Cancer in the National Cancer Institute inherited bone marrow failure syndrome cohort after fifteen years of follow-up

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ABSTRACT

The National Cancer Institute Inherited Bone Marrow Failure Syndromes Cohort enrolls patients with the four major syndromes: Fanconi anemia, dyskeratosis congenita, Diamond-Blackfan anemia, and Shwachman-Diamond syndrome, and follows them with a common comprehensive protocol. The current analysis includes more than double the numbers of patients and person-years since our first report, published in 2010. Patients with Fanconi anemia and dyskeratosis congenita developed head and neck and anogenital squamous cell carcinomas at rates that were hundreds-fold greater than those of the general population. In competing risk analyses the cumulative incidence of severe bone marrow failure, leading to stem cell transplantation or death, was more than 70% by age 60. Patients with Diamond-Blackfan anemia developed lung, colon, and cervical cancer at rates greater than those of the general population. The cumulative incidence of severe bone marrow failure in those with Diamond-Blackfan anemia was 50% by age 60. The smaller group, with Shwachman-Diamond syndrome, have not as yet developed a significant number of solid tumors, but 40% developed bone marrow failure by age 50. The risk of solid tumors following stem cell transplantation in Fanconi anemia and in dyskeratosis congenita was significantly higher than in non-transplanted patients. There was no clear association of genotype with cancer in any of the syndromes. Cancer was most common in Fanconi anemia, followed by dyskeratosis congenita; Diamond-Blackfan anemia and Shwachman-Diamond syndrome are less cancer-prone, but nonetheless all patients are at increased risks of bone marrow failure and specific cancers. *clinicaltrials.gov Identifier: 00027274*

Introduction

The inherited bone marrow failure syndromes (IBMFS) include specific genetic disorders with varying degrees of hematopoietic failure, birth defects, high risks of cancer, and mutations in genes in unique molecular pathways.[1] The most frequent syndrome is Fanconi anemia (FA), a primarily autosomal recessive disorder in which pathogenic variants have been recognized in at least 21 genes whose products collaborate in the FA/BRCA DNA repair pathway. Children with FA may have bone marrow failure (BMF) and characteristic physical findings, such as short stature, radial ray anomalies, microcephaly, abnormal kidneys, and others.[2] There is notable clinical heterogeneity, with diagnosis of adults with normal physical appearances and normal hematopoiesis, who are only identified when they develop typical FA cancers or through family studies. The diagnosis of FA is confirmed by the detection of increased chromosome breaks after culture of cells with a DNA cross-linker, such as diepoxybutane or mitomycin C.[3]

Case reports of malignancies in patients with FA were first reviewed in 1996, and the types and risks of cancer have been described in retrospective and contemporary cohorts, including patients from North America, France, Germany, Israel, and elsewhere.[4-9] We previously reported overall risks of leukemia and solid tumors in the National Cancer Institute (NCI) FA cohort of approximately 30- to 50-fold; the

highest risks were acute myeloid leukemia (AML), head and neck squamous cell carcinoma (HNSCC), and vulvar SCC;[8] risks were similar in the other FA cohorts.

The risks and types of cancer are not as well studied in the other IBMFS. Patients with dyskeratosis congenita (DC) may present with marrow failure and features of the diagnostic triad: dysplastic nails, lacy reticular pigmentation, and oral leukoplakia.[2] Others may have a variety of additional findings, such as pulmonary or hepatic fibrosis, avascular necrosis of the hips, or stenosis of lacrimal ducts, esophagus, and/or urethra.[10] The diagnostic test for DC is the presence of very short telomeres in blood leukocytes.[11] Pathogenic variants have been identified in more than 12 genes involved in telomere biology.[1] The cancer pattern in DC is similar to that of FA, with lower risks in DC.[8,12]

Patients with Diamond-Blackfan anemia (DBA) also have an increased risk of cancer.[13] Patients with DBA are usually diagnosed in infancy or childhood, with macrocytic anemia and elevated levels of red cell adenosine deaminase.[2,14] More than 14 primarily autosomal dominant genes have been identified in the ribosome biogenesis pathway.[15] The solid tumors in DBA are osteosarcoma, colon, and lung cancer, distinct from the other IBMFS.

Shwachman-Diamond syndrome (SDS) is characterized by exocrine pancreatic insufficiency and neutropenia.[2] Cancer information is limited to case reports and small series; cancer is primarily AML.[16] Most patients with SDS have biallelic pathogenic variants in *SBDS*, which is involved in ribosome biogenesis; a few patients have mutations in *DNAJC21* or *EFL1*.[17,18]

The potential impact of hematopoietic cell transplant (HCT) on cancer in IBMFS is a crucial question. We previously compared cancer in non-transplanted patients in the North American Survey with cancer in transplanted patients in the French FA cohort.[5] We found that the risk increased by more than 4-fold, while the median age dropped by 16 years. The potential contributions of total body irradiation and chronic graft-*versus*-host disease (GvHD) were not statistically significant in that study. There are no comparable age-dependent analyses from other FA cohorts or other types of IBMFS, although data from Europe suggest an increase in cancer based on the interval from transplantation in FA.[19]

Herein, we report a new analysis of the cumulative cancer experience of individuals enrolled in the NCI IBMFS Cohort. Our first report included 196 IBMFS patients and 4302 person-years among the four IBMFS enrolled and analyzed for seven years, up to and including 2008.[8] We now have more than double the number of affected individuals (530) and person-years (12607) after 15 years, with more precise estimates of hazard rates and relative risks. The current data permit stronger evidence-based counseling of patients with an IBMFS.

Methods

The NCI IBMFS Cohort opened in January 2002, and continues to accrue patients (protocol 02-C-0052, *clinicaltrials.gov Identifier: 00027274*). The study was approved by the NCI Institutional Review Board, and participants or their proxies sign consent and medical release forms. Enrollment is voluntary, with information provided directly by family members. A family contact initiates a telephone interview, followed by completion of a Family History Questionnaire. Each family member fills in an Individual Information Questionnaire, and biennial follow-up forms are sent.

First-degree family members are included in the study evaluations, in order to identify undiagnosed cases, determine carrier status, and provide genetic counseling.

All participants enroll in the "Field Cohort", and a subset (the "Clinical Center Cohort") is evaluated at the National Institutes of Health Warren G Magnuson Clinical Center in Bethesda MD, USA. Competing adverse events include severe BMF (sufficiently severe to lead to death or HCT);[4] myelodysplastic syndrome (MDS; severe cytopenia with dyspoietic morphology of marrow cells, with or without a cytogenetic clone);[20] acute leukemia, usually AML; or solid tumors or lymphomas.[8]

Patients in the current report enrolled from January 2002 through December 2015, with follow-up through May of 2016. Clinical diagnoses were validated by syndrome-specific tests (chromosome breakage for FA; telomere length by flow cytometry and fluorescent *in situ* hybridization for DC; red blood cell adenosine deaminase for DBA; and serum isoamylase and trypsinogen for SDS),[3,14,21,22] and confirmed by genetic testing whenever possible. Cancer diagnoses were provided by self-report or by proxy, and confirmed in 60% by review of medical records. Lack of a report of cancer led to the conservative assumption that the patient had not had cancer. The classification of a patient as having MDS was done according to the medical records provided by the patient. We were unable to do central review, and thus MDS may have been overreported according to minor dyspoieses or cytogenetic reports.

Analyses were done using Microsoft Excel Office 365 Proplus version 1609 (Microsoft, Redmond, WA, USA), Stata 14.2 (StataCorp, College Station, TX, USA), and MATLAB2017A (the MathWorks, Natick, MA, USA). Survival probabilities were calculated by the Kaplan-Meier method in the absence of competing risks with censoring at death.[23] Cumulative incidence and cause-specific hazards accounting for competing risks (BMF, leukemia, or solid tumors) were determined as described previously.[4] The ratio of observed-to-expected cancers (O/E) was derived from general population incidence data from the Surveillance, Epidemiology, and End Results (SEER) Program, adjusting for age, sex, race, and birth cohort.[24] Sex ratios were analyzed with the binomial test of comparison with a ratio of 1:1. Statistical tests were 2-sided, and *P*-values <0.05 were considered significant.

Results

We enrolled 360 families with at least one member with DBA, DC, FA, or SDS (Table 1), including 530 affected individuals and 12607 affected person-years. There was an excess of males among those with DC compared with the other syndromes. The median age in May 2016 (or age at death) was higher in DC, associated with diagnosis in older adults, while the median survival age was higher in patients with DBA (67 years) compared with 51 years in DC and 39 years in FA, due to the lower risk of death from BMF or cancer in DBA. More than 80% of the affected participants survived to adulthood (≥18 years of age). Thirty and 40% of those with DC and FA had received an HCT, compared with 7 and 25 % of those with DBA or SDS. The crude death rates were more than 30% in DC and FA, and around 10% in DBA and SDS.

Malignancies in patients who had not undergone HCT were more frequent in DC and FA (>10%) than in DBA and SDS (Table 2 and Table 3). Leukemia was reported in 3% of DC, FA, and SDS (and none of those with DBA), while solid tumors occurred in 5% of DBA, 7% of DC,

12% of FA, and 3% of those with SDS. Two patients with DBA, four with DC, and five with FA had multiple malignancies (Table 2).

The O/E ratio for any malignancy in non-transplanted patients was 2.5 for DBA, 4.2 for DC, 19 for FA, and 8.5 for SDS (Table 3). The significant cancers in DBA were

lung, colon, and cervix; in DC they were HNSCC (primarily tongue), AML, non-Hodgkin lymphoma (NHL), and anal SCC; in FA, HNSCC (primarily tongue), AML, vulva, esophagus, brain, and anal SCC were presented; and in SDS one ovarian cancer and one AML were statistically significant.

Table 1. Participants.

	DBA	DC	FA	SDS	Total
Number of Families	87	108	130	35	360
Number of Patients	135	197	163	35	530
Person-Years	3458	5655	2854	640	12607
Birth Years, Median (range)[†]	1996	1991	1996	1998	1993
	(1921-2014)	(1902-2014)	(1945-2014)	(1962-2012)	(1921-2014)
Male:Female	72:63	127:70	72:91	15:20	286:244
M:F ratio	1.1:1	1.8:1*	0.79:1	0.75:1	1.2:1
Number alive**	122 (90%)	127 (74%)	111 (68%)	31 (89%)	391 (74%)
Age alive, Median, years (range)**	19	25	17	17	20
	(1.5-87)	(4-79)	(2-64)	(4-39)	(1.5-87)
Number deceased**	13 (10%)	70 (36%)	52 (32%)	4 (11%)	139 (26%)
Age deceased, Median, years (range)**	33	29	20	31	26
	(0-70)	(1.3-82)	(0.2-58)	(13-53)	(0-82)
Age Median, years (range)**	20	26	17	18	22
	(0-87)	(1.3-82)	(0.2-64)	(4-53)	(0-87)
Median Survival, years (CI)***	67	51	39	41	–
	(57-70)	(46-55)	(35-44)	(32-50)	
% reached ≥18 years	95	90	80	95	–

[†]For birth year, DC older than DBA, FA or SDS. *$P<0.001$ for excess of males. **Status and age in May 2016 or at death. DC older than DBA or FA, DBA or SDS. ***From Kaplan-Meier survivals (see Figure 3). CI, 95% confidence interval. DBA: Diamond-Blackfan anemia; DC: dyskeratosis congenita; FA: Fanconi anemia; SDS: Shwachman-Diamond syndrome; M: male; F: female.

Table 2. Complications in participants.

	DBA	DC	FA	SDS	Total
No or pre-stem cell transplantation (HCT)					
Number	135	197	163	35	530
N with malignancy (%)[*]	7 (5)	20 (10)	21 (13)	2 (6)	50 (9.4)
N with leukemia (%)	0	6 (3)	5 (3.1)	1 (2.9)	12 (2.3)
N with solid tumor (%)	7 (5)	14 (7)	20 (12)	1 (2.9)	37 (7)
N with lymphoma (%)	0	2 (1)	0	0	2 (0.4)
N of malignancies among N with malignancy	9	27	26	2	55
N of leukemias	0	7	5	1	13
N of solid tumors	9	17	21	1	49
N of lymphomas	0	3	0	0	3
N with multiple malignancies (%)**	2 (1.5)	4 (2)	5 (3)	0	11 (2)
N with myelodysplastic syndrome (MDS) (%)	3 (2)	21 (11)	26 (16)	6 (17)	56 (11)
N died from malignancy, no HCT (%)	3 (2)	8 (4)	15 (9)	2 (6)	28 (5)
Post-stem cell transplantation (HCT)					
N who had HCT (% of total)	10 (7.4)	60 (30.5)	63 (38.7)	3 (8.6)	136 (25.7)
N with malignancy after HCT (%)***	1 (10)	3 (5)	7 (11)	0	11 (8)
N died after HCT related to HCT	2 (20)	17 (28)	16 (25)	–	35 (26)
N died from malignancy after HCT (%)	1 (10)	1 (1.7)	4 (6)	–	6 (4)

[*]Percent of number with no or pre-HCT. **Multiple malignancies in non-transplanted patients: DBA: Cervix and lung; colon and liver. DC: Leukemia, cervix and thyroid; non-Hodgkin lymphoma (NHL), lip, NHL; Acute lymphocytic leukemia (ALL), acute myeloid leukemia (AML) and soft tissue; mouth and pharynx. FA: mouth and mouth; tongue and esophagus; anus and vulva; cervix and vulva; tongue and lip. ***Percent of number who had HCT. DBA: Diamond-Blackfan anemia; DC: dyskeratosis congenita; FA: Fanconi anemia; SDS: Shwachman-Diamond syndrome; N: number; HCT: stem cell transplantation.

Following HCT, one of 10 patients with DBA developed post-transplant lymphoproliferative disease (PTLD); three of 60 patients with DC developed tongue SCC, Hodgkin disease, and PTLD, respectively; seven of 63 patients with FA developed a total of 12 malignancies, including HNSCC, pharyngeal SCC, vulvar SCC, thyroid cancer, and PTLD (Table 4). One brain tumor developed 10 months after HCT in a patient with *FANCD1/BRCA2*. The effect of HCT on increased cancer rates is reflected in the higher O/E ratios for the transplanted *vs.* the non-transplanted groups: DBA 81 *vs.* 2.5, DC 30 *vs.* 4.2, FA 55 *vs.* 19 (Table 3 and Table 4).

MDS was analyzed separately (Table 3), since it was based on self-report or medical records, but the slides were not centrally reviewed. The O/E ratio for MDS in DBA was 42, in DC 578, in FA 5669; there were no MDS cases in SDS.

Nonmelanoma skin cancer (Table 3) was rare in DBA and not reported in SDS, but quite frequent in DC and FA, with multiple cancers in a few patients, including both basal cell carcinomas (BCC) and skin SCC. There was one SCC and six BCC in DBA, 14 SCC and six BCC in DC, and 23 SCC and 12 BCC in FA. Nine out of 197 patients with DC and 11 out of 163 with FA had one or more skin cancers. Two patients with DC had two each, two had three each, and one had five skin cancers; while six patients with FA had two skin cancers each, one had three, and one reported 17 separate skin cancers.

The competing risks of adverse first events are demonstrated in Figure 1. The cumulative incidence of HCT for severe BMF or death without malignancy rose linearly in DC to more than 70% by age 70, due to an annual hazard rate of about 2% in childhood with a sharp rise at around age 50 that reached 10% by age 80 years. The cumulative incidence of severe BMF in FA leveled off at 70% by age 50, with a peak hazard rate of 4% at age 12-15, and a plateau of 5% at age 45. Severe BMF following the syndrome-specific red blood cell aplasia in DBA reached around 40% by age 70; the maximal level in SDS was 20%.
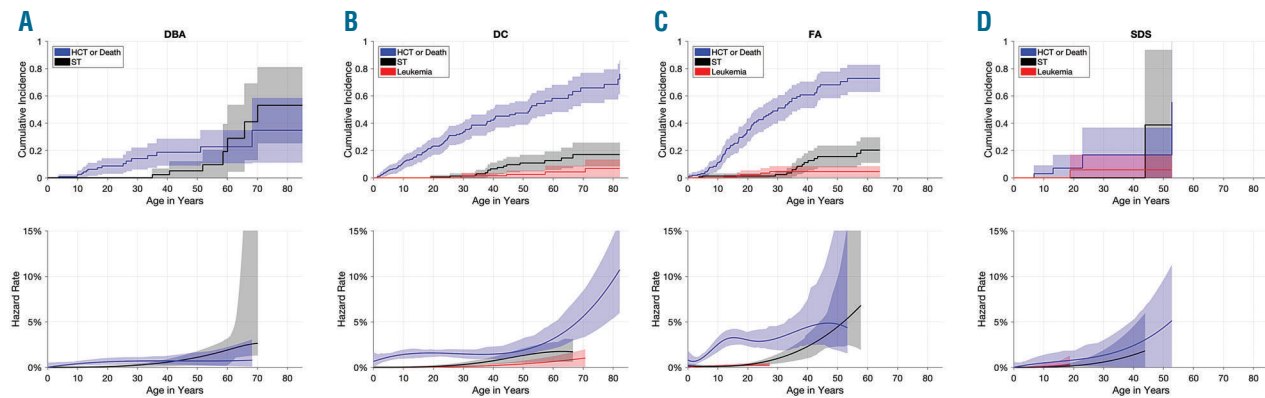

Figure 1. Complications in patients in the NCI inherited bone marrow failure syndromes cohort. (A-D) Cumulative incidence (top panels) and annual hazard rates (bottom panels) of competing adverse events by age in patients with (A) Diamond-Blackfan anemia (DBA), (B) Dyskeratosis congenita (DC), (C) Fanconi anemia (FA) and (D) Shwachman-Diamond syndrome (SDS). Adverse events are severe bone marrow failure leading to hematopoietic cell transplantation or death (HCT or death, blue), acute leukemia (red), or solid tumors (ST, black). Top panels, cumulative incidence of experiencing each event as the initial cause of failure (observed, stair-step lines) and 95% confidence intervals (shaded areas). Bottom panels, annual hazard rates (modeled, smooth curves), i.e., % per year who develop a given event type among subjects who are still susceptible, and 95% confidence intervals (shaded areas).
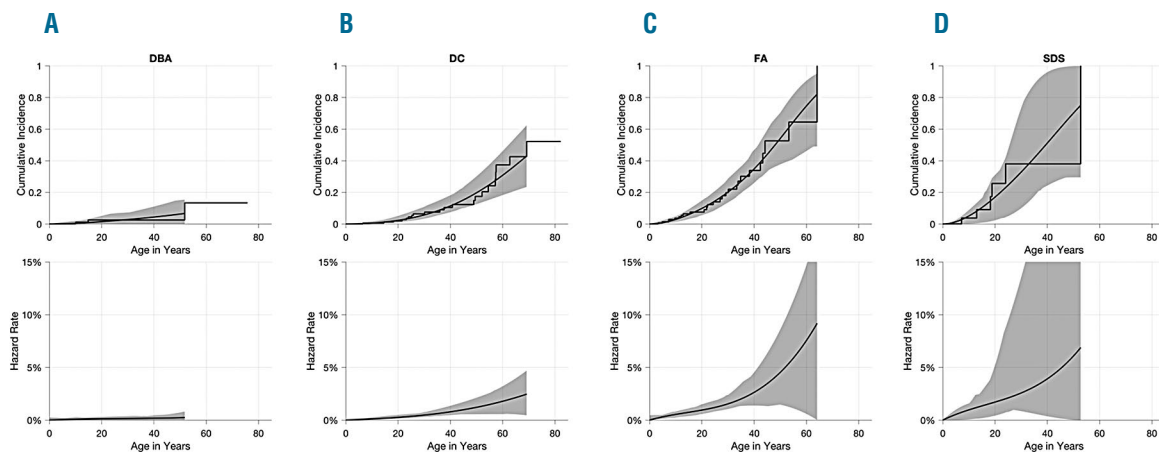

Figure 2. Cumulative incidence and annual hazard rates of myelodysplastic syndrome (MDS) by age. Patients with (A) Diamond-Blackfan anemia (DBA), (B) Dyskeratosis congenita (DC), (C) Fanconi anemia (FA) and (D) Shwachman-Diamond syndrome (SDS). Top panels show observed (stair-step lines) and modeled (smooth curves) cumulative incidence curves. Bottom panels show hazard rates (smooth curves) as described in Figure 1. MDS is not considered a competing risk.

The cumulative incidence of solid tumors was close to 20% by age 65 in both DC and FA (Figure 1). The contours of the hazard rates were different, essentially plateauing at 2% per year in DC, while the hazard rate increased more than exponentially in FA after age 30. Solid tumor cumu- lative incidence in DBA was more than 50% by age 70, and was around 40% in SDS by age 45. Leukemia had a cumulative incidence of under 10% in DC, and under 5% in FA and SDS, by ages 70, 30, and 20, respectively, with low hazard rates; no cases of AML were reported in our

Table 3. Cancers in patients in the NCI IBMFS cohort who did not have HCT

Syndrome	Cancer	Ages (years) Median (range)	Observed N	Expected N	O/E	95% CI
DBA	**All sites***	**49 (17-70)**	**9**	**3.68**	**2.5**	**1.1-4.7**
N=135	**Solid Tumors***	**49 (17-70)**	**9**	**3.12**	**2.9**	**1.3-5.5**
PY=3458	**Lung***	**53 (47-70)**	**4**	**0.34**	**12**	**3.2-30**
	Colon*	**34, 55**	**2**	**0.18**	**11**	**1.4-41**
	Cervix*	**17, 40**	**2**	**0.05**	**37**	**4.6-136**
	Liver	34	1	0.04	27	0.7-151
	MDS*	**13**	**1**	**0.02**	**42**	**1.1-234**
	Skin 8 ca, N = 5	45 (39-63)	8			
	1 NMS, N = 1		1			
	1 SCC, N = 1		1			
	6 BCC, N = 4		6			
DC	**All sites***	**38 (18-63)**	**27**	**6.47**	**4.2**	**2.8-6.1**
N=197	**Solid Tumors***	**38 (18-61)**	**17**	**5.57**	**3.1**	**1. 8-4.9**
PY=5655	**HNSCC***	**38 (18-61)**	**11**	**0.15**	**74**	**37-133**
	Tongue*	***33 (18-42)***	***8***	***0.04***	***216***	***94-427***
	Leukemia*	**40 (28-63)**	**7**	**0.3**	**24**	**9. 5-48**
	AML*	***40 (28-56)***	***5***	***0.07***	***73***	***23-169***
	NHL*	**57 (43-65)**	**3**	**0.29**	**11**	**2.2-30**
	Anus*	**34**	**1**	**0.02**	**47**	**1.2-262**
	Esophagus	38	1	0.04	28	0.7-157
	Rectum	37	1	0.16	6.4	0.2-36
	Cervix	37	1	0.14	7.3	0.2-41
	Thyroid	37	1	0.27	3.7	0.1-20
	MDS*	**31 (4-73)**	**18**	**0.03**	**578**	**343-914**
	Skin 22 ca, N = 9	35 (14-54)	22			
	2 NMS, N = 2		2			
	14 SCC, N = 7		14			
	6 BCC, N = 4		6			
FA	**All sites***	**30 (4-58)**	**26**	**1.37**	**19**	**12-28**
N=163	**Solid Tumors***	**34 (4-58)**	**21**	**1.1**	**19**	**12-29**
PY=2854	**HNSCC***	**37 (29-53)**	**10**	**0.02**	**527**	**253-970**
	Tongue*	***37 (29-42)***	***5***	***0***	***1054***	***342-2460***
	Leukemia*	**17 (12-27)**	**5**	**0.13**	**40**	**13-92**
	AML*	***17 (12-27)***	***5***	***0.02***	***213***	***69-496***
	Vulva*	**29 (24-39)**	**3**	**0.01**	**582**	**120-1702**
	Esophagus*	**34, 35**	**2**	**0**	**1266**	**153-576**
	Brain*	**3.7, 4.9**	**2**	**0.09**	**23**	**2.8-84**
	Anus*	**33**	**1**	**0**	**256**	**6.5-1427**
	Lung	58	1	0.04	26	0.7-144
	Cervix	22	1	0.05	20	0.5-109
	Breast	30	1	0.3	3.4	0.1-19
	MDS*	**13 (1-57)**	**26**	**0**	**5669**	**3703-8307**
	Skin 35 ca, N = 11	33 (26-41)	35			
	23 SCC, N = 10		23			
	12 BCC, N = 4		12			
SDS	**All sites***	**31 (19-43)**	**2**	**0.24**	**8.5**	**1.02-30**
N=35	Solid Tumors	43	1	0.18	5.5	0.1-31
PY=640	**Ovary***	**43**	**1**	**0.01**	**169**	**4.3-944**
	Leukemia	19	1	0.03	34	0.9-194
	AML*	***19***	***1***	***0***	***202***	***5-1126***

*Bold = significant at *P*<0.05. Italicized = subset of category in the row above. Age is age at malignancy; age at first skin cancer if multiple skin cancers. Pt: patient; N: number of patients; O/E: observed-to-expected; CI: confidence interval; SCC: squamous cell carcinoma; BCC: basal cell carcinoma; HNSCC: head and neck squamous cell carcinoma; ca: cancer; NMS: nonmelanoma skin cancer; DBA: Diamond-Blackfan anemia; DC: dyskeratosis congenita; FA: Fanconi anemia; SDS: Shwachman-Diamond syndrome; PY: person- years; MDS: myelodysplastic syndrome; AML: acute myeloid leukemia; NHL: non-Hodgkin lymphoma.

DBA cohort. MDS, a non-competing risk, was analyzed separately (Figure 2). By age 50 in each syndrome, the respective cumulative incidences (and confidence intervals) were 50% (35-65%) in FA, 20% in DC (15-25%), 65% (25-100%) in SDS, and 5% (0-15%) in DBA. Thus the cumulative incidence was higher in FA than in DC, and both were higher than in DBA.

The median overall survival was longest in DBA (67 years), followed by DC (51 years), SDS (41 years) and FA (39 years) [Figure 3 (arrows), Table 1]. These data include cases who were only identified as adults, such as patients with DC who had pulmonary fibrosis as older adults, patients with FA with solid tumors, or parents of affected children in dominant disorders such as DBA or some DC genotypes, who had the same pathogenic variant as their child. The most frequent causes of death in the non-transplanted patients were aplastic anemia and malignancies in FA and DC as well as pulmonary disease in DC, and iron overload and malignancies in DBA. Transplanted patients with FA developed solid tumors, pneumonia, GvHD, and renal disease, while those with DC developed solid tumors and pulmonary fibrosis.

The effect of HCT on cancer incidence is shown in Figure 4. Patients with FA had a striking increase in cumulative incidence of cancer and left shift to younger age (Figure 4A). These data are skewed by the medulloblastoma 10 months after HCT in the patient with *FANCD1/BRCA2*; the trend persisted after removal of that data point (Figure 4B).[25] Cancer cumulative incidence was also higher and earlier post-HCT in DC (Figure 4C).

We identified the genotype in 61% of the patients with DBA, 78% of those with DC, 73% of those with FA, and 82% of those with SDS (Table 5). Twenty-eight patients with DBA had pathogenic variants in *RPS19*, and 21 in *RPS29*. There were five cancers in four individuals from one DBA family among the 21 patients with mutations in *RPS29*. This significant association (P<0.001) is confounded by the familial component. The majority of the pathogenic variants in the patients with DC were in *DKC1, TERT, TERC, RTEL1* and *TINF2*; the number of patients with cancer were 5, 3, 5, 0 and 1, respectively. The excess of cancers in those with mutations in *DKC1, TERT*, or *TERC* was significant (P=0.02); however, the patients with pathogenic variants in *RTEL1* and *TINF2* (and fewer cancers) were generally younger than in the first three genotype groups. The most frequent genotypes in the patients with FA were *FANCA* and *FANCC*, and there was no association of genotype with cancer; there were 11 cancers in 70 patients with
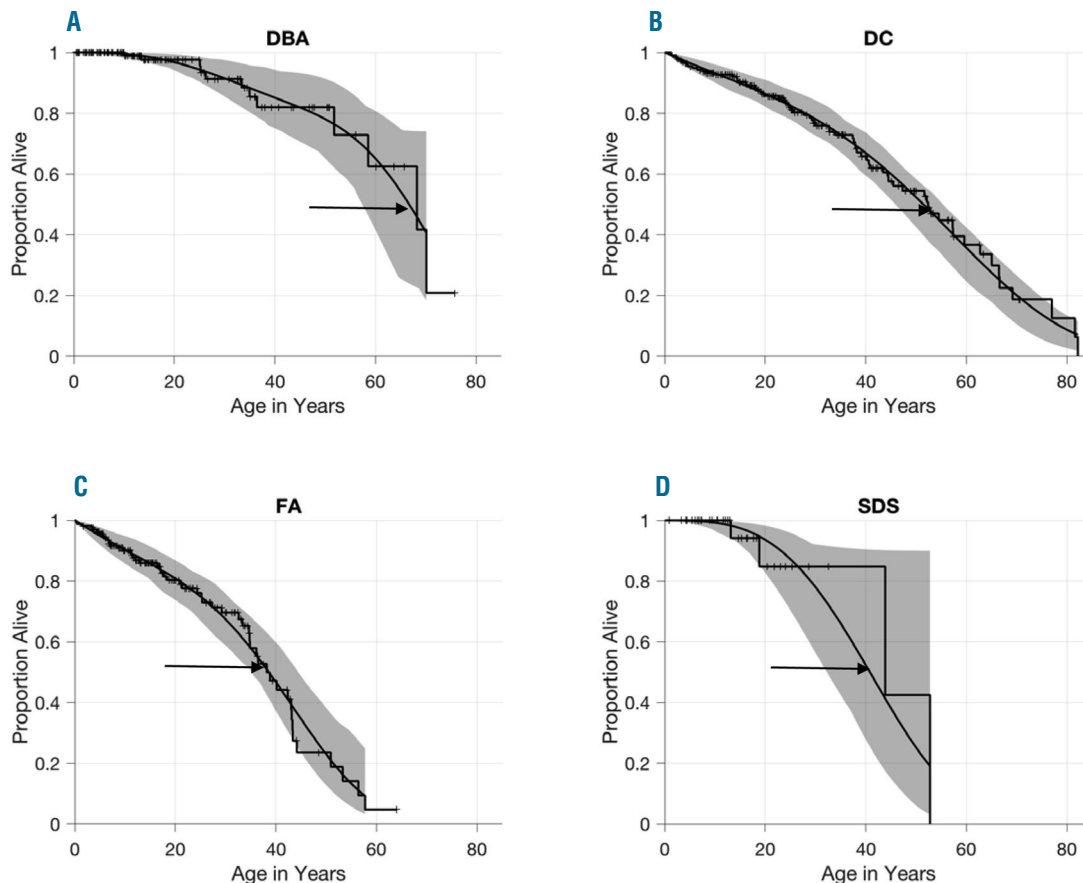

Figure 3. Survival curves for patients in the NCI IBMFS cohort, showing proportion alive by age in years. (A) Diamond-Blackfan anemia (DBA), (B) Dyskeratosis congenita (DC), (C) Fanconi anemia (FA) and (D) Shwachman-Diamond syndrome (SDS). Tick marks represent patients who were still alive at the time of analysis. Observed survival (stair-step lines) and smoothed survival (smooth curves) are shown; shaded areas show 95% confidence intervals for the smoothed curves.

FANCA, three in 19 patients with *FANCC*, and seven in 74 patients with other genotypes (global *P*=0.5).

Discussion

The NCI IBMFS cohort includes more than 500 patients with one of the four major rare IBMFS, more than twice the number discussed in our previous report.[8] The current estimates are more precise, extend through older ages, and cover all four syndromes (*versus* mostly FA and DC in the prior report). These significant data are most stable (narrower confidence intervals) for FA and DC, due to the larger number of events than in the other syndromes. The median survival improved by 5-10 years. The data reflect the additional eight years of follow-up, as well as
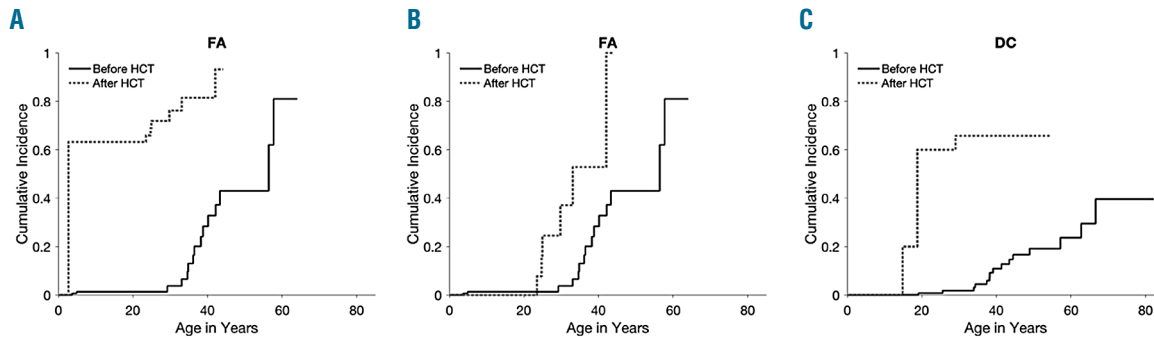

Figure 4. Cumulative incidence of solid tumors by age in non-transplanted and transplanted patients. Non-transplanted, blue and transplanted, red. (A) Fanconi anemia (FA), including one patient with *FANCD1/BRCA2* who developed a brain tumor at age 32 months, 21 months following a stem cell transplant. The rate ratio for transplanted/non-transplanted is 3.5 (95% CI 1.2 – 9.2). (B) FA, excluding the patient with *FANCD1/BRCA2*. The rate ratio is 3 (95% CI 0.97 - 8.2). (C) Dyskeratosis congenita (DC). The rate ratio is 5.7 (95% CI 1.1 – 20.3).

Table 4. Cancers in patients in the NCI IBMFS cohort, following transplant

Syndrome	Cancer	Ages (years) Median (range)	Observed N	Expected N	O/E	95% CI
DBA	**All sites, N = 1**	**10**	**1**	**0.01**	**81**	**2.05-451**
N=10	Solid Tumors		0	0.01	0	0-451
PY=53	**NHL***	**10**	**1**	**0**	**983**	**25-5474**
DC	**All sites, N = 3**	**19 (15-29)**	**3**	**0.1**	**30**	**6-87**
N=60	Solid Tumors	18.8	1	0.08	13	0.3-73
PY=248	**HNSCC**	**18.8**	**1**	**0**	**432**	**11-2404**
	Tongue	*18.8*	*1*	*0*	*1561*	*40-8699*
	Hodgkin	**14.9**	**1**	**0.01**	**164**	**4-914**
	NHL*	**29.1**	**1**	**0.01**	**141**	**4-786**
	Skin BCC	50	1			
	Skin SCC	35 (14-37)	3			
FA	**All sites, N = 7**	**31 (3-51)**	**12**	**0.22**	**55**	**29-97**
N=63	**Solid Tumors**	**33 (3-51)**	**11**	**0.16**	**67**	**33-120**
PY=544	**HNSCC**	**41 (25-51)**	**5**	**0.01**	**933**	**303-2178**
	Lip	*41*	*1*	*0*	*4,662*	*118-25975*
	Tongue	*25, 48*	*2*	*0*	*1,451*	*176-5244*
	Hypopharynx	*33, 51*	*2*	*0*	*16935*	*2051-61174*
	Larynx	**42**	**1**	**0**	**1383**	**35-7704**
	Vulva	**25, 28**	**2**	**0**	**6298**	**763-22752**
	Brain	**2.7**	**1**	**0.02**	**61**	**1.5-339**
	Thyroid	**24, 34**	**2**	**0.02**	**100**	**12-360**
	NHL*	**30**	**1**	**0.02**	**66**	**1.7-369**
	Skin BCC	32	9			
	Skin SCC	31 (30-41)	12			
SDS	All sites		0	0.01	0	0-579
N=3	Solid Tumors		0	0	0	0-908
PY=33						

Bold = significant at *P*<0.05. Italicized = subset of category in the row above. *NHL, probably post-transplant lymphoproliferative disease (PTLD). Age is age at malignancy; age at first skin cancer if multiple skin cancers. One patient with FA had 8 BCC and 9 SCC. Another with FA had 1 BCC and 1 SCC. Observed N: number of events; N: number of patients; O/E: observed-to-expected; CI: confidence interval; SCC: squamous cell carcinoma; BCC: basal cell carcinoma; HNSCC: head and neck squamous cell carcinoma; DBA: Diamond-Blackfan anemia; DC: dyskeratosis congenita; FA: Fanconi anemia; SDS: Shwachman-Diamond syndrome; PY: person-years; NHL: non-Hodgkin lymphoma.

improvements in clinical management, combined with diagnosis of affected individuals at older ages. The older participants increasingly comprise parents and grandparents in autosomal dominant disorders (DBA, some DC) who carry the same pathogenic variant as the proband. In addition, a growing number of individuals may have been diagnosed with DC as older adults, after the development of recently recognized complications such as pulmonary fibrosis or hepatic disease, coupled with the expansion of genetic testing and appreciation of a broader clinical spectrum. The diagnosis of FA is also occurring in older individuals, such as a patient who presented with a solid tumor at age 30,[26] or another identified as a potential HCT donor for an adult sibling with FA (*Alter, unpublished*). These two patients had hematopoietic somatic mosaicism which may have contributed to their survival free of BMF.[3]

More than one-third of the patients with DC or FA received an HCT. The cumulative incidence of receiving an HCT or dying from severe BMF by age 50 was 70% in FA and 50% in DC. The annual hazard rate for HCT or non-malignant death was 3-5% in FA by age 10, and 1-2% in children with DC, and rose after age 50 in DC. The hazard rate in FA appeared to level off in adults with FA, but kept increasing in adults with DC. HCT was reported in less than 10% of the participants with DBA or SDS, perhaps because some patients with DBA respond to corticosteroids or receive red blood cell transfusions and iron chelation. Those with SDS and neutropenia may have responded to treatment with granulocyte-colony stimulating factor (G-CSF), and did not develop pancytopenia.

All of the syndromes had relatively low frequencies of leukemia, but high O/E ratios for AML. The O/E for AML in the NCI patients with DC was 73, and was above 200 in those with FA, consistent with the O/E of 300 in our earlier report, but lower than the O/E of 600-1000 in three other FA cohorts.[4,6,7] High O/E ratios for AML reflect its development in patients with an IBMFS at a younger age than in sporadic adult cases. The confidence intervals are wide due to the small numbers of cases in the cohorts. The lower values in the current analysis compared with earlier studies may indicate regression to the mean, or may reflect enrolment biases toward patients with certain cancers in the earlier phase of our cohort. Some participants in our cohort were only diagnosed during family studies after a sibling developed leukemia or a solid tumor; the affected sibling with a malignancy was also enrolled in our cohort. Nevertheless, BMF as well as MDS remain major complications in adults with each of the four major IBMFS.

The major cancer sites in FA included HNSCC (primarily tongue), AML, vulva, esophagus, and brain, and are consistent in all the reported FA cohorts. Malignancies in DC were also HNSCC and AML as well as NHL. The unique cancer spectrum in DBA was lung, colon and cervix in the NCI DBA cohort, and lung, colon and osteosarcoma in the DBAR.[13] Hence, our new analysis supports the conclusion that the rate of development of cancer is highest in FA, less in DC, and substantially lower and of different types in DBA. Our SDS cohort is small, and the single cases of leukemia and ovarian cancer are too few to indicate an increased rate of these malignancies. The pathophysiology of HNSCC in these syndromes is not clear; the possible role of human papillomavirus in HNSCC is controversial,[27] but seems unlikely.[28,29]

Solid tumors following HCT were first quantified in our comparison of cancer incidence in patients with FA transplanted in Paris with non-transplanted patients in the North American Survey (NAS).[4,5] We now confirm a similarly increased risk and earlier median age for cancer in transplanted patients. Others had suggested a transplant-related increased risk of solid tumors in FA, although the cumulative incidence was not reported by age.[19,30] We found a similar transplant effect on cancer in patients with DC. We do not have sufficient data and the transplant reg-

Table 5. Genotypes according to frequency, total group and those with cancer without HCT.

DBA Genes	Total (CA)	DC Genes	Total (CA)	FA Genes	Total (CA)	SDS Genes	Total (CA)
RPS19	28 (1)	DKC1	35 (5)	FANCA	70 (11)	SBDS2***	26 (2)
RPS29*	21 (5)	TERT	34 (3)	FANCC	19 (3)	SBDS1***	1
RPL26	7	TERC	29 (5)	FANCD2	7		
RPL35A	7	RTEL1	23	FANCG	7 (1)		
RPL11	4	TINF2	22 (1)	FANCI	4		
RPS7	4	PARN	4	FANCJ	4		
RPS24	3	WRAP53	3 (1)	FANCD1*	3 (2)		
RPS10	3	CTC1	3	FANCB	2		
RPL5	3	ACD	1	FANCF	2		
RPS17	2			FANCR	1		
RPL15	1						
Unknown**	52 (1)		43 (5)		44 (4)		8
Total with Gene	83 (6)		154 (15)		119 (17)		27 (2)
Total Overall	135 (7)		197 (20)		163 (21)		33 (2)

All patients, not according to families, not age adjusted. Several were assigned a gene by pedigree. Columns indicate the number of patients with each genotype (number with cancer). *Significant association of cancer with genotype. Four people in one *RPS29* family had three lung, one cervix, and one colon cancer. **Incomplete or truly unknown and unknowable (no DNA available) ***SBDS1 means only one mutated allele identified. *SBDS2* means two mutated alleles. DBA: Diamond-Blackfan anemia; DC: dyskeratosis congenita; FA: Fanconi anemia; SDS: Shwachman-Diamond syndrome; CA: cancer

imens are too diverse to identify specific associations between cancer risk with the use of irradiation in the preparative regimens, or the development of GvHD. Future studies of radiation-free HCT, with careful documentation of GvHD, and focusing on the age of the patient rather than the interval following HCT, may shed light on this concern. The assumption must be that any patient with an IBMFS who has received an HCT will, at best, not be at less risk for a solid tumor, and thus cancer surveillance remains a high priority.

Our cohort remains too small to lead to significant conclusions about the relation of genotype to cancer. The association with *RPS29* in DBA is confounded by all five cancers occurring in a single family. The brain tumors in all three of the patients with *FANCD1/BRCA2*, two without HCT and one within 10 months following HCT (and thus probably not due to the HCT), are consistent with the known extremely high risk of this cancer in patients with that genotype.[25] The NCI cohort is too small to have large numbers of patients with each genotype (except *FANCA*). It will be important to consider whether a pathogenic variant leads to a null or a hypomorphic phenotype, since the former might be expected to be more severe than the latter.[31] Therefore, further follow-up and enrollment of additional subjects are necessary to determine additional genotype-phenotype associations in FA, DC, and DBA.

Limitations of our study include biased enrolment because of possible specific interest in cancer risks. Our participants are predominantly North American; specific genotypes and cancer risks may differ in other populations. Mild cases with less severe BMF and/or fewer birth defects, or with mosaicism, may not have been identified. There may have been left truncation of very severe patients, who died without a diagnosis and were not entered into our study retrospectively. We may be skewed toward adult patients, since we are not specifically a Children's hospital.

Strengths of our study include comparison of patients with the major IBMFS in a single cohort, with presumably similar participation biases. The magnitude of the risks of adverse events showed similar trends in other cohorts (e.g., the German FA Registry, Israel and NAS), and in our earlier report. Our participants are seen by the same medical consultants who now have more than 15 years of experience of dealing with these rare disorders, and studied in the same laboratories.

In summary, the NCI cohort is now sufficiently mature to provide more stable estimates of the types and risks of cancer in the major rare IBMFS. Continued follow-up will help identification of effective methods for cancer prevention and surveillance in these high-risk populations, and effective case management.

Since our data were locked-in as of May, 2016, we became aware of nine additional cancers in nine patients, eight who had no prior cancer. Three in DC, no HCT: tongue, thyroid, and skin. Three in DC, post-HCT: tongue, pharynx, and bladder. Two in FA, no HCT: AML, and skin. One in FA, post-HCT: bladder, in a patient with a prior tongue cancer.

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