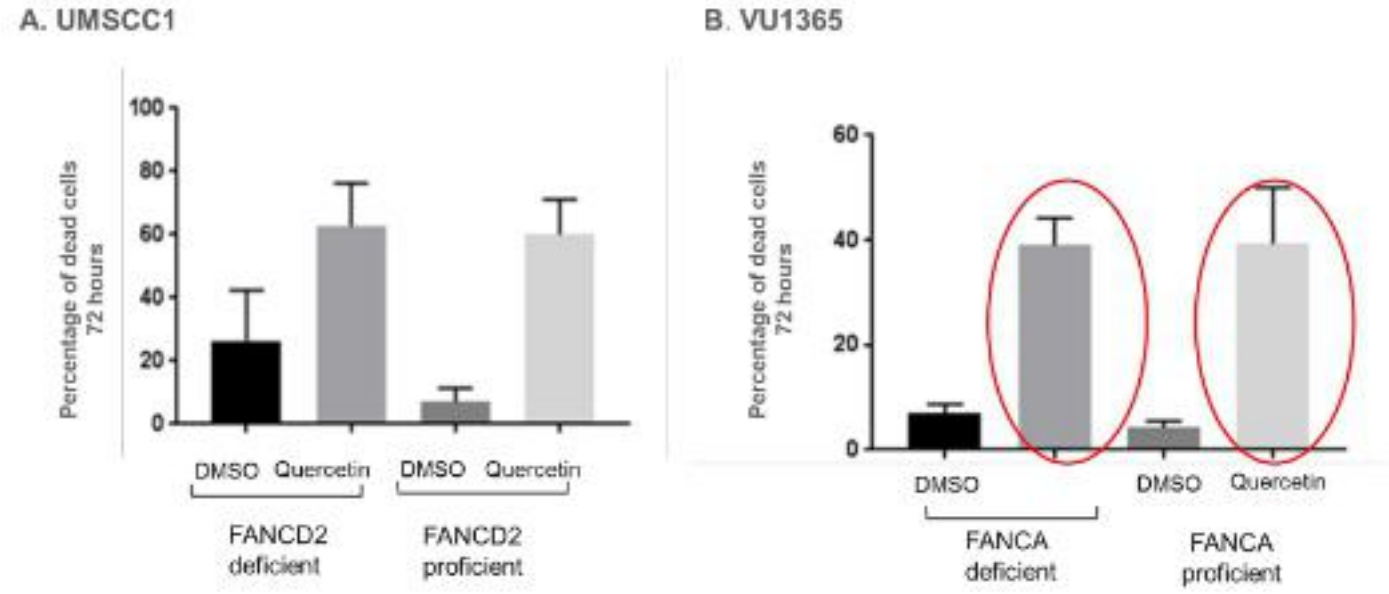


Fig. 6 Quercetin stimulates cell death in FA deficient and proficient cancer cells

Diminished growth of SCC cells occurred in part through apoptosis induction (**Fig 7**).

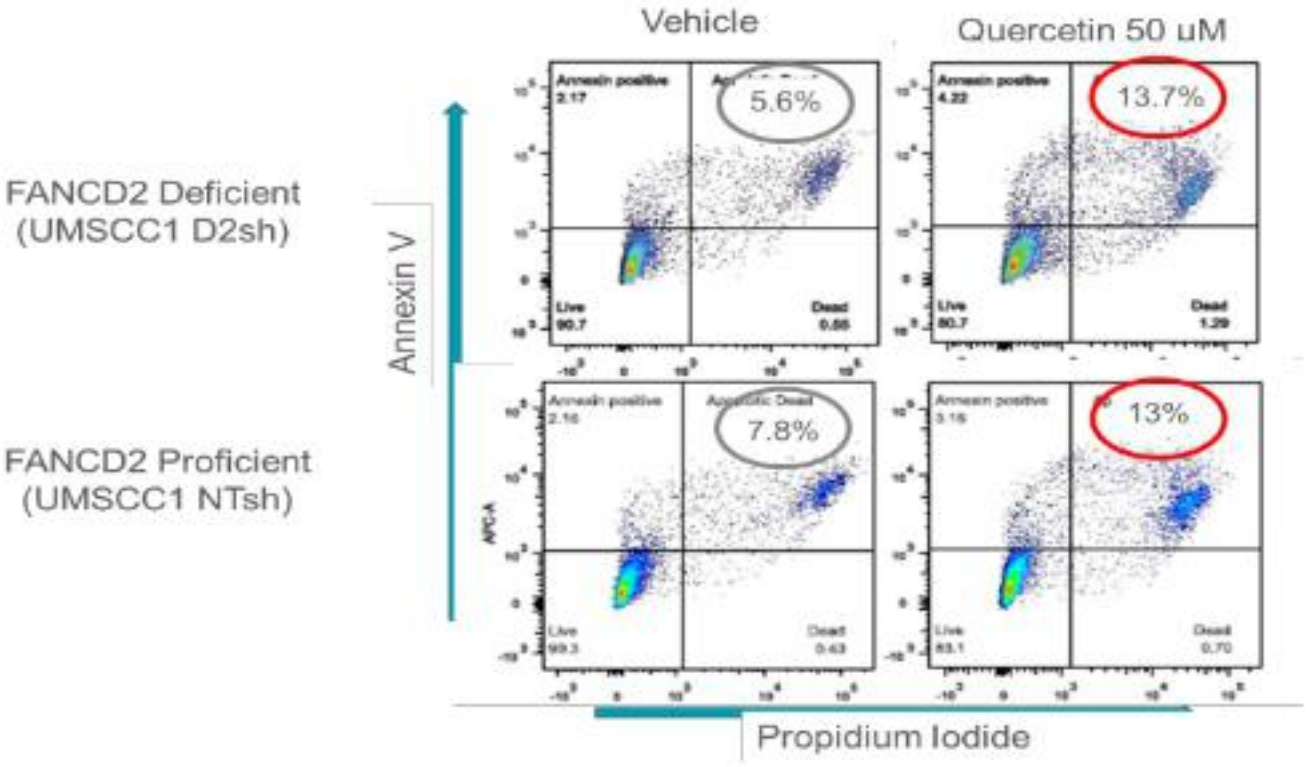


Fig 7. Quercetin induces apoptosis in FANCD2 deficient & proficient cancer cell lines

Based on these promising in vitro and in vivo data, we propose Quercetin chemoprevention for SCC in post-HCT patients with FA.

Quercetin

Quercetin (3, 30, 40, 5, 7-pentahydroxyflavone), is a flavonol that belongs to a group of polyphenolic compounds known as flavonoids [38] Flavonoids share a basic three-ring chemical structure, with two aromatic centers and a central oxygenated heterocyclic ring[39]. Quercetin is a common ingredient in dietary supplements and multivitamin preparations, and is a compound not generally thought to be harmful (as noted in the review of the compound by the FDA; see GRAS notices on the FDA website: http://www.accessdata.fda.gov/scripts/fcn/gras_notices/grn341-1.pdf http://www.accessdata.fda.gov/scripts/fcn/gras_notices/grn341-2.pdf).

Its richest dietary sources include onions, apples, tea and red wine[40] . Among dietary flavonols, quercetin is by far the most abundant, with the average daily intake of quercetin accounting for approximately 70% of the total flavonoid intake in Western diet[41]. The daily flavonoid intake (mainly from onions, apples, grapes, wine, tea, berries, herbs and spices) in the human diet is highly variable, estimations ranging from 23 mg/day (only flavonols + flavones), to more than 500 mg/day, and up to 1 gm/day (total flavonoids)[41-45] .

Quercetin has a wide range of biological activities including free radical scavenging, iron chelation, **anti-inflammation**, strong antioxidant, and **antineoplastic actions[46-50] .** Epidemiological studies have positively correlated flavonoid intake with decreased incidence of coronary heart diseases and common human cancers.

Glutathione (GSH) is a scavenger of free radicals. Quercetin can directly lower intracellular GSH and may cause a pro-oxidative state in cancer cells[51] . In vitro and in vivo studies showed, that quercetin may exert opposite effects; namely, anti- as well as pro-oxidant, that depend on the redox state of a cell[52-54]. Quercetin acts as a strong antioxidant scavenger of free radicals and simultaneously undergoes an oxidation process, giving rise to the formation of the semiquinone radical. A labile semiquinone radical undergoes a second oxidation reaction that leads to the formation of a quercetinquinone. Quinone reacts strongly with protein thiols and it is eliminated by glutathione, which, in turn, results in its clearance [52].

Pharmacokinetics of Quercetin

Quercetin is found in fruits and vegetables in a water-soluble form in which the quercetin molecule is conjugated to a sugar[39]. However, when quercetin is consumed, the sugar is cleaved off in the small intestine, allowing the lipid-soluble aglycone form of quercetin to passively diffuse into the cells lining the intestinal wall [55] . In the liver, aglycone quercetin is either methylated, sulfated or glucuronidated. Quercetin predominately circulates in the bloodstream in these conjugate forms, which differ not only from aglycone quercetin, but also from the glycosylated forms found in plant foods [56] .

Quercetin is available as both oral and intravenous preparations. It is recommended to store it at room temperature, away from heat, moisture, and direct light.

Quercetin is present in plasma as methyl, glucuronide and sulfate conjugates of quercetin, with very little unconjugated quercetin aglycone present [42,57].

Moon et al examined the pharmacokinetics of quercetin aglycone as well as its conjugated metabolites and developed a population pharmacokinetic model for quercetin that incorporates

enterohepatic recirculation. The oral clearance (CL/F) was high (3.5x104 l/h) with an average terminal half-life of 3.5 h (range 0.956–12.5 h) for quercetin. The plasma concentration versus time curves exhibited re-entry peaks. A one compartment model that included enterohepatic recirculation best described the plasma data[58].

Our study showed similar PK profile and exposure in children and adults with FA treated with oral Quercetin (see Table 1).

Quercetin has a strong affinity for normal plasma proteins, 99.1% +/- 0.5%. Serum albumin is the primary binding serum protein (99.4%). Binding by very low-density lipoproteins or alpha-1-acid glycoproteins represents less than 0.5% of the total binding [59,60] .

In a human study, orally administered rutin (quercetin rutinoside) and quercetin were not found in the urine or plasma in unaltered form, even if given at doses of 50 to 65 milligrams/kilogram. Fifty-three percent of quercetin was retrieved in the feces over the first 3 days, the rest was assumed to have undergone O-ring cleavage[40]. Metabolites of quercetin include 3,4,-dihydroxyphenylacetic acid (homoprotocatechuic acid), metahydroxyphenylaic acid, 4-hydroxy-3-methoxyphenylacetic acid (homovanillic acid). All are found in small amounts in urine with unknown activity[61] . Microorganisms in the bowel remove the glycosides and produce ring fission, leading to phenolic acids that are demethylated and dehydroxylated[62] .

Potential Toxicities of Quercetin

Quercetin is commonly used as a dietary supplement and generally not thought to be harmful. Studies performed in healthy normal adults as well as several therapeutic adult trials have revealed no known immediate toxicities of oral quercetin administration[56,63-69] . Planned dosing for this study is based on our own pilot study in patients with FA where highest dose level that achieved biological effect was tolerated extremely well[27] . We will utilize use of an allometric power model to calculate pediatric maintenance doses of drugs expressed as a percentage of total adult dose of 4000mg/day (Table 1).

In general, oral quercetin is well tolerated without any major side effects. Our study confirmed this benign side effect profile in patients with FA treated with Quercetin for up to 2 years. Four patients out of 12 gained weight while on Quercetin, a desired effect in most patients with FA, as they are usually unable to gain weight.

Similarly while *in vitro* studies have raised the concern for mutagenicity, extensive *in vivo* studies have not supported this finding. A number of studies concerning quercetin's effects such as DNA damage or its cytotoxic activity, carried out in animal models, have turned out to be negative [70,71]. In two studies however, after prolonged administration of this flavonoid, a significant increase in the rate of bladder and intestinal cancer incidence occurred at very high doses in Norway and F344 rats [72,73]. Importantly, it is not possible to achieve such high doses in humans due to relatively low bioavailability of quercetin. In addition, quercetin is extensively metabolized by methylation, glucuronidation and sulfation to non-mutagenic compounds, likely contributing significantly to the absence of carcinogenic or mutagenic activity in vivo. In addition to human clinical trials, epidemiologic studies have consistently demonstrated no correlation between dietary quercetin intake and cancer incidence. One study demonstrated an inverse association between total flavonoid intake (95% of which was determined to be quercetin) and overall cancer incidence [74]. Thus despite earlier concerns, long-term supplementation with high doses of quercetin has not been linked to any adverse effects in

rodents or human subjects and quercetin is considered a reasonably safe antioxidant in adults. These results are supported by our own data from the pilot study of Quercetin in children and adults with FA.

In terms of genotoxicity or DNA damage, despite concerns by some, most studies show that quercetin does not cause DNA damage (an important virtue, especially when considered for use in patients with FA)[75]. Çelik et al in fact showed that quercetin is highly effective in reducing the DNA damage caused by the antitumor agents, idarubicin and mitomycin C, following bioactivation by P450 reductase[76]. Our preliminary data show reduction of chromosomal fragility (ongoing DNA damage) in the buccal mucosa after Quercetin treatment (see **Table 2**).

The lack of genotoxicity of quercetin in vivo can be explained again by its low bioavailability and by degradation of its aglicone form caused by intestinal bacterial flora or O-methylation, glucuronidation and sulphatation in the gastrointestinal tract[77]. Importantly, quercetin in vivo is swiftly metabolized to non-mutagenic forms, i.e. 30-O- and 40-O-methylquercetin[78] .

Drug/food interactions reported with quercetin include the following. Digoxin: increased digoxin levels and risk of digoxin toxicity (sudden death, nausea, vomiting, arrhythmias) (animal data); and Fluroquinolones: reduced fluoroquinolone effectiveness (in vitro data) have been reported to cross react with quercetin.

Dosing:
In this study we propose to treat post-HCT children and adults with FA at weight adjusted total max dose of 4000mg/day (the highest dose found to be safe and achieve biologically meaningful levels in our previous study.

The results of the proposed project will use these data to support the development of a novel therapeutic approach for prevention and/or treatment of SCC in post-HCT patients with FA. We hypothesize that quercetin will prevent the development of SCC potentially obviating the need for adjuvant therapy with chemotherapy and/or radiation therapy and associated toxicities in these high risk patients. If found to be effective, treatment with quercetin can be included in standard clinical care of this patient population.

IV. RESEARCH PLAN

1a. How many subjects will be studied?

Potential subjects will be screened and approximately 45 eligible post-transplant subjects will be enrolled in this study. Additionally, patients without history of HCT will be allowed to participate at the discretion of the Principal Investigator. Approximately 10 subjects will be included in this second group. In both groups, patients with or without existing pre-malignant lesions or history of SCC will be allowed to participate, if they wish so and at the discretion of the PI. Some patients with small tumors may only need surgical excision as definitive therapy. These patients will be allowed to participate in the study if they wished so, once they recover from surgery, to potentially prevent recurrence. Patients receiving radiation therapy, chemotherapy or immunotherapy for treatment of SCC will not be eligible.

1b. How will subjects be selected for this study?

Patients attending the CCHMC Fanconi Anemia Comprehensive Care Center who meet the eligibility criteria will be offered the opportunity to participate in this research study. Patients who are referred by their primary physician from other institutions or are self-

referred may be offered opportunity for study participation. The study is open to all participants regardless of gender or ethnicity. Patients starting from 2 years of age, including adults who are post-transplant, will be enrolled on the study. Patients should be at least 6 months post-HCT as they are expected to have normalization of their metabolism and albumin (an important consideration given quercetin's affinity to bind to serum albumin).

2a. **Inclusion Criteria**

1) Diagnosis of FA
2) Able to take enteral medication
3) Patients ≥2 years

2b. **Exclusion Criteria**

1) Renal failure requiring dialysis
2) Total bilirubin >3 mg/dl and/or SGPT >200 at time of enrollment
3) Patients receiving digoxin therapy, who are unable to discontinue treatment due to medical reasons
4) Patients who are pregnant or breastfeeding or are at risk of pregnancy or fathering a baby and are unable to use acceptable methods of birth control during the length of the study
5) Patients who have received quercetin supplementation or other antioxidants within last 30 days.
6) Patients receiving radiation therapy, chemotherapy or immunotherapy for treatment of SCC.

3. **Study Design / Randomization**

This study is an open-label, single arm study.

Although randomization will be ideal, due to biological activity shown in our pilot study (see preliminary data), this study will not involve randomization. This study will enroll approximately 45 post-HCT patients with FA, and approximately 10 patients with FA without history of HCT. In both groups, patients with or without evidence of any pre-malignant lesions concerning for SCC or history of SCC may be allowed to participate if they wish so and at the discretion of the PI. Some patients with small tumors may only need surgical excision as definitive therapy. These patients will be allowed to participate in the study if they wished so, once they recover from surgery, to potentially prevent recurrence. Patients receiving radiation therapy, chemotherapy or immunotherapy for treatment of SCC will not be eligible.

All patients will be treated with oral quercetin for a total of 24 months +/- 30 days or until the study team receives confirmation from our collaborator Jordi Surrallés' lab (Barcelona, Spain) that the buccal samples collected at the 2 year visit are sufficient for analysis.

The quercetin will be administered at an adjusted dose calculated based on weight (see Table 3) for a maximum total daily dose of 4000mg/day. If the patient is 70 kg or more, the dose will automatically be assigned at the maximum dose of 4000mg/day. Patients will be followed carefully.

We will determine the efficacy of Quercetin in reducing buccal micronuclei (a surrogate marker of DNA damage and susceptibility to squamous cell carcinoma due to genomic instability) in post-HCT patients with fanconi anemia (FA). Based on our preliminary data – we anticipate improvement (decrease) in number of micronuclei with Quercetin treatment.

Additionally, from our recent pilot study, we have a valid pharmacodynamic endpoint of ROS, and we will use reduction in ROS as an indication that the levels achieved have biological activity. Our pre-clinical animal and preliminary clinical data in patients with FA support that modification of ROS will modify other surrogate endpoints.

We will utilize correlative biological studies to Measure the impact of Quercetin therapy on additional potential surrogate markers:

1. Peripheral blood ROS and salivary ROS
2. Salivary total antioxidant capacity
3. Biomarkers measured via Exhaled Breath Condensate (EBC) - anti-oxidants, aldehydes etc.
4. Oral microbiome
5. Skin elasticity (see Recommended Observation Table).

After completing 24 months (+/- 30 days or until the study team receives confirmation from our collaborator Jordi Surrallés' lab (Barcelona, Spain) that the buccal samples collected at the 2 year visit are sufficient for analysis) of therapy, all participants enrolled will have an option to continue Quercetin (if elected to do so) as a part of their clinical care.

4. Procedures

Quercetin Administration

Informed consent from patient/parent will be obtained before screening patients for enrollment on this study. The following tests will be performed to determine patient eligibility:

Blood will be drawn for the following: chemistries (liver and renal functions). For females of childbearing potential, pregnancy testing will be performed prior to the first dose of quercetin.

Tests/procedures that are done as part of routine clinical care will not be repeated for the study.

All patients enrolled in this study will be treated with quercetin at an adjusted dose calculated, based on weight (see **Table 3**). Oral quercetin will be administered twice a day for 24 months +/- 30 days or until the study team receives confirmation from our collaborator Jordi Surrallés' lab (Barcelona, Spain) that the buccal samples collected at the 2 year visit are sufficient for analysis.

The dose utilized in this study is already shown to be safe and well tolerated in children and adults with FA. Moreover it is also known to achieve the desired exposure [27] .

Approximate Age	Weight (kg)	Percentage of adult dose	Fraction of adult dose
Birth	3.2	5	1/20
2 months	4.5	13	1/8
4 months	6.5	17	
12 months	10	23	1/4
18 months	11	25	
5 years	18	36	
7 years	23	43.5	
10 years	30	53	1/2
11 years	36	61	
12 years	40	66	
14 years	45	72	3/4
16 years	54	82	
Adult	70	100	1

Modified from Holford and Anderson (1997) Pediatric dosages, In: New ethicals catalogue. Adis International. Auckland, New Zealand, C9.
Note: The neonatal estimate based on size has been reduced further by 50% to account for age-related maturational changes of clearance.

Table 3. Pediatric maintenance doses of drugs expressed as a percentage of adult dose using an allometric 34 power model.

Preparation and Administration of Study Agent

Quercetin is routinely available as an over the counter product due to it being a nutritional supplement. However, for the purpose of the study, it will be purchased in the powder form from PCCA (supplied as 96% Quercetin dehydrate), and stored and distributed by the investigational pharmacy at CCHMC using standard operational procedures. The lot number of the investigational product is to be documented according to institutional standard of practice in the Investigational Drug Service.

Quercetin is administered as an oral medication, supplied in powder form. Quercetin will be stored at room temperature. The product will be dispensed for home administration. Each packet will be labeled in accordance with applicable regulatory requirements.

Quercetin should be administered twice a day at approximately the same time of day (± 4 hours) every day. If any dose of quercetin is missed, every effort should be made to resume the original dosing schedule. Subjects or their caregivers will be instructed by study staff on the proper dosing and storage/handling of the study agent.

Compliance in Investigational Product Administration

When quercetin is dispensed for administration to the subject during the study, the investigator or responsible person will determine the level of compliance with the administration of the investigational product.

The subject's investigational product compliance (e.g., amount used/amount expected to be used in interval between visits) will be recorded by review of the dosing documentation during follow up communication and at each visit. Ongoing participation

will be assessed based on compliance. Patients who are <60% compliant will be considered non-evaluable and not included in the final analysis. These patients may be replaced at the discretion of the principal investigator to ensure optimal number of evaluable patients.

Additional measures are being introduced to promote adherence as described in detail below.

Measures

Barriers Assessment Tool (BAT): The BAT is a 20-item scale developed specifically to assess barriers specific to pediatric patients and assesses perceived barriers to their medication regimen. There is a patient and parent version of the measure. From a list of barriers, respondents are asked to indicate via checkbox next to each item which barriers have gotten in the way of taking their medicine. The design of the BAT was based on prior work in other pediatric sub-specialties (see citations below) and was developed so that it could be quickly and easily administered and scored in a clinical setting. The types of barriers include logistical issues (e.g., forgetting, inconvenience), ingestion difficulties (e.g., swallowing, taste), efficacy (e.g., feel I don't need it), financial difficulties, regimen characteristics (e.g., too many medications, side effects), and patient-specific issues (e.g., refusal by child, embarrassment). We modified the BAT using an iterative process incorporating feedback from patients and their caregivers, healthcare providers, and patient education specialists.

1. Simons LE, Blount RL. Identifying barriers to medication adherence in adolescent transplant recipients. J Pediatr Psychol. 2007;32:831-844.

2. Simons LE, McCormick ML, Mee LL, Blount RL. Parent and patient perspectives on barriers to medication adherence in adolescent transplant recipients. Pediatr Transplant. 2009;13:338-347.

3. Simons LE, McCormick ML, Devine K, Blount RL. Medication barriers predict adolescent transplant recipients' adherence and clinical outcomes at 18-month follow-up. J Pediatr Psychol. 2010;35:1038-1048.

4. Hommel KA, Baldassano RN. Brief report: Barriers to treatment adherence in pediatric inflammatory bowel disease. J Pediatr Psychol. 2010;35:1005-1010.

5. Modi AC, Crosby LE, Guilfoyle SM, Lemanek KL, Witherspoon D, Mitchell MJ. Barriers to treatment adherence for pediatric patients with sickle cell disease and their families. Child Health Care. 2009;38:107-122.

6. Modi AC, Monahan S, Daniels D, Glauser TA. Development and validation of the pediatric epilepsy medication self-management questionnaire. Epilepsy Behav. 2010;18:94-99.

MedActionPlan is a HIPAA compliant mobile application that allows patients to track when they have taken their medications. The patients access their medications through the MyMedSchedule Plus interface. In an objective, peer reviewed assessment of

adherence applications the MedActionPlan app was rated as the top application in overall quality, subjective quality and perceived impact (Carmody et al., 2019). Patients will be asked to indicate each time they have taken their medication on the MyMedSchedule Plus app. Patients who have not reported taking their medication will receive a notification on their phone reminding them to take their medication. If the patient and caregiver choose, the caregiver can also receive a reminder for the patient to take their medication. The study team will have access to patient reports of medication taken through the HIPAA compliant MedActionPlan provider portal.

The MedActionPlan app portal will be utilized for logging, tracking, and monitoring quercetin dosing. The MyMedSchedule Plus app will be utilized in place of the drug diary, except in rare situations based on individual circumstances and inability to utilize the app. Data will be reviewed regularly by the study staff, in consultation with Dr. Pai/designee and patients may be contacted in the event of compliance issues to determine barriers and provide support to improve adherence. Patients/caregivers must log doses taken twice dailyin the app close to the time taken and must do so 75% to meet compliance criteria each month.

Carmody, J. K., Denson, L. A., & Hommel, K. A. (2019). Content and usability evaluation of medication adherence mobile applications for use in pediatrics. Journal of Pediatric Psychology, 44(3), 333-342.

Adherence Support Sessions: The Adherence Support Session will focus on providing adherence education and adherence feedback from the MedActionPlan application to the patient and if applicable, the caregiver. Specifically, patients and caregivers will be provided with feedback regarding their adherence since the last visit using the MedActionPlan data. Next, based on the barriers endorsed by the patients and their caregivers individually tailored adherence intervention will be provided including problem-solving barriers and setting small goals to overcome those barriers. Specifically, the patient and the caregiver will meet with the study psychologist or her designee. The psychologist will 1) teach the patient and/or their caregiver to brainstorm several creative solutions for overcoming barriers they are encountering with medication taking, 2) select the most viable solution for implementation, and 3) develop a specific plan to mitigate the impact of the barriers on their adherence. These adherence sessions will be conducted by Dr. Ahna Pai or her designee, who will meet with participants during/after the study visits (at baseline, 6 months, 1 year, and 2 years) in-person or virtually, as feasible. If participants are not able to meet with Dr. Pai or her designee during the study visit due to scheduling or any other reason, the adherence session may be completed virtually up to 30 days after the visit. Additionally, Dr. Pai or designee will contact the patient/caregiver to check in after the first month of taking quercetin and again at 18 months, to see how the patient is doing and/or give support as needed. In the event a patient demonstrates compliance issues during the course of the study, Dr. Pai/designee in conjunction with the study coordinator or nurse will contact the patient to assess for barriers and provide support to improve adherence as needed. Study staff will do their due diligence to schedule adherence sessions/check ins with participants. If participants are unable to complete the sessions/check ins due to scheduling or any other reason this will be documented but will not be considered a protocol deviation.

During and at the end of study participation, quercetin containers and any remaining supply will be returned to investigator or responsible person when subject returns to CCHMC for study visits. Investigator or designee will return containers and remaining supply to Investigational Drug Pharmacy at CCHMC who will then log the returned amount of drug using standard operational procedures.

Dosing Schema

Quercetin (age and wt. appropriate dose – see table 3) will be given orally on a twice a day schedule starting with weight adjusted maximum total daily dose of 4000 mg/day. If any of the patients is 70 kg or more, the starting dose will be automatically assigned at the maximum dose of 4000 mg/day.

Recommended Observations

Patients will be monitored as shown in the Recommended Observations table (Table 4). Basic blood tests will be allowed to be drawn at local center and results sent to us. For some of the specialized blood tests, we will allow shipping in of the blood samples (drawn locally), if the patients live long distance.

Table 4. Recommended Observations

Tests	Prior to First Dose	1 month	6 months	12 months (1 Year)	24 months (2 Years)	30 months (Optional)
Standard of Care Evaluations						
History[+]/PE, including HT&WT, ENT and GYN (if applicable) visit	X		X	X	X	X
BUN, creatinine, electrolytes	X					
ALT, AST,	X					
Research Evaluations*#						
Pregnancy testing	X					
Adherence Support Sessions	X[€]	X°	X[€]	X[€]	X°	
PB-ROS assessment	X	X	X	X	X	X
Salivary ROS assessment	X	X	X	X	X	X
Antioxidant measurement - Saliva	X	X	X	X	X	X
Buccal micronuclei[^]	X	X	X	X	X	X
Exhaled Breath Condensate - for anti-oxidants, aldehydes etc.	X	X	X	X	X	X
Oral microbiome	X		X	X	X	X
Skin elasticity	X		X	X	X	X
Extra blood sample (up to 10-12 mL) will be collected for additional tests in future (e.g. aldehyde load)	X		X	X	X	X

Study visits to be conducted within 30 days (+/-) of listed study dates

***** If a clinical visit occurs within 30 days (+/-) of the time points noted within the table, research samples may be collected at that visit. Data from standard of care (SOC) (e.g., ENT or GYN visits) and/or clinical procedures (e.g. CBC, chemistries, endocrine testing, etc.), including those done locally, will be collected for the study at all visits, as available.

One month research samples when possible to transport, will be accepted by mail. Similarly, in the event study visit/s are cancelled (for unforeseen circumstances), other research samples will be obtained via mail if possible. Local patients (living in Tristate or surrounding area) may choose to come in person to submit the one month samples. Samples may need to be repeated if there is a problem with the quality, transport, and/or shipping of the sample. If a repeat sample is necessary, it may be collected via mail, in person (local patients), or in conjunction with an already scheduled visit.

[+] As part of history, we collect information about conditioning regimen for transplant (exposure to radiation vs no radiation), and occurrence of mucositis and/or oral/gastrointestinal/vaginal graft vs host disease.

[^] COVID-19 testing may be completed at each timepoint in which buccal samples are collected. This testing will be completed as necessary to ensure the safety of patients and those collecting and processing the samples. This testing will be paid for by the study.

[€]Patients/caregivers will complete Barriers Assessment Tools during the Adherence Sessions.

°Patients will be contacted at one month and 18 months via phone or virtual platform for follow up.

Follow-up will be at 6, 12, 24 and 30 months (from the start of therapy). **Six month visit may be research only visit, and 30 month visit will be optional**. If standard of care procedures are performed clinically around the study visittime points, the results may be used for the study. Up to 10-12 mL blood will be collected for the extra research sample. The amount collected will be based on a safe blood volume based on the participant's weight. All research procedures will be completed as feasible (i.e., if a participant is unable to produce enough saliva or is unable to complete the breath test due to physical limitations, the tests will be omitted for that visit).

In addition to the above observations, the below questionnaires may be completed by patients and/or parents, or the information collected by study staff, prior to the first dose of quercetin, and at 6 months, 1 year, and 2 years:

- PROMIS and Hope scales
- Exhaled Breath Condensate (EBC) Survey.

PROMIS Profiles assess seven patient quality of life domains: functional mobility, fatigue, anxiety, depression, peer relationships, pain intensity and pain interference. The 29-item Adult Form will be completed by all adult participants. The 25-item Parent-Proxy Form will be completed by all parents of children under the age of 18 years. Children between 8-17 years of age will also complete the 25-item Pediatric Profile Form to obtain multiple reports of the patient's quality of life. These profiles demonstrate excellent reliability and validity both in general and pediatric oncology populations [79-81.]

Additionally, we will also measure hope in children using the Children's Hope Scale (CHS) [82]. Children between 8-17 years of age will complete the six-item Children's Hope Scale that assesses two components of a child's dispositional hope a) their perception that they can take action to move towards a desired goal and b) that they are capable of attaining goals. The CHS has good internal consistency (a = .77) and strong construct validity.

For patients with a significant score on the depression section, we will alert the caregiver and clinical care team, and inform the patient that their assigned social worker will contact them.

An additional quality of life questionnaire will be completed (by the patient or study staff) at 6 months, 1 year and 2 years. This questionnaire was developed based on parent reporting of changes noted in physical and emotional well-being from our previous dose-finding pilot study of Quercetin.

These scales or questionnaires will be completed only when feasible.

Study staff will follow up with patients/parents on a monthly basis for the first 6 months of treatment with quercetin. The MedActionPlan app will also be utilized for logging, tracking, and monitoring quercetin dosing (as detailed earlier in this section). Dr. Pai/designee will contact the patient/parent via phone or virtually to check in after taking quercetin for one month and again at 18 months. After 6 months, communication with patients/parents will occur every other month until 1 year. From 1 year through 30 days post last dose of investigational quercetin, communication will occur every 3 months. The frequency of communication may be increased as needed in the event there are compliance or other

issues.

For patients who elect to continue quercetin as a part of their clinical care - we will continue to follow and collect data on outcomes.

V. EVALUATION CRITERIA

1. The **first primary endpoint** of this study is to determine the efficacy of Quercetin in reducing buccal micronuclei (a surrogate marker of DNA damage and susceptibility to squamous cell carcinoma due to genomic instability) in post-HCT patients with fanconi anemia (FA).

   Forty-five patients with FA will be treated with twice daily oral quercetin for a total of 24 months +/- 30 days. Appropriate dosing will be calculated based on body weight as a percentage of adult dose, using an allometric power model (to a maximum total adult dose of 4000mg/day) which has been shown to be safe and well tolerated in pre-transplant patients with FA [27]. Additionally, it is shown to be safe and well tolerated in children and adults with FA also [27].

   We will measure the reduction of micronuclei (in vivo marker of chromosome fragility/ongoing DNA damage) in the buccal brushings from patients with FA after Quercetin therapy.

   Buccal brushings from both cheeks at each time point will be collected. These will be shipped to Dr Jordi Surrallés' lab in Barcelona for quantification.

   Based on our limited preliminary data, we will utilize average number of total micronuclei (average of right and left side) and follow these serially.

   We will collect and analyze these at baseline, and again 1 month, 6 months, 1 yr and 2 yr time points. These samples will be allowed to be mailed in if needed.

   Based on our preliminary data – we expect to see improvement (decrease) in number of micronuclei from buccal brushings following quercetin treatment. **A 20% reduction in the average total number of micronuclei will be considered a success of the intervention.**

   Formal sample size calculation was not done due to limited number patients with this rare disease. We will try to enroll as many patients as possible, however for now have chosen approximately 45 post-transplant patients for the study.

2. The **secondary endpoint** of this study is to measure the impact of Quercetin therapy on additional potential surrogate markers:
   1. Peripheral blood ROS and salivary ROS
   2. Salivary total antioxidant capacity
   3. Biomarkers measured via Exhaled Breath Condensate (EBC) - anti-oxidants, aldehydes etc.
   4. Oral microbiome
   5. Skin elasticity - in patients with FA, who are post HCT.

3. **Correlative tests** for the study will be evaluated as follows:

   a. Measure the impact of quercetin therapy on ROS (peripheral blood and saliva) and total anti-oxidant capacity (saliva).

      We will assess this, by prospectively performing serial measurements of ROS levels in peripheral blood and saliva from patients treated with quercetin (Drs. Davies, Pang and Cancelas-Perez, CCHMC). These will be measured before, during, and at the end of therapy at least and when available, post completion of therapy as outlined in the Recommended Observations, Table 4.

      ROS analysis is known to have significant inter-individual and temporal changes. With this limitation in mind, it will be assessed using quantitative measurement.

      We anticipate observing decline in ROS levels over time. Based on our preliminary data, ≥25% reduction in ROS level compared to baseline will be considered optimal.

      Similarly serial measurements of antioxidants (quantitative) will be performed on the saliva samples (Table 4) as an assessment of change in oxidant/antioxidant balance with Quercetin therapy.

      These laboratory observations will be used as surrogate markers for delay/prevention of SCC in patients with FA.

   b. Measure the impact of quercetin therapy on biomarkers collected using Exhaled Breath condensation (EBC). Samples will be analyzed for serial measurements of anti-oxidants, aldehyde load etc. These samples will be analyzed in collaboration with Dr Lindsey Romick-Rosendale's lab in CCHMC.

   c. Oral microbiome changes post Quercetin treatment will be measured serially. Despite suggestions that oral microbiome plays a role in pathogenesis of SCC in patients with FA, there are limited data on baseline microbiome in these patients, so again this will be an exploratory endpoint. These will be measured and analyzed in collaboration with Dr David Haslam from Infectious Disease at CCHMC.

   d. Skin integrity will be measured at baseline and serially again after quercetin therapy (**Table 4**). Based on our preliminary data, we know that skin in FA is not normal (submitted) and patients with FA will have shorter time to blister compared to controls. However, lack of preliminary data on effect of quercetin on skin elasticity will make this an exploratory endpoint also.

Additional important assessments include **ENT and GYN exams** (when indicated depending on the age) performed at baseline and at each of the subsequent study visits. If patients are being followed by local ENT and/or GYN physicians, clinical information and progress notes from those visits will be allowed for the study. Although natural history of SCC in post-HCT patients remain highly variable, we anticipate that for most patients, we will see stable exam and no new lesions. We will look for improvement in the suspicious or pre-malignant lesions (when present at baseline) after quercetin therapy. If new suspicious lesion/s is/are observed during the span of the study – patients will receive standard

recommended clinical care. His/her continued participation will be determined based on the required additional therapy for the lesion and discussion between the patient/parent and the PI.

As we don't expect to address direct efficacy, above observations will be recorded carefully and analyzed in detail, similar to other correlative studies.

In addition we will assess effect of quercetin in improving insulin sensitivity/glucose tolerance obtained as a part of their clinical care in post-HCT patients with FA, if available. These results will be interpreted with the help of Dr. Jonathan Howell from Division of Endocrinology at CCHMC.

Patients without history of HCT will be analyzed separately from the post-transplant patients.

For all patients, PROMIS and Hope scales from 6 months, 1 year and 2 year visits (when available) will be compared to baseline assessments (pre-quercetin). We anticipate that average patient quality of life (QOL) and hope at the one and two year time points (post-Quercetin) will be significantly higher than average baseline QOL and hope (pre-Quercetin).

VI. OFF STUDY CRITERIA/CRITERIA FOR WITHDRAWING SUBJECTS

Subjects (or parents or legal guardians) have the right to withdraw from the study at any time and for any reason without prejudice to his or her future medical care by the physician or the institution.

Any subject who needs to come off study early due to personal reasons or PI recommendation will be asked to discontinue treatment as soon as possible. The subject may be asked to complete an end of study visit. Every effort will be made to complete this visit in conjunction with an already scheduled clinical visit. If a subject decides to discontinue treatment outside of a scheduled clinical visit, the subject may be asked to come to CCHMC to provide samples (local subjects) or samples may be provided via mail. This visit is not required and will be completed at the convenience of the subject only. If the subject does not come to CCHMC for the end of study visit, a follow up phone call will be made by the study coordinator to review general health status, the reason for withdrawal (if applicable), and plan to return any remaining study drug and study diaries. The subject will be followed for at least 30 days after last dose of quercetin.

<u>Criteria for Removal from Protocol Therapy:</u>
Patient withdrawal from protocol therapy

<u>Off Study Criteria:</u>
a) Completion of study endpoints.
b) Death
c) Lost to follow-up
d) Entry into another therapeutic study for FA.

VII. STATISTICAL CONSIDERATIONS

The goal of this study is to assess the impact of oral quercetin therapy in prevention of SCC in post-HCT patients with FA, using surrogate markers of efficacy, i.e. reduction of micronuclei (marker of DNA damage) in the oral mucosa (buccal brushings) in post-

HCT patients with FA. The primary endpoint is the efficacy of oral quercetin therapy defined as a 20% reduction in micronuclei from baseline to 1-yr.

We anticipate that the results of our pilot study will show that long term oral quercetin therapy decreases micronuclei measured via buccal brushings in post-transplant patients with FA.

This study will enroll approximately 45 post-transplant patients. Data for approximately 10 patients who have not yet undergone transplant, will be analyzed separately and reported using descriptive analyses.

1. Sample Size and Study Duration:
A Simon two-stage design will be used to assess the primary endpoint. The advantage of the Simon two-stage design is that it allows for a planned interim analysis for the termination of the study if there is no evidence of efficacy. The null hypothesis is that the rate of response is 20%. This will be tested against a one-sided alternative that the true rate of response is greater than 20%. The first stage will consist of 14 evaluable patients. If 3 **or fewer** patients in the first 14 have an efficacy the trial will stop for futility. If 4 **or more** have an efficacy an additional 31 patients will be enrolled (for a total of approximately 45 patients). If 12 **or more** out of 45 have an efficacy the null hypothesis will be rejected in favor of the alternative. This design has a type I error rate less than 0.05 and a power of 0.80 if the true response rate is 40%.

Due to the length of follow-up required to assess the primary endpoint study enrollment will not pause in between stages. However, the determination of stopping for futility will be assessed based on the first 14 enrolled chronologically.

We follow >140 FA patients at CCHMC. Review of the entry rate into previous FA studies along with the fact that quercetin is now shown to be a safe in patients with FA except a small possibility of weight gain (usually considered a positive change for patients with FA), we anticipate no obstacles in participation. Although no serious toxicities have previously been seen and quercetin is generally safe, in patients with FA, the expected rate will be low in post-HCT FA patients also.

We anticipate completion of the study within a total of 4-5 years, including data analyses and manuscript preparation.

2. Statistical Considerations for Data Analysis:
The primary endpoint will be assessed by the previously described Simon two-stage design.

The secondary endpoint of 25% ROS reduction from baseline will be assessed using a Chi-square test under the null hypothesis that the rate of ROS response is 20%.

All other secondary objectives will be descriptive in nature. Summary measures such as means or medians for each particular outcome at each time point will be reported.

Given the exploratory nature of the quality of life assessments and the proposed sample size, descriptive analysis will be calculated to assess changes in quality of life and hope between time points when available.

VIII. REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

The protocol and informed consent document for this study must be approved in writing by the Institutional Review Board (IRB) prior to any patient being registered on the study.

Changes to the protocol, must also be approved by the IRB. Records of the Institutional Review Board review and approval of all documents pertaining to this study must be kept on file by the investigator and are subject to FDA inspection at any time during the study. Periodic status reports will be submitted to the Institutional Review Board at least yearly, as well as notification of completion of the study and a final report within 6-12 months of study completion or termination.

Informed consent will be obtained prior to the treatment of participants.

This protocol will be performed under our currently existing investigator initiated **IND# 113343.**

1. Data Safety Monitoring Plan:

There are no known toxicities associated with oral quercetin when given to non-FA adults, or children and adults with FA except a small possibility of weight gain (usually considered a positive change for patients with FA).

The study will be monitored closely by a medical monitor. This will be a qualified physician not associated with this particular protocol. The monitor will have expertise in basic hematology and conduction of clinical trials, and will have prior regulatory experience for similar studies. The monitor will work closely with the PI to monitor the participants on study. He/she will meet with the PI and review study data/progress, approximately every 6 months; more often at the discretion of the PI and/or medical monitor.

The medical monitor is required to review all unanticipated problems involving risk to subjects or others, unanticipated serious adverse events and all subject deaths associated with the protocol and provide an unbiased written report of the review.

At a minimum, the medical monitor should comment on the relationship of the event or problem, to participation in the study. Based on the review of these events, the monitor should make a recommendation regarding study continuation. Although no serious toxicities have previously been seen and quercetin is generally safe, in patients with FA, the expected rate will be low in post-HCT FA patients also. However, any unanticipated attributable serious adverse event will be discussed with the medical monitor. If there are any attributable Serious Adverse Events, the study will stop accrual pending review by the medical monitor. The study will resume patient accrual only if written approval is given by the medical monitor.

Disease progression (i.e., marrow failure, or development of myelodysplastic syndrome,

leukemia or squamous cell carcinoma) will not be captured as adverse events. These complications are part of the natural history of the disease of Fanconi anemia and there is no evidence suggesting that quercetin contributes to the development or progression of any of these conditions. However, in effort to do due diligence, we will perform an individual assessment of any future incidences of these conditions and discuss them with the medical monitor as they occur.

All decisions regarding study continuation, modification, or termination will be reported immediately or annually, as appropriate, to the IRB and other appropriate agencies. Reports for events determined by either the investigator or medical monitor to be possible or definitely related to study participation and reports of events resulting in death should be forwarded in compliance with current IRB policy and applicable federal regulations**.**

All reports from Medical monitor will be forwarded to Cincinnati Children's Hospital Medical Center IRB and other regulatory agencies as appropriate.

2. Blood Specimens

Collection volumes listed in **Appendix A**. Any research samples will be obtained in accordance with institutional standards to ensure patient safety and without compromising needed diagnostic/clinical blood samples or patient health. The total amount of blood drawn will not exceed 5ml/kg of body weight in any 24 hour period. The total volume of blood in any 24 hour period will include blood drawn for clinical, research and waste samples combined.

Residual clinical samples and/or residual samples obtained for other related research studies (e.g. the BMF repository, IRB# 2008-1393 or the FA-HPV Study, IRB # 2010-3354) may be utilized for this study to avoid an additional needle stick or testing for patients consented to those studies. Any residual samples remaining after study procedures have been completed may be placed in the BMF Repository as well.

3. Other Previously Approved Research Studies in Which the Projected Patient Population May Also Be Involved

Patients eligible for this study are not currently eligible for other open therapeutic studies at CCHMC or elsewhere.

4. Data Collection and Monitoring:

Data for the study will be entered into a secure, password protected database by the study staff.

Monitoring and auditing procedures will be followed to ensure that the study is conducted, documented, and reported in accordance with the IRB approved protocol, all applicable federal regulations and guidelines, and applicable regulatory requirements of Cincinnati Children’s Hospital Medical Center.

Verification of eligibility will be performed and appropriate documentation of informed consent will be documented for all subjects enrolled into the study. The timeliness of Serious Adverse Event reporting will be monitored to ensure regulatory compliance. All case report forms (CRF) for the first subject enrolled into the study will be monitored for

completeness and quality by comparing data in the case report forms to data in the source documents. Thereafter, a minimum of 10% of enrolled subjects' CRFs will be monitored for completeness and quality by comparing data in the case report forms to data in the source documents.

5. Facilities Utilized in the Study

Study participants will be cared for by the Division of Bone Marrow Transplantation and Immune Deficiency physicians on the Bone Marrow Transplant inpatient and outpatients units at Cincinnati Children's Hospital Medical Center.

6. Special Considerations

a) Investigational Drugs or Devices: quercetin is a commercially available dietary supplement. However, in this study it will be used under our existing investigator-initiated IND for use in pre-HCT patients with FA.

b) IND (Investigational New Drug): In this study, Quercetin will be used under our existing investigator-initiated IND for use in pre-HCT patients with FA – IND# 113343.

c) CCHMC Pharmacy: The Investigational Drug Service at CCHMC will be responsible for the purchase, storage and dispensing of quercetin.

IX. POTENTIAL BENEFITS

There may be no benefit to enrollment in this study. Quercetin has the potential to prevent SCC and associated complications without the need for chemotherapy and radiation treatment.

X. POTENTIAL RISKS, DISCOMFORTS, INCONVENIENCES AND PRECAUTIONS

1. Potential risks, discomforts, inconveniences specific to the study agent administration and research evaluations include:

- Drug administration requiring oral ingestion, can be associated with compliance issues with long term administration especially in adolescent and young adults. Mild to moderate weight gain has been seen in a few subjects currently receiving quercetin. This weight gain is generally well tolerated. This is considered a positive effect for patients with Fanconi Anemia, who are generally small and have difficulty gaining weight.
- Physical Exams: Physical exams may be associated with minor physical discomfort.
- Laboratory tests requiring venipuncture and buccal brushings: Venipuncture may be associated with pain or discomfort at the needle site, bruising, or infection. Buccal brushings may be associated with mild discomfort in the cheek area.

Every effort will be made to minimize this discomfort based on the participant's developmental stage.

2. Drug Interactions

Drug/food interactions reported with quercetin include the following. Digoxin: increased digoxin levels and risk of digoxin toxicity (sudden death, nausea, vomiting, arrhythmias) (animal data); and Fluroquinolones: reduced fluoroquinolone effectiveness (in vitro data) have been reported due to cross reactivity with quercetin.

Digoxin use is included in the exclusion criteria. If any of the patients require fluoroquinolones, an alternative agent of equivocal potency will be used instead.

3. Carcinogenesis, Mutagenesis, and Impairment of Fertility

Current available data (from animal and human studies) do not support the carcinogenic potential of quercetin. There are no studies addressing its effect on fertility. Mutagenesis studies (both in vitro and in vivo) show no confirmed evidence of mutagenic activity.

4. Pregnancy/Lactation

Pregnancy/lactation: Quercetin is safe in general without any major adverse effects. Developmental toxicity studies have been performed in rats and rabbits at doses ranging from 60- to 100-fold higher than the human dose and have revealed no evidence of harm to the fetus due to quercetin. There are, however, no studies in pregnant women. For females of childbearing potential, pregnancy testing will be conducted at baseline prior to the first dose of quercetin. It is not known whether quercetin is excreted in human milk or absorbed systemically after ingestion. Because many drugs and immunoglobulins are excreted in human milk, and the potential for unknown adverse reactions in nursing infants, these risks will be discussed in detail during consent.

5. Pediatric Use

Studies performed in healthy normal adults as well as several therapeutic adult trials have revealed no known immediate toxicities of oral quercetin administration [56,63-69] . Our own experience from our pilot study (including the ongoing expansion cohort) showed that quercetin is safe and well tolerated in children and adults with FA [27] . Patients from 3 years to 16 years old were enrolled in the study and everyone tolerated quercetin well without any major adverse events. Based on our experience and additional data of consumption of food containing quercetin by children (see below), we expect patients to tolerate quercetin well, even when taken for extended time periods.

Also, as discussed above in section III, among dietary flavonols, quercetin is by far the most abundant, with the average daily intake of quercetin accounting for approximately 70% of the total flavonoid intake in Western diet[41] . The daily flavonoid intake (mainly from onions, apples, grapes, wine, tea, berries, herbs and spices) in the human diet is highly variable, estimations ranging from 23 mg/day (only flavonols + flavones), to more than 500 mg/day, and up to 1 gm/day (total flavonoids) [41-45].

Estimates of quercetin intake in normal daily diet have been projected based on data included in the National Center for Health Statistics (NCHS), national Health and Nutrition Examination Surveys (NHANES) (CDC 2006; USDA, 2009a,b). Within the individual population groups, the largest percentage of potential consumers of quercetin was identified in children where 55.9% of the population group reported consuming foods in which quercetin is present. When heavy consumers (90^{th} percentile) were assessed, the estimate for the all-person intake and all-user intake of quercetin was observed to be largest in male teenagers at 450mg/person/day and 668 mg/person/day, respectively as shown below in **Table 5**. On a body weight basis, the estimates for the all-person and all-user 90^{th} percentile intakes of quercetin were largest in infants with values of 12 and 53 mg/kg/day, respectively.

Table 5. 2 **Summary of the Estimated Daily Per Kilogram Body Weight Intakes of Quercetin Based on all Proposed Food-Uses and Use-Levels in the United States (NHANES 2003-2004, 2005-2006)**

Population Group	Age (Years)	% Users	Actual # of Total Users	All-Person Consumption (mg/kg bw/day)		All-User Consumption (mg/kg bw/day)	
				Mean	90th Percentile	Mean	90th Percentile
Infants	0 to 2	28.9	552	7	12	22	53
Children	3 to 11	55.9	1,528	4	9	7	13
Female Teenagers	12 to 19	47.8	949	1	4	3	6
Male Teenagers	12 to 19	49.7	964	2	7	5	11
Female Adults	> 20	27.3	1,167	1	1	2	4
Male Adults	> 20	28.4	1,092	1	2	3	7
Total Population	All ages	37.4	6,252	1	4	4	9

Derived using data and individual food codes from the National Health and Nutrition Examination Surveys (NHANES) 2003-2004, 2005-2006 (CDC, 2006; USDA, 2009a,b).

Based on these data it is clear that quercetin can be consumed on daily basis without any side effects. Similarly, studies in adult volunteers and our previous experience in pre-HCT patients with FA [27] ,it is expected that quercetin will be well tolerated without any major complications in post-HCT patients with FA.

6. Adverse reactions

Studies performed in healthy normal adults as well as several therapeutic adult trials have revealed no known immediate toxicities of oral quercetin administration. Mild to moderate weight gain has been seen in a few subjects in our phase 1 study of Quercetin in pre-HCT FA patients. This is generally well tolerated. This is considered a positive effect for patients with Fanconi Anemia, who are generally small and have difficulty gaining weight.

7. Precautions that will be taken to monitor and avoid the above mentioned risks, discomforts, and inconveniences.

During the clinic visits, any reported new symptoms will be addressed and investigated promptly. Patients will be instructed to contact study physician immediately if they develop any new signs and symptoms in between visits, to determine whether these are related to study agent.

8. Adverse Events

Unanticipated severe adverse event/s must be reported to the principal investigator who is responsible for the reporting to the Cincinnati Children's Hospital Medical Center IRB, FDA, and the medical monitor as applicable. Adverse events will be assessed according to the Common Terminology Criteria for Adverse Events v5.0 (CTCAE published November 27, 2017).

All Adverse Event reporting is to comply with the current CCHMC IRB policy and

applicable federal regulations. If any serious adverse events occur, current guidelines will be followed for expedited reporting to the IRB, FDA and/or the medical monitor.

The investigator should notify the IRB of serious adverse events occurring at the site, in accordance with local procedures. All serious and medically significant adverse events considered related to quercetin by the investigator will be followed until resolved or considered stable.

Disease progression (i.e., marrow failure, or development of myelodysplastic syndrome, leukemia or squamous cell carcinoma) will not be captured as adverse events. These complications are part of the natural history of the disease of Fanconi anemia and there is no evidence suggesting that quercetin contributes to the development or progression of any of these conditions. However, in effort to do due diligence, we will perform an individual assessment of any future incidences of these conditions and discuss them with the medical monitor as they occur.

Definitions:

Adverse Event

An adverse event is any new, undesirable medical occurrence or change of an existing condition in a subject that occurs during treatment (which may include a specified post treatment period), whether or not considered to be product related. Abnormal laboratory findings considered by the investigator to be clinically significant, e.g. those that are unusual or unusually severe for the population being studied, should be recorded as adverse events.

Serious Adverse Event

A serious adverse event is defined by regulatory/clinical criteria. It is one that suggests a significant hazard or side effect, regardless of the investigator's or sponsor's opinion on the relationship to study material. This includes, but may not be limited to, any event that (at any dose):

- is fatal;
- is life threatening (places the subject at immediate risk of death);
- requires in-patient hospitalization or prolongation of existing hospitalization;
- is persistent and results in significant disability/incapacity; or
- is a congenital anomaly/birth defect.

Important medical events that may not be immediately life threatening or result in death or hospitalization, but may jeopardize the patient or require intervention to prevent one of the outcomes listed above, or result in urgent investigation, may be considered serious.

If the adverse event is sufficiently severe in the investigator's judgment, the subject should be removed from treatment and a termination assessment performed. The subject should be given appropriate care under medical supervision until symptoms cease or stabilize.

9. Risk assessment recommendation

- ☐ Minimal risk but with direct benefit to participants
- ☐ Minimal risk but without direct benefit to participants
- ☒ More than minimal risk but with potential for direct benefit to participants
- ☐ More than minimal risk but without direct benefit to participant

XI. CONFIDENTIALITY

All records and documents pertaining to the study will be maintained by the Investigator and designee(s), and will be available for inspection by the Cincinnati Children's Hospital Institutional Review Board and the Medical monitor. All documents will be kept in a locked cabinet or on password protected computers in the office of the Clinical Management Research Support Core (CMRSC). Access to the files will be limited to the Principal Investigator and designees. All information provided to the investigator dealing with the study and information obtained during the course of the study will be regarded as confidential.

XII. PERIOD OF TIME ESTIMATED TO COMPLETE PROJECT AS DESCRIBED

The period of time to complete enrollment for this study is estimated to be approximately 2-3 years. Total time for completion of follow up is estimated to be 5 years.

XIII. FUNDING

1. **Name of funding agency or private sponsors**
   Research grant support from Fanconi anemia research fund, Gateway for Cancer Research and FDA are used for study expenses.

2. **The institution to which funding will be made**
   CCHMC

XIV. PAYMENT FOR STUDIES

1. **Third party payers**
   All costs associated with the routine clinical care of patients will be the responsibility of the patient or their insurance carrier. All other costs and expenses related to the conduct of this study will be paid from research grant and/or divisional funds (including the study agent quercetin, research labs/expenses related to 6 month study visit). Visits/Tests/procedures that are done as part of routine clinical care will not be repeated for the study. The results of these tests will be used for the study.

2. **Reimbursement to participants**
   To the extent possible, all study visits will be scheduled at the same time clinical visits are planned. All costs and expenses related to the conduct of this study that are research related/experimental, will be paid from research grant (including the cost of study agent quercetin, research labs at 1 month/other time points and expenses related to 6-month study visit) and divisional funds. Travel assistance will be offered for the 6 month visit.

   Quercetin compliance and reporting will be reviewed approximately monthly, and subjects will receive $15 per month if they meet 75% compliance (at Months 1-5, 7-11 and 13-23). Subjects will be sent payment via ClinCard and the ClinCard will be reloaded at each compliance review if the subject meets compliance requirements.

Additionally, during the in-person follow up visits (Month 6, 1 Year and 2 Year), subjects will receive $50 at the 6-month visit, $60 at the 1-year visit, and $75 at the 2 year visit for continued and successful participation in the study including meeting compliance requirements (75%) for the month preceding each visit.

The subject's quercetin compliance (e.g., amount used/amount expected to be used in interval between visits) will be determined by regular review of the MedActionPlan app (or drug diary in rare situations). Patients/caregivers must log doses taken twice daily in the app on the date taken and close to the time taken and must do so 75% of the time each month to meet compliance criteria.

ClinCard will be utilized for payment. If a subject does not have a social security number or tax identification number, he/she will be offered gift cards in the amount specified above. Subjects may decline to receive compensation. This will be documented in the subject's research chart but will not be considered a protocol deviation.

XV. METHOD TO BE USED IN PROCURING CONSENT OF SUBJECTS

All prospective patients will have the study explained by a member of the research team. All the potential hazards and possible adverse reactions will be explained to patient/parent.

Prior to the initiation of the study, acknowledgement of the receipt of this information and the subject's freely tendered offer to participate will be obtained in writing from each subject in the study. Assent for subjects 7-10 years old will not be obtained due to the complex nature of the therapy and the limited capability of the minor to reasonably consider all the implications associated with this treatment that may be of direct benefit to the subject. Assent will be obtained for subjects aged 11 and older.

The consent process may be conducted via telephone according to Clinical Management and Research Support Core (CMRSC) SOP. No research procedures will occur prior to getting documented consent. The same process will be followed for the assent process for subjects aged 11 and older.

This protocol, informed consent, assent form, and any amendments to the protocol will be reviewed and approved by the CHMCC IRB prior to initiation. The study will not be initiated without the approval of the IRB.

XVI. SCHUBERT RESEARCH CLINIC SERVICES

The Schubert Research Clinic Services may be utilized for certain study visits as required to accommodate better patient flow for the research procedures, as approved by the Schubert Research Clinic.

XVII. REFERENCES

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Appendix A: Blood Volumes (Study Visits Screening through End of Treatment)

Tests	Prior to First Dose	1 month	6 months	12 months (1 Year)	24 months (2 Years)	30 months (Optional)	Maximum Total
BUN, creatinine, electrolytes	1 ml						1ml
ALT, AST,	1 ml						1ml
PB-ROS assessment	5-7mls	5-7mls*	5-7mls	5-7mls	5-7mls	5-7mls	42mls
Additional blood sample for future studies	5-12 mls		5-12 mls	5-12 mls	5-12 mls	5-12 mls	25-52mls
Blood Volume Sub-total	12-21mls	5-7mls	10-19mls	10-19mls	10-19mls	10-19mls	69- 96 mls

*PB ROS will be collected for subjects seen at CCHMC for study visits only (fresh sample required), as feasible. If patient unable to return to CCHMC for study visit, then we will accept PB ROS sample collected and shipped to CCHMC within approximately 24 hours after collection if feasible.
Data from standard of care (SOC) procedures (e.g. Counts, etc) will be collected for the study, as available (no additional blood will be collected).