haematologica
Journal of the Ferrata Storti Foundation

Genomic profiling of pediatric hematologic malignancies and diagnosis of cancer predisposition syndromes: tumor-only *versus* paired tumor-normal sequencing

by Haley Newman, Mary Egan Clark, Derek Wong, Jinhua Wu, Garrett M. Brodeur, Stephen P. Hunger, Sarah K. Tasian, Timothy Olson, Julia T. Warren, David T. Teachey, Kira Bona, Jeffrey Schubert, Netta Golenberg, Maha Patel, Elizabeth H. Denenberg, Elizabeth A. Fanning, Jiani Chen, Tamara Luke, Sarah Charles, Daniel Gallo, Kajia Cao, Weixuan Fu, Zhiqian Fan, Lea F. Surrey, Gerald Wertheim, Minjie Luo, Suzanne P. MacFarland, Marilyn M. Li, and Yiming Zhong

Received: December 12, 2023.
Accepted: February 14, 2024.

Citation: Haley Newman, Mary Egan Clark, Derek Wong, Jinhua Wu, Garrett M. Brodeur, Stephen P. Hunger, Sarah K. Tasian, Timothy Olson, Julia T. Warren, David T. Teachey, Kira Bona, Jeffrey Schubert, Netta Golenberg, Maha Patel, Elizabeth H. Denenberg, Elizabeth A. Fanning, Jiani Chen, Tamara Luke, Sarah Charles, Daniel Gallo, Kajia Cao, Weixuan Fu, Zhiqian Fan, Lea F. Surrey, Gerald Wertheim, Minjie Luo, Suzanne P. MacFarland, Marilyn M. Li, and Yiming Zhong. Genomic profiling of pediatric hematologic malignancies and diagnosis of cancer predisposition syndromes: tumor-only versus paired tumor-normal sequencing. Haematologica. 2024 Feb 22. doi: 10.3324/haematol.2023.284855 [Epub ahead of print]

Genomic profiling of pediatric hematologic malignancies and diagnosis of cancer predisposition syndromes: tumor-only *versus* paired tumor-normal sequencing

Haley Newman[1,2]; Mary Egan Clark[1]; Derek Wong[3]; Jinhua Wu[3;] Garrett M Brodeur[1,2,4]; Stephen P Hunger[1,2,4]; Sarah K Tasian[1,2,4]; Timothy Olson[1,2,4]; Julia T. Warren[2,5]; David T Teachey[1,2,4]; Kira Bona[6]; Jeffrey Schubert[3]; Netta Golenberg[3]; Maha Patel[3]; Elizabeth H Denenberg[3]; Elizabeth A Fanning[3]; Jiani Chen[3]; Tamara Luke[3]; Sarah Charles[3]; Daniel Gallo[3]; Kajia Cao[3]; Weixuan Fu[3]; Zhiqian Fan[3]; Lea F Surrey[2,3]; Gerald Wertheim[2,3]; Minjie Luo[2,3;] Suzanne P MacFarland[1,2,4]; Marilyn M Li[1,2,3]; Yiming Zhong[2,3]

Affiliations:

1. Division of Oncology, Department of Pediatrics, Children's Hospital of Philadelphia, Philadelphia, PA
2. Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA
3. Department of Pathology and Laboratory Medicine, Children's Hospital of Philadelphia, Philadelphia, PA
4. Center for Childhood Cancer Research, Children's Hospital of Philadelphia, Philadelphia, PA
5. Division of Hematology, Department of Pediatrics, Children's Hospital of Philadelphia, Philadelphia, PA
6. Department of Pediatric Oncology, Division of Population Sciences, Dana-Farber Cancer Institute, Boston, MA

Corresponding author:
Yiming Zhong, PhD
zhongy1@chop.edu
Department of Pathology and Laboratory Medicine
The Children's Hospital of Philadelphia
Perelman School of Medicine, University of Pennsylvania
3615 Civic Center Boulevard, Philadelphia, PA

Data sharing statement: The datasets generated and analyzed for this study are available from the corresponding author on reasonable request.

Funding: This investigation was supported by 5K12CA076931-24 to HN from the National Institute of Health. SKT is a Scholar of the Leukemia & Lymphoma Society and holds the Joshua Kahan Endowed Chair in Pediatric Leukemia Research at the Children's Hospital of Philadelphia.

Disclosures: SPH owns common stock in Amgen and has received honoraria or consulting fees from Amgen, Jazz, Novartis and Servier. SKT receives/d research funding from Kura Oncology and Incyte Corporation, serves/d on scientific advisory boards for Aleta Biotherapeutics, AstraZeneca, Jazz Pharmaceuticals, Kura Oncology, and Syndax Pharmaceuticals, and has received travel support from Amgen (all for unrelated studies).

Contributions: HN, MEC, DW, YZ and ML conceived and designed the study, analyzed the data, and wrote initial version of the manuscript. All authors (HN, MEC, DW, JW, GMB, SPH, SKT, TO, JW, DTT, KB, JS, NG, MP, EHD, EAF, JC, TL, SC, DG, KC, WF, ZF, LFS,GW, ML, SPM, MML, YZ) interpreted the results and reviewed and contributed to the final version of the manuscript.

Next-generation sequencing (NGS) technology has dramatically enhanced genomic characterization of hematologic malignancies, aiding in diagnosis, risk stratification, and treatment.[1-3] Simultaneously, enhanced sequencing and more accessible germline testing has revealed that cancer predisposition syndrome (CPS) accounts for a greater proportion of pediatric cancer diagnoses than previously appreciated.[4,5] The diagnosis of a CPS in children is critical for informed treatment decision-making, future cancer surveillance, testing for family members, and family planning.[7-9] Although somatic DNA-based panel results may suggest the presence of a germline variant associated with cancer predisposition based on variant allele fraction (VAF) and/or other features of the variant and gene,[6] tumor-only sequencing cannot definitively distinguish somatic and germline alterations. Thus, tumor-only sequencing requires follow-up testing for assessment of potential germline mutations. Alternatively, up-front paired tumor and germline-referred to herein as tumor-normal (T/N)—testing at the time of diagnosis utilizes DNA isolated from both cancer cells and non-malignant cells (usually from skin biopsy), sequenced at the same time and on the same platform such that data from tumor and normal DNA of the same individual can be analyzed together. This approach not only enhances precision of identifying somatic alterations by comparing the patient's cancer genome to their own constitutional genome instead of a generic human reference genome, but also can simultaneously identify germline cancer predisposition within genes being sequenced.[10] We herein describe the implementation of DNA-based paired T/N testing at a large pediatric cancer program, compare diagnostic yield of tumor-only testing followed by germline confirmation versus paired T/N testing, assess clinical implications for patients diagnosed with a CPS, and examine benefits and detriments of these two sequencing approaches.

A total of 1,190 pediatric and adolescent and young adult (AYA) patients (age 0 to 35 years) were retrospectively included in this cohort, 1,034 of whom underwent tumor-only testing between June 2016 and October 2022, and 156 patients of whom underwent paired T/N testing between January 2021 and August 2023 (**Figure 1**). This study was approved by the Children's Hospital of Philadelphia Institutional Review Board. Our targeted hematologic cancer panel (HEMEP)[2] and comprehensive hematologic panel (COHEM) interrogate 117 known cancer genes associated with hematologic malignancies for SNV, indel, and CNV. The COHEM panel also includes RNA-based fusion analysis for over 700 exons of 117 cancer genes for known and novel fusions.[2, 11] The identified variants were categorized according to the guidelines.[12,13] Demographics and clinical characteristics of this patient cohort are described in **Supplemental Table 1**. The mean age was 9 years. The most common diagnosis was B-cell acute lymphoblastic leukemia (B-ALL; 58%), followed by acute myeloid leukemia (AML)/myeloid sarcoma (18%), and T-cell acute lymphoblastic leukemia/lymphoblastic lymphoma (T-ALL/T-LL; 9%). Most cases (1068/1190, 90%) underwent COHEM panel testing, while a smaller proportion had HEMEP panel sequencing (122/1190, 10%).

Among 1,034 patients who initially underwent tumor-only molecular sequencing, 31 (3%) of patients were found to have genomic alterations consistent with a pre-existing/known CPS. An additional 111 (11%) patients without a known CPS met criteria for follow-up germline testing as recommended on their diagnostic tumor NGS reports (**Figure 1**). Within the cohort of 111 patients recommended for germline follow-up testing, 47 cases were submitted from outside institutions and had no follow-up clinical data available. Notably, none of these cases had germline specimens submitted to the CHOP diagnostic genomics laboratory. In the cohort of

patients treated at our institution (n=64) recommended for follow up testing, 29 (45%) had subsequent confirmatory germline testing facilitated by the cancer predisposition team or the patient's primary oncologist, whereas 35 patients (55%) had no documented confirmatory testing. Of these 35 patients, 16 died within six months of somatic testing, which may have precluded the possibility or desire to perform recommended follow-up testing. Notably, this high mortality rate may in part reflect the overrepresentation of children and AYAs at our institution with relapsed/refractory disease, referred for early-phase clinical trial participation or other salvage therapies, who undergo molecular testing. One patient has germline testing pending insurance authorization, and another patient was recently referred to the cancer predisposition clinic. For the remaining 17 patients, germline predisposition was mentioned only in genomics reports, suggesting the information may have potentially been missed by clinicians and families. Among the 29 patients who initially underwent somatic-only tumor testing and subsequent recommended germline testing, 10 (34%) were confirmed to have a CPS, including genetic mutations associated with Noonan syndrome-like disorder (*CBL*), DNMT3A overgrowth syndrome and predisposition to hematologic malignancy/Tatton-Brown-Rahman syndrome (*DNMT3A*), ETV6 thrombocytopenia and leukemia predisposition syndrome (*ETV6*), GATA2 deficiency syndrome (*GATA2*), Lynch syndrome (*MSH2*), Noonan syndrome (*PTPN11)*, Mirage syndrome (*SAMD9*), and LFS (*TP53*).

Among 156 patients who underwent paired T/N testing, ten patients (6%) were found to have genomic alterations consistent with a pre-existing/known CPS. Six patients (4%) were diagnosed with a new CPS (**Figure 1**), including CDNK2A associated predisposition (*CDKN2A*), CEBPA-associated predisposition to AML (*CEBPA*), ETV6 thrombocytopenia and leukemia predisposition syndrome (*ETV6*), GATA2 deficiency syndrome (*GATA2*), IKZF1-assocated leukemia predisposition (*IKZF1*), and RUNX1 familial platelet disorder with associated myeloid malignancies (*RUNX1*). If tumor only testing, instead of T/N, were performed on those six patients with a new CPS, the results would trigger a germline testing.

Potential or confirmed germline variants were identified in 35 cancer predisposition genes from 117 patients (**Figure 2**; **Supplemental Table 2**). Potential germline variants were most frequently identified in *TP53* (n=36), *NF1* (n=15) and *PTPN11* (n=10). Among patients diagnosed with a CPS, there were several immediate, clinically relevant implications (**Table 1**). Two patients with germline *CBL*- or *PTPN11*-mutant juvenile myelomonocytic leukemia (JMML) were treated according to guidelines for patients with germline predisposition to JMML, which differ from guidelines for children with somatic Ras pathway mutation-driven JMML.[12] An additional three patients were diagnosed with germline GATA2 deficiency syndrome, one with AML and two with monosomy 7 MDS. All three were recommended for hematopoietic stem cell transplantation (HSCT) with genetic testing of family members for optimal *GATA2* wild-type transplant donor selection and predisposition screening. Two patients were diagnosed with LFS portending lifelong increased risk of malignancy and prompting referral to the cancer predisposition team for optimal tumor surveillance, genetic counseling, and cascade testing for at-risk family members.

Of the 16 patients with hematologic malignancies and a CPS newly diagnosed from genomic testing (Figure 1), there was no known cancer predisposition or early onset cancer in any other immediate family members. To date, 33 of 48 (69%) family members recommended for

germline testing based upon diagnoses of a CPS in their probands have undergone testing (**Supplemental Figure 1**). Among those tested, 12 family members tested positive for germline mutations and a CPS (including *CEBPA, ETV6, GATA2, IKZF1, SAMD9*) with indications for follow up in the pediatric or adult cancer predisposition clinic, and in some instances referral for HSCT.

Overall, 9% of patients in this cohort who underwent tumor-only testing with follow-up germline confirmation or upfront paired T/N testing (10 of 29 and 6 of 156, respectively) at our institution were diagnosed with a new CPS, consistent with reported frequency in pediatric cancer.[5] Among patients with suspected germline predisposition from somatic tumor-only testing, a substantial number (32%) did not undergo confirmatory germline testing.

The results of our study suggest that paired T/N testing for pediatric and AYA patients with hematologic malignancies has several advantages over somatic tumor-only testing. From a patient care perspective, this approach identifies somatic variants and hereditary predisposition simultaneously[10] – obviating barriers to germline testing, mitigating loss to follow-up, and reducing undue anxiety for patients with findings suggestive of germline alteration that are in fact somatic events and do not have familial implications. Even with improvement in data reporting and recognition of a possible CPS, challenges with insurance approval for testing and additional clinical visits create substantial barriers to obtaining follow-up germline testing for many patients. Furthermore, with declining sequencing costs, there is now minimal extra cost of sequencing a paired normal sample on a single, streamlined platform. Thus, at our institution, the cost of up-front paired T/N is less than the cumulative cost of tumor-only sequencing followed by confirmatory sequencing with specific primer design and lab implementation. From a genomic perspective, paired T/N sequencing allows for the subtraction of variants in matched normal tissue from tumor tissue to reveal acquired genetic alterations that are truly somatic in origin and aid in variant classification.

Our study suggests that up front paired T/N testing should be pursued, when possible, with thorough pre-test counseling[15], as the impact on clinical decision making and long-term management is significant when a CPS is identified. Furthermore, this strategy can potentially alleviate undue emotional burden and mitigate economic barriers associated with follow-up testing in most patients with somatic mutation-driven cancers who do not merit further germline testing. Future studies should explore patient and family experiences utilizing mixed methods approaches incorporating qualitative data, as well as assess implementation of broader predisposition genomic panels in the context of pediatric malignancies. At present, our comprehensive hematologic panel does not include all cancer predisposition-related genes. We are currently working to implement a more comprehensive panel, as declining sequencing costs enable expanded genomic profiling without additional expense.

References

1. Duncavage EJ, Bagg A, Hasserjian RP, et al. Genomic profiling for clinical decision making in myeloid neoplasms and acute leukemia. Blood. 2022;140(21): 2228-2247.

2. Surrey LF, MacFarland SP, Chang F, et al. Clinical utility of custom-designed NGS panel testing in pediatric tumors. Genome Med. 2019;11(1):32.

3. Zhong Y, Xu F, Wu J, Schubert J, Li MM. Application of Next Generation Sequencing in Laboratory Medicine. Ann Lab Med. 2021; 41(1): 25-43.

4. Mody RJ, Wu YM, Lonigro RJ, et al. Integrative Clinical Sequencing in the Management of Refractory or Relapsed Cancer in Youth. JAMA. 2015;314(9):913-925.

5. Zhang J, Walsh MF, Wu G, et al. Germline Mutations in Predisposition Genes in Pediatric Cancer. N Engl J Med. 2015;373(24):2336-2346.

6. MacFarland SP, Zelley K, Surrey LF, et al. Pediatric Somatic Tumor Sequencing Identifies Underlying Cancer Predisposition. JCO Precis Oncol. 2019;3:PO.19.00062.

7. Kratz CP, Achatz MI, Brugières L, et al. Cancer Screening Recommendations for Individuals with Li-Fraumeni Syndrome. Clin Cancer Res. 2017;23(11):e38-e45.

8. Tabori U, Hansford JR, Achatz MI, et al. Clinical Management and Tumor Surveillance Recommendations of Inherited Mismatch Repair Deficiency in Childhood. Clin Cancer Res. 2017;23(11):e32-e37.

9. Xiao H, Shi J, Luo Y, et al. First report of multiple CEBPA mutations contributing to donor origin of leukemia relapse after allogeneic hematopoietic stem cell transplantation. Blood. 2011;117(19):5257-5260.

10. Mandelker D, Ceyhan-Birsoy O. Evolving Significance of Tumor-Normal Sequencing in Cancer Care. Trends Cancer. 2020;6(1):31-39.

11. Chang F, Lin F, Cao K, et al. Development and Clinical Validation of a Large Fusion Gene Panel for Pediatric Cancers. J Mol Diagn. 2019;21(5):873-883.

12. Li MM, Datto M, Duncavage EJ, et al. Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer: A Joint Consensus Recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists. J Mol Diagn. 2017;19(1):4-23.

13. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17(5):405-24.

14. Wintering A, Dvorak CC, Stieglitz E, Loh ML. Juvenile myelomonocytic leukemia in the molecular era: a clinician's guide to diagnosis, risk stratification, and treatment. Blood Adv. 2021;5(22):4783-4793.

15. Botkin JR. Ethical issues in pediatric genetic testing and screening. Curr Opin Pediatr. 2016;28(6):700-704.

Table 1. Clinical vignettes of patients diagnosed with Cancer Predisposition Syndrome

Cases diagnosed from somatic testing with follow up germline testing

Case	Syndrome	Gene	Diagnosis	Age of onset (years)	Clinical vignette	Clinical implications of knowing CPS
1	Noonan syndrome-like disorder	*CBL*	JMML	1	Patient presented with splenomegaly and thrombocytopenia as a toddler. Genetic testing notable for *CBL* mutation in tumor and subsequently heterozygous germline specimen. Treated with cytarabine and fludarabine for two cycles and required no additional treatment; remains in remission.	-Management of JMML impacted by knowing germline predisposition -Connected to other multidisciplinary subspecialists.
2	DNMT3A overgrowth syndrome and predisposition to hematologic malignancy (Tatton-Brown-Rahman)	*DNMT3A*	T-ALL	2	Patient had a history of history of paraspinal neuroblastoma and subsequently presented with cervical adenopathy and a mediastinal mass consistent with T-cell lymphoblastic lymphoma. Progressed during therapy and subsequently died from disease progression.	-Connected to genetics team for follow up and familial testing
3	ETV6 thrombocytopenia and leukemia predisposition syndrome	*ETV6*	B-ALL	16	Teenager presented with flu like symptoms, diagnosed with high-risk ALL. Mother and sibling also had a history of B-ALL; both underwent testing and found to have the same pathogenic mutation. Patient is currently in remission.	-Follows with CPP -Guidance provided on bleeding phenotype in some patients with this syndrome -Cascade testing performed
4	GATA2 deficiency syndrome	*GATA2*	MDS	10	Patient initially presented with lymphedema, neutropenia and monocytopenia. Bone marrow biopsy revealed monosomy 7 MDS and *GATA2* mutation, which was then confirmed as germline. Recommended for HSCT.	-Identification of predisposition influenced recommendation for HSCT. Also guided familial cascade testing to identify the optimal donor.
5	GATA2 deficiency syndrome	*GATA2*	MDS	10	Patient presented with fevers and pancytopenia, found to have monosomy 7 MDS with *GATA2* mutation which was then confirmed as germline. Underwent bone marrow transplant, in remission and doing well.	-Identification of predisposition influenced recommendation for HSCT. Also guided familial cascade testing to identify the optimal donor.
6	Lynch syndrome	*MSH2*	B-ALL	19	Presented with abdominal pain, found to have high risk B-ALL. Somatic testing showed a *MSH2* variant which was confirmed as germline. In remission and doing well.	-Follows with CPP -Family members underwent genetic testing -Connected with gastroenterology for colonoscopies and dermatology for regular skin exams
7	Noonan syndrome	*PTPN11*	B-ALL	8 weeks	Presented with JMML in infancy and on presentation noted to have heart murmur and dysmorphic features. Cancer panel demonstrated *PTPN11* variant, confirmed as germline. JMML spontaneously resolved. Patient in remission and doing well.	-Management of JMML impacted by knowing germline predisposition -Connected to subspecialists (genetics, cardiology, urology, endocrine, ophthalmology)
8	Mirage syndrome	*SAMD9*	MDS	6	Presented with lymphedema, neutropenia and monocytopenia. Bone marrow biopsy revealed hypocellular marrow with a chromosomal abnormality in small fraction of cells (46, XX der(1;7)) resulting in gain of 1q and loss of 7q. NGS testing revealed a pathogenic *GATA2* mutation consistent with GATA2 haploinsufficiency and monosomy 7 myelodysplasia. Given monosomy 7 MDS, patient was referred for bone marrow transplant. In remission post-transplant and	-Identification of predisposition influenced recommendation for HSCT. Also guided familial cascade testing to identify the optimal donor. -Family cascade testing performed

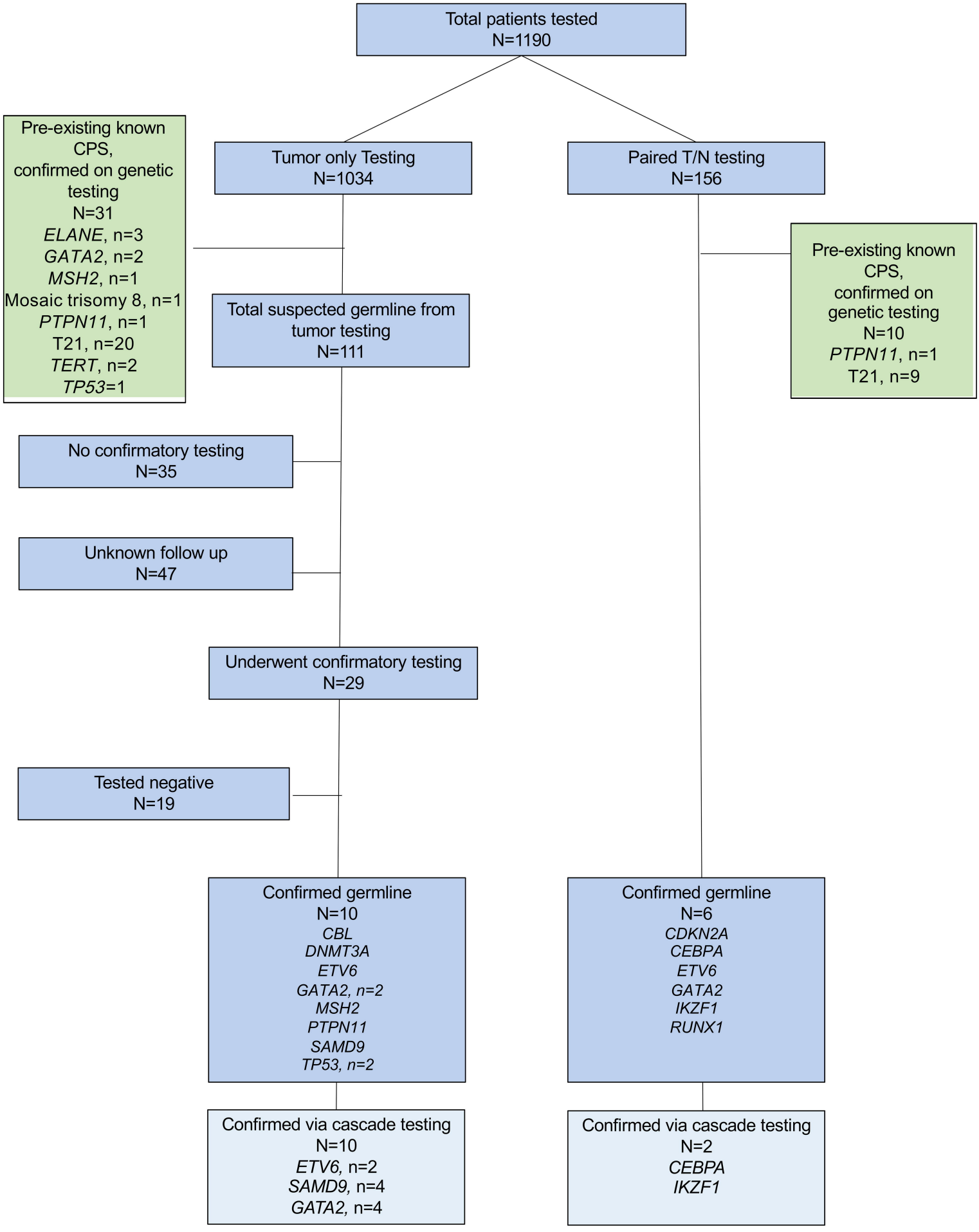
					doing well.	
9	Li Fraumeni syndrome	*TP53*	B-ALL	12	Presented with fevers, diagnosed with low hypodiploid B-ALL. Now off therapy and in remission, doing well.	-Follows with CPP and undergoes routine LFS surveillance. -Family members underwent cascade testing
10	Li Fraumeni syndrome	*TP53*	B-ALL	10	Presented with weight loss, fevers and diagnosed with hypodiploid B-ALL. Somatic testing showed TP53 variant in tumor, confirmed in germline and thought to be mosaic given low VAF.	-Follows with CPP - Will undergo routine LFS surveillance
Cases diagnosed from paired T/N testing						
1	CDKN2A associated predisposition	*CDKN2A*	B-ALL	15	Presented with fever, bleeding gums and diagnosed with B-ALL. Found to have germline *CDKN2A* variant.	-Recommended for follow up with CPP
2	CEBPA-associated predisposition to AML	*CEBPA*	AML	2	Presented with fever and increased work of breathing, diagnosed with AML. T/N testing showed a germline mutation in *CEBPA* and additional *CEBPA* mutation in the tumor only. In remission and doing well.	-Follows with CPP -Family underwent cascade testing
3	ETV6 thrombocytopenia and leukemia predisposition syndrome	*ETV6*	B-ALL	6	Presented with fatigue and pallor and diagnosed with B-ALL. T/N demonstrated pathogenic *ETV6* variant in germline and tumor, hyperdiploid ALL. Now in remission and doing well.	-Follows with CPP -Guidance provided on bleeding phenotype in some patients with this syndrome -Cascade testing performed
4	GATA2 deficiency syndrome	*GATA2*	AML	14	Presented with splenomegaly, petechiae and purpura, diagnosed with AML. T/N testing demonstrated somatic and germline *GATA2* (0.50 tumor/0.47 germline) and additional somatic *CEBPA* mutation. Given *GATA2* predisposition, patient was recommended for HSCT. Underwent transplant and is now in remission.	-Identification of predisposition influenced recommendation for HSCT. Also guided familial cascade testing to identify the optimal donor.
5	IKZF1 associated leukemia predisposition	*IKZF1*	B-ALL	2	Presented with prolonged fevers, diagnosed with high risk B-ALL. Somatic testing demonstrated *P2RY8::CRLF2* fusion; paired T/N with IKZF1 variant in the skin and tumor specimen. Currently in remission.	-Follows with CPP -Family underwent cascade testing; parent referred to adult CPP program.
6	RUNX1 familial platelet disorder with associated myeloid malignancy	*RUNX1*	AML	2	Initially followed by hematology for congenital thrombocytopenia and subsequently diagnosed with AML. Genetic testing demonstrated germline *RUNX1* pathogenic variant. Received HSCT, in remission and doing well.	-Identification of predisposition influenced recommendation for HSCT. Also guided familial cascade testing to identify the optimal donor -Follows with CPP.

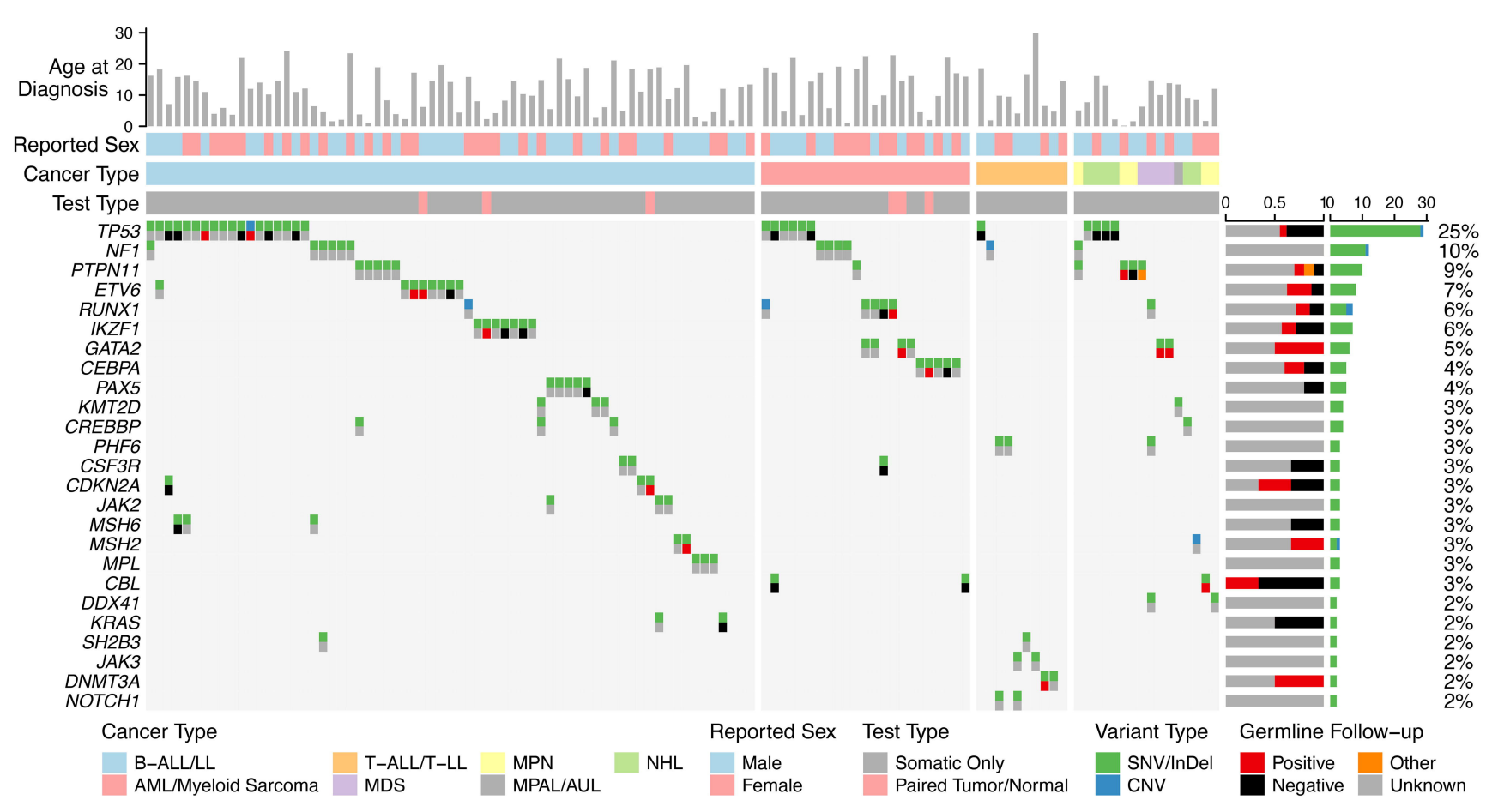
CPP: cancer predisposition program; CPS: cancer predisposition syndrome; HSCT: Hematopoietic stem cell transplantation; LFS: Li-Fraumeni Syndrome; T/N: tumor/normal; VAF: variant allele fraction

Figure Legends

Figure 1. Consort diagram of patients undergoing genomic testing with tumor-only or paired tumor-normal testing and cancer predisposition syndrome diagnoses.

Figure 2. Oncoprint of variants recommended for germline follow-up (identified through somatic testing) or confirmed germline variants identified through paired tumor/normal (T/N) testing. Top icon demonstrates variant type (SNV/indel, CNV) and bottom icon demonstrates germline follow-up result. Annotations for age at diagnosis, sex, cancer type, and test type (somatic vs T/N) are displayed on the top of the oncoprint. Barcharts to the right show the proportion of patients with a germline variant identified that underwent follow up by gene (left) and the frequency of each gene within the whole cohort (right).


Total patients tested
N=1190
Pre-existing known CPS, confirmed on genetic testing
N=31
ELANE, n=3
GATA2, n=2
MSH2, n=1
Mosaic trisomy 8, n=1
PTPN11, n=1
T21, n=20
TERT, n=2
TP53=1
Tumor only Testing
N=1034
Paired T/N testing
N=156
Pre-existing known CPS, confirmed on genetic testing
N=10
PTPN11, n=1
T21, n=9
Total suspected germline from tumor testing
N=111
No confirmatory testing
N=35
Unknown follow up
N=47
Underwent confirmatory testing
N=29
Tested negative
N=19
Confirmed germline
N=10
CBL
DNMT3A
ETV6
GATA2, n=2
MSH2
PTPN11
SAMD9
TP53, n=2
Confirmed germline
N=6
CDKN2A
CEBPA
ETV6
GATA2
IKZF1
RUNX1
Confirmed via cascade testing
N=10
ETV6, n=2
SAMD9, n=4
GATA2, n=4
Confirmed via cascade testing
N=2
CEBPA
IKZF1


Age at Diagnosis
30
20
10
0
Reported Sex
Cancer Type
Test Type
0
0.5
10
10
20
30
TP53 25%
NF1 10%
PTPN11 9%
ETV6 7%
RUNX1 6%
IKZF1 6%
GATA2 5%
CEBPA 4%
PAX5 4%
KMT2D 3%
CREBBP 3%
PHF6 3%
CSF3R 3%
CDKN2A 3%
JAK2 3%
MSH6 3%
MSH2 3%
MPL 3%
CBL 3%
DDX41 2%
KRAS 2%
SH2B3 2%
JAK3 2%
DNMT3A 2%
NOTCH1 2%
Cancer Type
B–ALL/LL
AML/Myeloid Sarcoma
T–ALL/T–LL
MDS
MPN
MPAL/AUL
NHL
Reported Sex
Male
Female
Test Type
Somatic Only
Paired Tumor/Normal
Variant Type
SNV/InDel
CNV
Germline Follow–up
Positive
Negative
Other
Unknown

Supplemental Table 1. Demographics of study cohort

	All N=1190	Tumor testing N=1034	Paired Tumor-Normal testing N=156
Age			
Median age, years (range)	9.2 (0-32)	9.5 (0-32)	6.4 (0-22)
Sex, No (%)			
Female	546(46)	470 (46)	76 (49)
Male	643(54)	563 (55)	80 (51)
Race, No (%)[a]			
White	449 (38)	363 (35)	86 (55)
Black or African American	114 (10)	88 (9)	26 (17)
Asian	34 (3)	25 (2)	9 (6)
Other	160 (13)	125 (2)	35 (22)
Diagnosis, No (%)			
B-ALL	704 (59)	609 (59)	95 (61)
AML/myeloid sarcoma	212 (18)	178 (17)	34 (22)
T-ALL/T-TLL	108 (9)	95 (9)	13 (8)
NHL[b]	68 (6)	63 (6)	5 (3)
Predisposition to MDS/Leukemia[c]	27 (2)	26 (3)	1 (1)
MPN[d]	36 (3)	30 (3)	6(4)
Other[e]	35 (3)	33 (3)	2(1)

[a]432 patients with tumor-only testing missing self-identified race data

[b]NHL=Diffuse large B-cell Lymphoma (DLBCL), Follicular lymphoma, Anaplastic large cell lymphoma (ALCL), Primary mediastinal b-cell lymphoma (PMBCL)

[c]Predisposition to MDS/leukemia= Schwachman Diamond Syndrome, Fanconi anemia, Telomere biology disorder, Severe Congenital Neutropenia with *ELANE* mutation, mosaic Trisomy 8, Diamond-Blackfan anemia, *GATA2* haploinsufficiency syndrome, Noonan Syndrome *PTPN11*, MECOM-associated bone marrow failure syndrome

[d]MPN=Chronic myeloid leukemia (CML), Transient myeloid leukemia of Downs Syndrome (TMD), Juvenile monomyelocytic anemia (JMML)

[e]Other = myelodysplasia (MDS), mixed phenotype acute leukemia/ambiguous lineage leukemia (MPAL/AUL), Hodgkin lymphoma

Supplemental table 2. All variants identified by somatic testing as potentially germline or confirmed as germline on Tumor/Normal (T/N) testing.

Gene	Transcript	Nucleotide	Amino Acid	Mutation type	VAF	Test (somatic or T/N)	Germline Confirmation
TP53	NM_000546.5	c.659A>G	p.Tyr220Cys	missense	0.44	somatic	Unknown
TP53	NM_000546.5	c.1009C>T	p.Arg337Cys	missense	0.51	somatic	Unknown
NRAS	NM_002524.4	c.178G>A	p.Gly60Arg	missense	0.43	somatic	Unknown
DDX41	NM_016222.3	c.4G>T	p.Glu2*	nonsense	0.23	somatic	Unknown
PHF6	NM_032458.2	c.890G>A	p.Cys297Tyr	missense	0.38	somatic	Unknown
RUNX1	NM_001754.4	c.334del	p.Leu112Cysfs*10	frameshift	0.42	somatic	Unknown
PTPN11	NM_002834.4	c.181G>A	p.Asp61Asn	missense	0.45	somatic	Positive
IKZF1	NM_006060.5	c.475A>T	p.Asn159Tyr	missense	0.42	somatic	Unknown
RB1	NM_000321.2	c.1362C>G	p.Tyr454*	nonsense	0.39	somatic	Unknown
GATA2	NM_001145661.1	c.521del	p.Pro174Hisfs*44	frameshift	0.44	somatic	Unknown
RUNX1	NM_001754.4	c.805+1del	p.?	splice_site	0.44	somatic	Unknown
CSF3R	NM_000760.3	c.1640G>A	p.Trp547*	nonsense	0.47	somatic	Unknown
ETV6	NM_001987.4	c.865C>T	p.Gln289*	nonsense	0.49	somatic	Unknown
ETV6	NM_001987.4	c.1014_1020del	p.Arg339Phefs*30	frameshift	0.68	somatic	Unknown
TP53	NM_000546.5	c.614A>G	p.Tyr205Cys	missense	0.83	somatic	Unknown
NF1	NM_001042492.2	c.6855C>A	p.Tyr2285*	nonsense	0.94	somatic	Unknown
RTEL1	NM_016434.3	c.897del	p.Phe299Leufs*10	frameshift	0.39	somatic	Unknown
ETV6	NM_001987.4	c.1202A>G	p.Tyr401Cys	missense	0.61	somatic	Positive
CDKN2A	NM_000077.4	c.377T>A	p.Val126Asp	missense	0.74	somatic	Unknown
ETV6	NM_001987.4	c.416_417del	p.Ser139Tyrfs*14	frameshift	0.54	TN	Positive
GATA2	NM_001145661.1	c.1085G>A	p.Arg362Gln	missense	0.5	TN	Positive
NF1	NM_001042492.2	c.1260+1del	p.?	splice_site	0.25	somatic	Unknown
TP53	NM_000546.5	c.1045G>T	p.Glu349*	nonsense	0.83	somatic	Unknown
CEBPA	NM_004364.4	c.162dup	p.Ile55Hisfs*53	frameshift	0.45	somatic	Unknown
CEBPA	NM_004364.4	c.938_939insTCT	p.Lys313delinsAsnLeu	inframe	0.4	somatic	Unknown
WT1	NM_024426.5	c.1297T>A	p.Cys433Ser	missense	0.76	somatic	Unknown

GATA2	NM_001145661.1	c.989_992dup	p.Leu332Thrfs*53	frameshift	0.41	somatic	Positive
PTPN11	NM_002834.4	c.1504T>C	p.Ser502Pro	missense	0.34	somatic	Unknown
PTPN11	NM_002834.4	c.182A>C	p.Asp61Ala	missense	0.47	somatic	Unknown
RUNX1	NM_001754.4	c.437A>T	p.Asn146Ile	missense	0.45	TN	Positive
CEBPA	NM_004364.5	c.68dup	p.His24Thrfs*136	frameshift	0.34	TN	Positive
JAK2	NM_004972.4	c.2600G>A	p.Arg867Gln	missense	0.37	somatic	Unknown
KRAS	NM_004985.5	c.35G>C	p.Gly12Ala	missense	0.38	somatic	Unknown
SH2B3	NM_005475.3	c.622G>C	p.Glu208Gln	missense	0.52	somatic	Unknown
NF1	NM_001042492.3	c.6855C>A	p.Tyr2285*	nonsense	0.45	somatic	Unknown
TP53	NM_000546.6	c.976G>T	p.Glu326*	nonsense	0.76	somatic	Unknown
PAX5	NM_016734.3	c.295dup	p.Ile99Asnfs*3	frameshift	0.32	somatic	Unknown
GATA2	NM_001145661.1	c.604_624delinsACTT	p.Ala202Thrfs*2	frameshift	0.22	somatic	Positive
JAK3	NM_000215.4	c.307C>T	p.Arg103Cys	missense	0.45	somatic	Unknown
KMT2D	NM_003482.4	c.7228C>T	p.Arg2410*	nonsense	0.41	somatic	Unknown
TP53	NM_000546.6	c.818G>A	p.Arg273His	missense	0.39	somatic	Positive
CREBBP	NM_004380.3	c.4416G>C	p.Trp1472Cys	missense	0.45	somatic	Unknown
PTPN11	NM_002834.5	c.226G>A	p.Glu76Lys	missense	0.43	somatic	Unknown
IKZF1	NM_006060.6	c.331C>T	p.Arg111*	nonsense	0.48	TN	Positive
ETV6	NM_001987.5	c.163+1G>A	p.?	splice_site	0.48	somatic	Unknown
NF1	NM_001042492.2	c.205-2A>T	p.?	splice_site	0.36	somatic	Unknown
NF1	NM_001042492.2	c.2033dupC	p.I679Dfs*21	frameshift	0.35	somatic	Unknown
NF1	NM_001042492.2	c.7909C>T	p.R2637*	nonsense	0.21	somatic	Unknown
PTPN11	NM_002834.4	c.214G>A	p.A72T	missense	0.41	somatic	Unknown
PAX5	NM_016734.2	c.749dupT	p.T251Hfs*38	frameshift	0.45	somatic	Unknown
MSH6	NM_000179.2	c.3261dupC	p.F1088Lfs*5	frameshift	0.46	somatic	Negative
TP53	NM_000546.5	c.646G>A	p.V216M	missense	0.91	somatic	Negative
MSH2	NM_000251.2	c.1861C>T	p.R621*	nonsense	0.87	somatic	Unknown
TP53	NM_000546.5	c.743G>A	p.R248Q	missense	0.88	somatic	Unknown
TP53	NM_000546.5	c.789_833delinsCCCT	p.Leu264Profs*28	frameshift	0.78	somatic	Unknown
IKZF1	NM_006060.5	c.568_583delinsTTTA	p.G190_H195delinsFN	inframe	0.48	somatic	Unknown
KMT2D	NM_003482.3	c.4163G>T	p.Arg1388Leu	missense	0.55	somatic	Unknown

MPL	NM_005373.2	c.117G>T	p.Lys39Asn	missense	0.48	somatic	Unknown
JAK2	NM_004972.3	c.3188G>A	p.Arg1063His	missense	0.43	somatic	Unknown
PTPN11	NM_002834.4	c.923A>G	p.Asn308Ser	missense	0.46	somatic	Unknown
MPL	NM_005373.2	c.407C>T	p.Pro136Leu	missense	0.43	somatic	Unknown
TP53	NM_000546.5	c.827C>A	p.Ala276Asp	missense	0.91	somatic	Unknown
CSFR3	NM_000760.3	c.2221C>T	p.Gln741*	nonsense	0.78	somatic	Negative
RUNX1	NM_001754.4	c.484A>G	p.Arg162Gly	missense	0.38	somatic	Negative
CBL	NM_005188.3	c.1141T>C	p.Cys381Arg	missense	0.98	somatic	Positive
NF1	NM_001042492.2	c.2709G>A	p.Val903Val	missense	0.27	somatic	Unknown
NF1	NM_001042492.2	c.6147+1G>A	p.?	splice_site	0.2	somatic	Unknown
PTPN11	NM_002834.4	c.181G>T	p.Asp61Tyr	missense	0.43	somatic	Negative
TP53	NM_000546.5	c.733G>A	p.Gly245Ser	missense	0.51	somatic	Unknown
PAX5	NM_016734.2	c.910+1G>A	p.?	splice_site	0.48	somatic	Unknown
ETV6	NM_001987.4	c.463+1G>A	p.?	splice_site	0.6	somatic	Unknown
IKZF1	NM_006060.5	c.484C>T	p.Arg162Trp	missense	0.9	somatic	Negative
CBL	NM_005188.3	c.1100A>C	p.Gln367Pro	missense	0.92	somatic	Negative
TP53	NM_000546.5	c.743G>A	p.Arg248Gln	missense	0.2	somatic	Negative
CEBPA	NM_004364.4	c.52_61dup	p.Ser21Thrfs*90	frameshift	0.23	somatic	Unknown
PAX5	NM_016734.2	c.239C>G	p.Pro80Arg	missense	0.38	somatic	Negative
PAX5	NM_016734.2	c.103del	p.Asp35Metfs*2	frameshift	0.41	somatic	Negative
ETV6	NM_001987.4	c.613dup	p.Leu205Profs*12	frameshift	0.39	somatic	Negative
DNMT3A	NM_022552.4	c.2645G>A	p.Arg882His	missense	0.45	somatic	Positive
TP53	NM_000546.5	NA	whole_gene	deletion	1	somatic	Positive
NF1	NM_001042492.2	c.5327C>A	p.Ser1776*	nonsense	0.89	somatic	Unknown
SH2B3	NM_005475.2	c.622G>C	p.Glu208Gln	missense	0.95	somatic	Unknown
DNM2	NM_001005360.2	c.1684_1686del	p.Lys562del	inframe	0.33	somatic	Unknown
GATA2	NM_001145661.1	c.1085G>A	p.Arg362Gln	missense	0.51	somatic	Unknown
DDX41	NM_016222.3	c.844C>T	p.Arg282Cys	missense	0.47	somatic	Unknown
TP53	NM_000546.5	c.524G>A	p.Arg175His	missense	0.9	somatic	Unknown
PHF6	NM_032458.2	c.494del	p.Gly165Glufs*53	frameshift	0.51	somatic	Unknown
IKZF1	NM_006060.5	c.91A>G	p.Met31Val	missense	0.5	somatic	Unknown

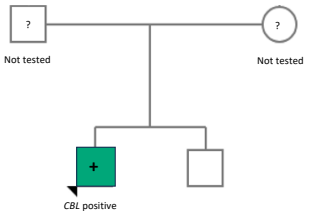
DNMT3A	NM_022552.4	c.2645G>A	p.Arg882His	missense	0.47	somatic	Unknown
KMT2D	NM_003482.3	c.10441-1G>A	p.?	splice_site	0.44	somatic	Unknown
EP300	NM_001429.3	c.5403C>A	p.Cys1801*	nonsense	0.39	somatic	Unknown
NOTCH1	NM_017617.4	c.5033T>C	p.Leu1678Pro	missense	0.48	somatic	Unknown
PHF6	NM_032458.2	c.820C>T	p.Arg274*	nonsense	0.38	somatic	Unknown
TP53	NM_000546.5	c.916C>T	p.Arg306*	nonsense	0.36	somatic	Negative
TP53	NM_000546.5	c.1024C>T	p.Arg342*	nonsense	0.26	somatic	Negative
TP53	NM_000546.5	c.646G>A	p.Val216Met	missense	0.97	somatic	Negative
MPL	NM_005373.2	c.815G>A	p.Trp272*	nonsense	0.46	somatic	Unknown
RELN	NM_005045.3	c.8433del	p.Pro2812Glnfs*16	frameshift	0.46	somatic	Unknown
PTPN11	NM_002834.4	c.226G>A	p.Glu76Lys	missense	0.43	somatic	Unknown
ETV6	NM_001987.4	c.460_461dup	p.Asp155Lysfs*55	frameshift	0.15	somatic	Unknown
NF1	NM_001042492.2	c.7271_7272del	p.Arg2424Lysfs*3	frameshift	0.34	somatic	Unknown
NF1	NM_001042492.2	c.7271_7272del	p.Arg2424Lysfs*3	frameshift	0.34	somatic	Unknown
TP53	NM_000546.5	c.742C>T	p.Arg248Trp	missense	0.38	somatic	Unknown
TP53	NM_000546.5	c.742C>T	p.Arg248Trp	missense	0.38	somatic	Unknown
CREBBP	NM_004380.2	c.1409T>A	p.Leu470*	nonsense	0.35	somatic	Unknown
TP53	NM_000546.5	c.818G>A	p.Arg273His	missense	0.62	somatic	Negative
BCL11B	NM_138576.3	c.1942_1956delinsCCGGTCGCATT	p.Gly648Profs*74	frameshift	0.38	somatic	Unknown
JAK3	NM_000215.3	c.1718C>T	p.Ala573Val	missense	0.43	somatic	Unknown
NOTCH1	NM_017617.4	c.7072G>A	p.Ala2358Thr	missense	0.53	somatic	Unknown
NOTCH1	NM_017617.4	c.6392del	p.Gly2131Alafs*117	frameshift	0.41	somatic	Unknown
KRAS	NM_004985.4	c.187G>A	p.Glu63Lys	missense	0.89	somatic	Negative
IKZF1	NM_006060.5	c.475A>T	p.Asn159Tyr	missense	0.47	somatic	Negative
CBL	NM_005188.3	c.1228-2A>G	p.?	splice_site	0.9	somatic	Negative
TP53	NM_000546.5	c.818G>A	p.Arg273His	missense	0.37	somatic	Negative
NF1	NM_001042492.2	c.7189G>T	p.Gly2397Trp	missense	0.78	somatic	Unknown
PTPN11	NM_002834.4	c.215C>T	p.Ala72Val	missense	0.44	somatic	Unknown
CEBPA	NM_004364.4	c.333_334dup	p.Pro112Argfs*49	frameshift	0.48	somatic	Negative
CEBPA	NM_004364.4	c.941_942ins15	p.Val314_Leu315insSerSerGlnLysVal	inframe	0.34	somatic	Negative
CEBPA	NM_004364.4	c.937_939dup	p.Lys313dup	inframe	0.38	somatic	Unknown

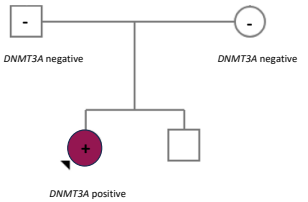
CEBPA	NM_004364.4	c.245del	p.Phe82Serfs*78	frameshift	0.4	somatic	Unknown
MSH2	NM_000251.2	NA	exon_12_16	deletion	0.5	somatic	Unknown
CSF3R	NM_000760.3	c.799del	p.Glu267Serfs*61	frameshift	0.48	somatic	Unknown
IKZF1	NM_006060.5	c.484C>T	p.Arg162Trp	missense	0.44	somatic	Unknown
JAK2	NM_004972.3	c.2047A>G	p.Arg683Gly	missense	0.38	somatic	Unknown
PAX5	NM_016734.2	c.239C>G	p.Pro80Arg	missense	0.44	somatic	Unknown
SETD2	NM_014159.6	c.5735T>A	p.Leu1912*	nonsense	0.73	somatic	Unknown
TP53	NM_000546.5	c.1009C>T	p.Arg337Cys	missense	0.11	somatic	Unknown
TP53	NM_000546.5	c.846_847insGCCCAGG	p.Arg283Alafs*25	frameshift	0.68	somatic	Negative
TP53	NM_000546.5	c.716A>G	p.Asn239Ser	missense	0.53	somatic	Unknown
TP53	NM_000546.5	c.707A>G	p.Tyr236Cys	missense	0.43	somatic	Unknown
CREBBP	NM_004380.2	c.5039_5041del	p.Ser1680del	inframe	0.41	somatic	Unknown
KMT2D	NM_003482.3	c.15784+2T>C	p.?	splice_site	0.44	somatic	Unknown
MSH2	NM_000251.2	c.2087C>T	p.Pro696Leu	missense	0.47	somatic	Positive
TP53	NM_000546.5	c.845G>C	p.Arg282Pro	missense	0.81	somatic	Unknown
TP53	NM_000546.5	c.670G>A	p.Glu224Lys	missense	0.36	somatic	Negative
TP53	NM_000546.5	c.738G>A	p.Met246Ile	missense	0.65	somatic	Negative
NF1	NM_001042492.2	NA	exon_16_36	deletion	0.5	somatic	Unknown
TP53	NM_000546.5	c.743G>C	p.Arg248Pro	missense	0.7	somatic	Unknown
GATA2	NM_001145661.1	c.1085G>A	p.Arg362Gln	missense	0.29	somatic	Unknown
RUNX1	NM_001754.4	c.634_635insT	p.Asp212Valfs*16	frameshift	0.2	somatic	Unknown
RUNX1	NM_001754.4	NA	exon_4_7	deletion	0.5	somatic	Unknown
TP53	NM_000546.5	c.718A>G	p.Ser240Gly	missense	0.7	somatic	Unknown
CDKN2A	NM_000077.4	c.172C>T	p.Arg58*	nonsense	0.41	somatic	Negative
TP53	NM_000546.5	c.743G>A	p.Arg248Gln	missense	0.3	somatic	Negative
TP53	NM_000546.5	c.817C>T	p.Arg273Cys	missense	0.39	somatic	Negative
CREBBP	NM_004380.2	c.4361T>G	p.Leu1454Arg	missense	0.67	somatic	Unknown
MSH6	NM_000179.2	c.1483C>T	p.Arg495*	nonsense	0.32	somatic	Unknown
MSH6	NM_000179.2	c.1444C>T	p.Arg482*	nonsense	0.3	somatic	Unknown
TP53	NM_000546.5	c.847delinsGAGGCGA	p.Arg283delinsGluAlaSer	inframe	0.44	somatic	Unknown
MSH6	NM_000179.2	c.2731C>T	p.Arg911*	nonsense	0.47	somatic	Unknown

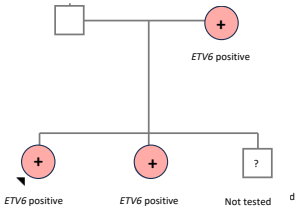
NF1	NM_001042492.2	c.574C>T	p.Arg192*	nonsense	0.41	somatic	Unknown
PTPN11	NM_002834.4	c.172A>T	p.Asn58Tyr	missense	0.19	somatic	Positive
NF1	NM_001042492.2	c.2033dup	p.Ile679Aspfs*21	frameshift	0.66	somatic	Unknown
RUNX1	NM_001754.4	NA	exon_3_5	deletion	0.5	somatic	Unknown
TP53	NM_000546.5	c.743G>A	p.Arg248Gln	missense	0.68	somatic	Negative
TP53	NM_000546.5	c.379T>C	p.Ser127Pro	missense	0.46	somatic	Negative
TP53	NM_000546.5	c.427G>A	p.Val143Met	missense	0.41	somatic	Negative
CDKN2A	NM_000077.4	c.303_304insC	p.Ala102Argfs*18	frameshift	0.49	TN	Positive

Figure Legends

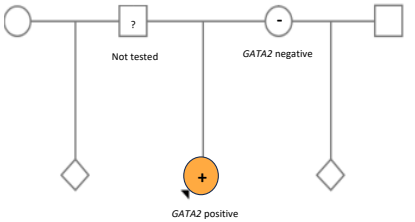
Supplemental Figure 1. Pedigrees of patients diagnosed with cancer predisposition syndromes, highlighting cascade testing. Twelve family members in five pedigrees diagnosed with germline predisposition.

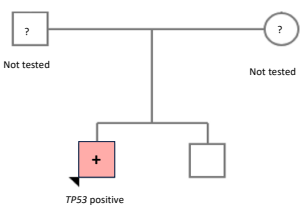


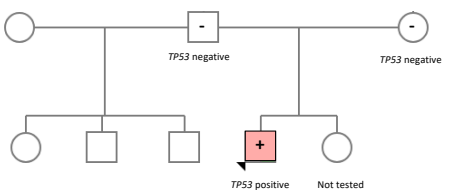


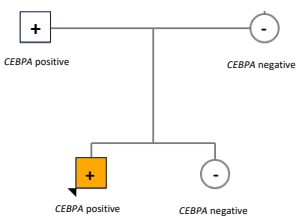


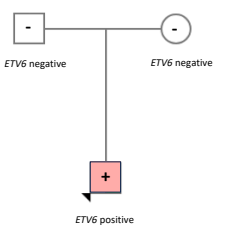
d

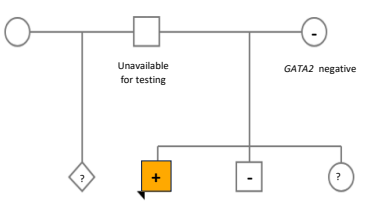


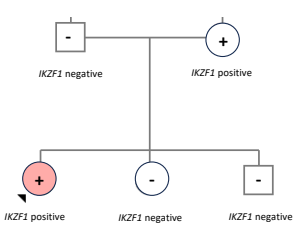


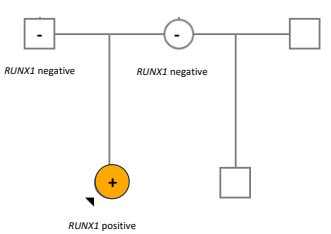


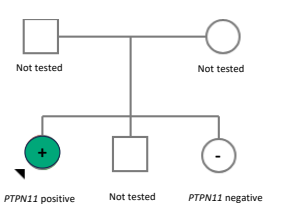












Diagnosis	
	T-ALL
	JMML
	B-ALL
	AML
	MDS
Genetic testing	
+	Positive
-	Negative
?	Recommended for testing but not yet tested