**RARE GERMLINE VARIANT CONTRIBUTIONS TO MYELOID
MALIGNANCY SUSCEPTIBILITY**

by

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Rare Germline Variant Contributions to Myeloid Malignancy Susceptibility

Abstract

By

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Myeloid malignancies are rare but severe clonal disorders found in hematopoietic stem or progenitor cells. Both genetic and epigenetic changes can contribute to the disease by disturbing some key aspects in cells such as self-renewal, proliferation and differentiation. Therefore, it is important to know which genes are associated with these diseases in order to increase our understanding assess disease risk and develop therapeutic strategies to treat the disease. Recurrent somatic mutations in some genes, such as *RUNX1*, *IDH1/2* and *FLT3*, are known to be associated with development of these diseases. However, in finding the association between genes and myeloid malignancies, there is still a question as to the degree to which germline rare variants contribute to the disease phenotype.

In chapter 3, we will present our findings from 690 myeloid malignancies patients' whole-exome sequence data to elucidate the contribution to disease susceptibility of rare ($< 1\%$ population allele frequency) protein-altering alleles. We estimate that 3-4% of patients have rare variants in genes that would classify the patients as having germline predisposition under current guidelines. We also identify *MPO* as a new candidate predisposition gene, with 19 patients (2.8%) harboring rare pathogenic *MPO* variants, and significantly higher pathogenic allele frequencies in cases as compared to population controls. An elevated rare-variant burden is observed in autosomal recessive genes, particularly Fanconi anemia (FA) genes. The proportion of rare variants that are truncating is substantially higher in autosomal recessive and FA genes than in other. Even after accounting for numbers of rare variants, inherited pairs of such variants in the same patient and same FA gene (presumably affecting both parental homologs) are observed at a rate significantly higher than would be expected by chance, suggesting biparental inheritance of rare alleles in FA genes as a risk factor for myeloid malignancy. The FA gene *BRCA2* is particularly enriched for biparental inheritance, and rare germline *BRCA2* variants are associated with poor overall survival.

Chapter 1. Background and Significance

Chapter 1-1: Production of blood cells – Hematopoiesis

Hematopoiesis refers to the normal blood cell production. It is an important pathway as this process produces the majority of the cells in human body. Blood cells originate with pluripotent stem cells, which have the ability to both differentiate into other types of stem cells and renew as the same type of cell. Pluripotent stem cells can then differentiate into hematopoietic stem cells (HSCs), which are the origin of all types of blood cells. This type of cell also has the capability for renewal. Notably, about 1 in every 10,000 to 15,000 bone marrow cells is thought to be a stem cell while this proportion falls to 1 in 100,000 blood cells outside bone marrow (National Institutes of Health, 2016).

Figure 1: Hematopoietic stem cells differentiation pathway

Figure 1: Overview of Hematopoietic stem cells differentiation pathway. Adapted from (Pirahmadian, 2016)

HSCs can then both create more HSCs and differentiate themselves into the myeloid lineage or lymphoid lineage of blood cells. In the myeloid lineage, common myeloid progenitor (CMP) cells can then differentiate into different myeloid precursor cells, which are known as blast cells, such as megakaryoblasts, myeloblasts and proerythroblasts. The myeloblast progenitors can further differentiate into various types of mature blood cells including eosinophils, basophils, neutrophils and monocytes. On the other hand, the lymphoid lineage is started with common lymphoid progenitor (CLP), and it can renew itself and further differentiate into either B-cells or T-cells through the lymphocyte precursor (Harrison, 1982) (Figure1).

Chapter 1-2: What if things go wrong in hematopoiesis?

Blood cancers arise if any of the differentiation steps goes wrong during hematopoiesis. There are three main types of blood cancers: leukemia, lymphoma and myeloma. The term leukemia comes from the Greek white (leukos) and blood (haima). It generally refers to the abnormal production of immature blood cells, which cannot further differentiate into other mature blood cells, and it can be found in patients' blood and bone marrow. For leukemia patients, there are large amounts of immature blood cells circulating throughout the body which impair normal functions, including normal blood cell production and immune system activation. Based on the origin of the cell type affected, leukemia can be categorized into two main branches, myeloid and lymphoid.

Chapter 1-3: Oncogenes

In the past, all human cancer development was believed to be triggered by oncogenes, which were considered to originate from external factors such as retroviruses inserting fragments of viral DNA into the human body. This is partly due to the results of the experimental work conducted by Peyton Rous, which found out that cancer would present in healthy chickens if they were injected cell-free extract from chicken tumor cells. It was later found out that the extract encompassed the Rous sarcoma retrovirus (RSV), which was responsible for the development of sarcomas in chickens (Rous, 1911). However, the actual virus was not the transmissible agent, instead *v-SRC*, which contained in the RSV, is responsible for the tumor development and associated with nucleotide

sequences in the DNA of healthy chickens. This hypothesis was confirmed by Stehelin et al. (Stehelin, 1976) as he identified the first oncogene *SRC*. Since then, researchers found that oncogenes are possessed by many organisms, and those genes are mainly responsible for the regulation of cell development and division. Oncogenes can be activated by genetic abnormalities, including insertion of new enhancers or promoter regions to a gene region (e.g. in Burkitt's lymphoma, the juxtaposition of *MYC* to the human immunoglobulin heavy chain enhancer regions occurs) (Joos, 1992). Also, translocations give rise to new gene fusions, such as *BCR-ABL* (Salesse, 2002) and *EWS-ERG* (Kaneko, 1997). Furthermore, gene amplification, such as *MYCN* in neuroblastoma (Yoshimoto, 1999), or activating mutations in *RAF* or *RAS* genes may also stimulate oncogenes (Downward, 2003). The activation of oncogenes usually promotes abnormal cell growth and inhibits regulated apoptosis, which results in cancer.

Chapter 1-4: Tumor suppressor genes

The notion of tumor suppressor genes was first outlined by Knudson (Knudson, 1971) in retinoblastoma. Retinoblastoma can be either unilateral or bilateral. In Knudson's hereditary retinoblastoma study, he collected data for retinoblastoma occurring in both unilateral and bilateral cases and found that only one somatic mutation was necessary in the case of bilateral retinoblastoma, as a germline mutation was already present in the cells, while two somatic mutations were necessary for unilateral retinoblastoma to occur. These observations later

became the basis for the well-known ‘two-hit hypothesis’. Later, Friend et al. (Friend, 1986) validated the hypothesis by discovering recessive mutations in both alleles of the *Rb1* gene, which was the first tumor suppressor gene that was successfully replicated.

However, there are conditions which are exceptions to the ‘two-hit’ hypothesis. For example, the hereditary Li-Fraumeni syndrome which is associated with increased risk of breast, lung, colorectum and brain tumors, is an autosomal dominant condition which only needs one allele of *TP53* to be mutated to have disease phenotype. The p53 protein is considered as an important protein not only because it coordinates responses throughout the cell cycle and mechanisms of DNA repair, but it also determines both cell development and apoptosis (Finlay, 1989) (Knudson, 2001). Inactivation of both *Rb1* and *TP53* pathways are directly correlated to the occurrence of most of the human cancers. Other than *Rb1* and *TP53*, there are numerous tumor suppressor genes in humans, shown in Table 1 (Cooper, 2000).

Table 1: Tumor suppressor genes list

Gene	Type of cancer
<i>APC</i>	Colon/rectum carcinoma
<i>BRCA1</i>	Breast and ovarian carcinomas
<i>BRCA2</i>	Breast carcinoma
<i>DPC4</i>	Pancreatic carcinoma
<i>INK4</i>	Melanoma, lung carcinoma, brain tumors, leukemias, lymphomas
<i>MADR2</i>	Colon/rectum carcinoma
<i>NF1</i>	Neurofibrosarcoma
<i>NF2</i>	Meningioma

<i>p53</i>	Brain tumors; breast, colon/rectum, esophageal, liver, and lung carcinomas; sarcomas; leukemias and lymphomas
<i>PTC</i>	Basal cell carcinoma
<i>PTEN</i>	Brain tumors; melanoma; prostate, endometrial, kidney, and lung carcinomas
<i>Rb</i>	Retinoblastoma; sarcomas; bladder, breast, and lung carcinomas
<i>VHL</i>	Renal cell carcinoma
<i>WT1</i>	Wilms' tumor

Chapter 1-5: The two-hit model applies to leukaemogenesis

The multiplication, hematopoietic differentiation and death of acute myeloid leukemia (AML) stem cells are aberrant due to genomic damage. It is possible that gene rearrangements and mutations can be beneficial to cell proliferation, differentiation and/or survival, and cells which possess these mutations can have advantages when compared with other cells (Fröhling, 2005) (Kelly, 2002).

Activation mutations in cell-surface receptors like RAS or tyrosine kinases like *FLT3* can enhance AML stem cell clonal expansion (Fröhling, 2005) (Moreau-Gachelin, 2006) (Kosmider, 2006). Hematopoietic differentiation is also disrupted by core-binding-factor gene rearrangements caused by t (8;21), inv (16), or t (16;16), or by the *PML–RARA* and *MLL* gene rearrangements (Fröhling, 2005) (Moreau-Gachelin, 2006) (Kosmider, 2006). The two-hit model may provide reasonable explanations for the observation of *FLT3-ITDs* cytogenetic aberrations in AML patients who have t(15;17). (Thiede, 2002) (Schnittger, 2002) (Schnittger, 2011), as well as for the occurrence of t (8;21) and

inv (16) AML, both of which are frequently associated with *KIT* mutations (Dash, 2001) (Care, 2003) (Park, 2011).

Table 2: Common cytogenetic abnormalities in AML

Common Abnormality
t (8;21) (q22;q22)
t (15;17) (q24.1;q21)
inv (16)/t (16;16) (p13.1;q22)
t (6;9) (p23;q34)
t (9;22) (q34;q11.2)
inv (3) (q21.3;q26.2)
11q23 rearrangement
+8
5q-/5
7q-/7
20q-

Chapter 1-6: The World Health Organization (WHO) classification of AML

In 1999, The World Health organization created a classification system for AML which focuses on the immunological, cytogenetic, morphological and clinical characteristics of the disease (Harris, 1999). The FAB system was developed and published in the revised (2008) World Health Organization classification system, as the organization highlights that the field of hemato-oncology is changing towards a more comprehensive system (Vardiman, 2009). According to the WHO classification, AMLs are grouped into four categories: (i) AML with recurrent genetic abnormalities; (ii) AML with multilineage dysplasia; (iii) therapy-related disorders; therapy-related AML (t-AML) and therapy-related myelodysplastic syndromes (t-MDS); and (iv) AML not otherwise categorized.

The WHO classification system identified 108 new diagnostic entities in hematopathology, involving 50 new or provisional leukaemia entries (Betz, 2010).

Chapter 1-7: Somatic mutations in myeloid malignancies

In a previous study, Grimwade *et al.* noted that there were approximately half of the AML patients will have abnormal cytogenetics karyotypes which can be observed on standard karyotype analysis (Grimwade, 2010). Cytogenetic observations which are considered as favorable risk include t (15;17) (q24.1;q21), *PML-RARA*; t (8;21) (q22;q22), *RUNX1- RUNX1T1*; and inv (16)/t (16;16) (p13.1;q22) or t (16;16) (p13.1;q22), *CBFB-MYH1*, and those patients usually have a good response to chemotherapy. In contrast, patients with unfavorable cytogenetics observations, such as complex karyotype, -5, del (5q), -7, and abnormalities of 3q, usually have a less favorable outcome and survival. Treatments for those patients often require allogeneic stem cell transplant in order to prolong survival (Grimwade, 2016) (He, 2015).

Table 3: Genes which are recurrently somatically mutated in myeloid malignancies grouped by their functional class

Functional class	Specific example mutations
Signaling and kinase pathway	<i>FLT3</i> , <i>KRAS</i> , <i>NRAS</i> , <i>KIT</i> , <i>PTPN11</i> , and <i>NF1</i>

Epigenetic modifiers (DNA methylation and chromatin modification)	<i>DNMT3A, IDH1, IDH2, TET2, ASXL1, EZH2, and MLL/KMT2A</i>
Transcription factors	<i>CEBPA, RUNX1, and GATA2</i>
Tumor suppressors	<i>TP53</i>
Spliceosome complex	<i>SRSF2, U2AF1, SF3B1, and ZRSR2</i>
Cohesin complex	<i>RAD21, STAG1, STAG2, SMC1A, and SMC3</i>

In recent years, researchers have gained plethora of knowledge about the genetic basis of AML, thanks to the advancement of diagnosing technology such as fluorescence in situ hybridization (FISH), genome array, Sanger sequencing, whole exome sequencing (WES), whole genome sequencing (WGS), and RNA sequencing. To date, there are ~52 genes that are shown to have associations with myeloid malignancy (Grimwade, 2016). Genes that are mostly mutated in myeloid malignancies can be grouped into at least six groups: 1. Signaling and kinase pathway mutations; 2. Epigenetic modifiers; 3. Transcription factors; 4. Tumor suppressors; 5. Spliceosome complex and 6. Cohesin complex (DiNardo, 2016). Myeloid malignancies are known to frequently have mutations in specific genes, such as *RUNX1* and *TET2* (Harada, 2015) (Figueroa, 2010). *FLT3* mutations are known to cause de novo AML, and *TET2* mutations are known to have association with MDS.

Genes that are important for malignancy onset and progression tend to be those that acquire mutations somatically, or that are subject to somatic duplication or deletion events. As previously mentioned, germline variants can interact with somatic mutations, which is similar to Knudson's "two-hit" hypothesis. For example, *RUNX1* is frequently mutated somatically (Harada, 2004) in myeloid malignancy patients, and also harbors known germline predisposition variants (Walker, 2002). Similarly, *CEBPA* can carry germline and somatic mutations, often in the same acute myeloid leukemia (AML) patient (Pabst and Mueller 2009). Makishima et al. recently reported (Makishima, Yoshida et al. 2013) somatic *SETBP1* mutations in secondary AML that affect precisely the same amino acid as do known germline mutations causing Schinzel-Gideon syndrome, a congenital disease carrying an elevated likelihood of tumors. Another example of germline-somatic convergence is the presence of *PRPF8* somatic mutations and hemizygous deletions in MDS and related conditions (Kurtovic-Kozaric, Przychodzen et al. 2014). *PRPF8* is the gene in which germline mutations cause retinitis pigmentosa. More recently, Polprasert *et al.* have observed both somatic mutations and germline frameshift mutations in *DDX41* in MDS patients (Polprasert, 2015).

Chapter 1-8: Myeloid Malignancies: Knowns and Unknowns

Myeloid malignancies include leukemias or preleukemias that originate from the myeloid lineage in the hematopoietic stem cell differentiation pathway. They are clonal diseases, and their causes include genetic and epigenetic alterations that

disturb key processes of cells such as growth, regeneration and death (Murati, 2012). Diseases included in myeloid malignancies are primary and secondary acute myeloid leukemia (pAML and sAML), myelodysplastic syndrome (MDS), myeloproliferative neoplasms (MPNs), and myelodysplastic syndrome/myeloproliferative neoplasms (MDS/MPN).

The completion of the human genome project (Lander, 2001) (Venter, 2001) opened a new era of genetic studies. It allows scientists to conduct genome wide research projects and can change clinicians' therapeutic method from chemotherapy or radiotherapy to personalized medicine. Furthermore, with the revolution in next-generation sequencing and the sequencing of the human genome, the approach has been to sequence cancer genomes to find mutations across the scale spectrum, from large translocations to base-pair changes. Large-scale projects such as the Cancer Genome Atlas (TCGA) (Cancer Genome Atlas Research, 2013), The Therapeutically Applicable Research to Generate Effective Treatments (*TARGET*) (Farrar, 2016), the International Cancer Genome Consortium (ICGC) (International Cancer Genome, 2010), and others have created numerous databases with a plethora of sequencing data available to researchers. Using those data, researchers have been able to find new insights in numerous types of cancer such as ovarian (Bolton, 2010), breast (Purrington, 2014), and colon (Won, 2012) and therefore our knowledge about cancer has been expanded at a rapid rate.

Instead of generalizing about all patients based on the origin of a tumor, researchers are now focusing on the genomic profile of individual tumors. This has led to changes in drug development for cancer from targeting specific cancer types to targeting specific changes or mutations that cancer cells have. One successful example is Gleevec which specifically targets chronic myelogenous leukemia (CML) which is caused by the *BCR-ABL* translocation. The estimated overall survival rate for patients is 89%, with a relapse rate of only about 17% in CML patients after Gleevec treatment (Druker, 2006). Therefore, exploring the opportunity of precision medicine can be beneficial to patients as this can provide the most effective treatment based on individual differences. It can also increase our understanding of cancer.

While there is extensive research on somatic mutations, we have little information about germline variants in myeloid malignancy. Genome wide association studies (GWAS) have been performed to find associations between particular SNPs and other disease phenotypes. Many GWAS have been successful in certain diseases, such as in cystic fibrosis (CF) (Wright, 2011), age-related macular degeneration (AMD) (Naj, 2013) (Arakawa, 2011) (Klein, 2005), diabetes (Blackman, 2013) (Ma, 2014) (Hanson, 2014), and multiple sclerosis (Leone, 2013) (Martinelli-Boneschi, 2012). Although GWAS find a good number of association in many human diseases, the percent trait variance explained by GWAS signals is not enough to explain all the heritability. This is called "missing heritability". In the case of myeloid malignancy, there are only a

few GWAS publications on acute myeloid leukemia or myelodysplastic syndrome. There was almost no association found between common SNPs and disease phenotype, and the heritable component for myeloid malignancy remains undiscovered.

Chapter 1-9: The importance of rare variants

Exome-wide and genome-wide sequencing studies has given researchers insight to understand the effects of rare variants. By understanding rare variants, researchers may be able to explain some of the missing heritability of many human traits (Agarwala, 2013). Also, differential selective pressures acting on variants across the allele frequency spectrum tend to be skewed in favor of rare variants for traits most strongly influenced by natural selection, compared with quantitative phenotypes or late-onset diseases (Park, 2011). By discovering the associations driven by low-frequency and rare variants through genome sequencing and large-scale imputation efforts, researchers can make continuous improvements on trait heritability across the frequency spectrum (Kryukov, 2009).

It is also worth noting that rare variants in or near gene targets have a larger effect on phenotype (Consortium, 2015) (Bouatia-Naji, 2009). Understanding these variants through the next generation sequencing of protein-coding regions will bring advancements in annotating genetic associations with complex diseases and describing the functional consequences of human sequence variation.

Chapter 1-10: Difficulties in conducting GWAS in myeloid malignancies

Myeloid malignancies are rare diseases, as their incident rate is less than 15 per 100,000 persons. This creates a big challenge for researchers who want to conduct genome wide association studies on these diseases because small numbers of patients translates into insufficient statistical power. Nevertheless, several studies such as TCGA, TARGET, and St Jude's Pediatric Cancer Genome Project (PCGP) are trying to gather many patients with myeloid malignancies and set up large databases in order to ease the sample size problem. Those studies also provide sequencing data and results for researchers to download and reanalyze, and hence facilitate the advancement on myeloid malignancy research.

Apart from sample size, confounding effects caused by genetic admixture is another problem in many GWAS. Genetic admixture occurs when individuals from different populations interbreed. As a result, the genomes of the individuals in an admixed population become a mixture, in which chromosomal segments can be coming from different ancestral populations. Thus, each chromosomal segments have their own identity, or "local ancestry", and an individual would have proportion of different ancestry in his/her genome. (Liu, 2013).

In addition, cross contamination within individuals is also a source of false positive in sequencing analysis. As cancer (or pre-cancer) cells have the ability to invade healthy compartments, acquiring pure normal tissue from patients can be a problem for all cancer association studies (Cancer Genome Atlas

Research, 2013) (Taylor-Weiner, 2018) (Stieglitz, 2015) (Wei, 2016). Therefore, DNA which originates from normal healthy samples may sometimes contain tumor cells and this type of contamination has been reported in various diseases such as leukemias (Stieglitz, 2015) (Welch, 2012) (Landau, 2015), and breast, bladder, and gastric cancers (Deng, 1996) (Försti, 2001) (Leung, 2001), among others. This type of contamination creates false positives, which decrease the accuracy for germline variant detection through next generation sequencing methods. Therefore, avoiding cross contamination within individuals is a crucial quality control step for all association studies in cancer (Taylor-Weiner, 2018).

Chapter 1-11: Rationale for this study

For nearly 15 years, genome wide association studies (GWAS) have been conducted to find associations between inherited polymorphisms and disease phenotypes. Although the approach has seen success in many human malignancies, there has been no published GWAS of acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS). This is despite the fact that several studies (Richkind, Loew et al. 1998, Lichtenstein, Holm et al. 2000, Kerber and O'Brien 2005, Goldin, Kristinsson et al. 2012) show a heritable component in myeloid malignancy. Indeed, first-degree relatives of MDS carriers have double the likelihood of acquiring the disease (Strom, Gu et al. 2005), and leukemias in general affect children at a higher rate than do any other cancer. However, AML (excepting pediatric forms) and MDS are typically very late age-of-onset diseases, which complicates efforts to identify susceptibility variants. Case-control studies

may be hampered by a lack of certainty that the controls will not eventually acquire the disease.

The limited number of variants that have been implicated in myeloid malignancy susceptibility are mostly rare and have largely been identified in families (Churpek, Pyrtel et al. 2015, Godley and Shimamura 2017). Newfound predisposition alleles are also likely to be rare, given the dearth of published common susceptibility alleles and the nature of known susceptibility variants. Identifying new variants will enable surveillance and intervention for carriers and will inform appropriate choices for hematopoietic stem cell transplantation. Causal inherited alleles also serve as potential targets for therapeutics. There have been recent successes in this regard in solid tumors. Carriers of germline *BRCA1/2* mutations were shown to be responsive to poly (ADP-ribose) polymerase (PARP) inhibitors for multiple cancer types (Kaufman, Shapira-Frommer et al. 2015), and the PARP inhibitor olaparib has achieved regulatory approval for ovarian cancer patients. Furthermore, potentially targetable inherited variants in DNA-repair genes were shown to be frequent in men with metastatic prostate cancer (Pritchard, Mateo et al. 2016).

In this study, we analyze whole-exome sequence data from the germlines of 690 patients with myeloid malignancies. Much of this data has been obtained from studies that focused exclusively on the somatic mutational landscape. These studies used the germline exomes only to distinguish somatic mutations from inherited variants. We therefore exploit data that is largely discarded and was not mined for clues to inherited contributors to disease. We assess the presence and

impact of rare variants in genes with previously implicated susceptibility alleles, as well as in genes with known or putative roles in myeloid neoplasms or cancer in general. In an effort to identify new germline contributors, we also focus particularly on genes that are known to demonstrate an autosomal recessive inheritance pattern, including Fanconi anemia genes. The hypothesis here is that germline contributors may be identified by their appearing frequently paired, in the same patient, in autosomal recessive genes, impacting both parental homologs. Synergies both among rare variants and with somatic mutations are also investigated, under the hypothesis that a wild-type copy paired with a germline-disrupted copy may itself be disrupted somatically.

Chapter 2: Patient collections, variant calling and description of variants

Chapter 2-1: Germline whole exome sequencing and ancillary data collection

We obtained whole-exome germline bam files from myeloid malignancy patients from the Cleveland Clinic and the Josep Carreras Leukaemia Research Institute in Barcelona, Spain. We also downloaded whole exome bam files from online sources (Table 5), yielding a total of 690 cases. For all cases, DNA was collected from either buccal mucosa or paired T-lymphocytes (CD3⁺ cells). We obtained available somatic mutation, cytogenetic, and clinical information for the patient datasets.

Table 4: Patient sample sources

Data source	Accession ID/ Database	Number of patient samples	Diagnoses	Publication (PubMed ID)
Cleveland Clinic	PRJNA203580 (RA)	195	AML, MDS, MDS/MPN, MPN, pAML, PNH, sAML	27992414
Josep Carreras Leukaemia Research Institute	EGAS00001001011	37	MDS	25242043
St. Jude Children's Research Hospital	EGAS00001000349	65	pAML	27798625
St. Jude Children's Research Hospital	EGAS00001002183	21	pAML	28112737

Multiple origins	phs001027.v1.p1 (dbGaP)	47	pAML, sAML	27322744
Gustave Roussy Cancer Center	EGAD00001001853	39	MDS/MPN	26908133
International Cancer Genome Consortium (ICGC)	EGAD00001000123	50	MPN	NA
ICGC	EGAD00001000106	27	MPN	NA
ICGC	EGAD00001000117	39	MDS	25957392
Institutions throughout Japan	EGAS00001000521	6	MDS/MPN	23832011
Therapeutically Applicable Research To Generate Effective Treatments (TARGET)	GDC	22	pAML	26941285
The Cancer Genome Atlas (TCGA)	GDC	142	pAML	23634996

Table 5: Patient summary

	Karyotype							Gender (Male %)
	n (%)	complex ^a	inv16	-5	-7/7q-	trisomy 8	Mean age in years (1st quartile - 3rd quartile)	
MDS	190 (27.5)	18.8%	0%	50.9%	17.0%	8.0%	68 (62-78)	56.0%
MDS/MPN	83 (12)	0.8%	0%	5.1%	7.6%	7.6%	64 (63-77)	69.2%
MPN	82 (11.9)	0%	0%	0%	0.0%	0%	62 (60-64)	29.1%
pAML	300 (43.5)	15.8%	13.0%	6.5%	8.9%	10.3%	39 (13-61)	58.0%
PNH	7 (1)	0%	0%	0%	0.0%	0%	47 (37-59)	42.9%
sAML	28 (4)	33.3%	0%	38.1%	19.0%	14.3%	72 (67-78)	71.4%
Overall	690	15.2%	7.4%	17.2%	10.7%	9.4%	51 (33-72)	55.2%

^a Three or more cytogenetic lesions

Table 6: Patient cohort

<u>Patient ID</u>	<u>Age at diagnosis</u>	<u>Gender</u>	<u>Diagnosis</u>	<u>Status</u> (0 = dead, 1 = alive, NA = not available)	<u>Overall survival</u> (mos)	<u>Karyotype</u>
AML_074	71	M	pAML	NA	NA	49,XY,+13,+13,+19 [21]
AML_075	49	F	pAML	NA	NA	46,XX [22]
AML_078	31	F	pAML	NA	NA	46,XX,inv (16) (p13q22)[8]/47,XX,+8,inv (16) (p13q22)[12]
AML_079	54	F	pAML	NA	NA	47, XX, +21 [21]/46, XX [7]
AML_080	33	M	pAML	NA	NA	47, XY, +11 [43]/46, XY [5]
AML_081	43	M	pAML	NA	NA	47, XY, +11 [14]/ 46, XY [13]
AML_082	37	M	pAML	NA	NA	46, XY, del (11) (q14 q25) [3]/46, XY [60]
AML_083	62	M	pAML	NA	NA	46, XY [68]
AML_084	37	M	pAML	NA	NA	47,XY,+13[11]/46,XY[28]
AML_086	30	M	pAML	NA	NA	46, XY [26]
AML_088	30	F	pAML	NA	NA	46,XX[21]
AML_089	60	F	pAML	NA	NA	46, XX [24]
AML_090	42	M	pAML	NA	NA	46,XY[20]
AML_091	45	M	pAML	NA	NA	46,XY,add (7) (q22)[4]/46,XY[21]
AML_092	41	M	pAML	NA	NA	46,XY,inv (16) (p13q22)[21]
AML_093	53	M	pAML	NA	NA	46,XY[23]
AML_094	50	F	pAML	NA	NA	46,XX[24]
AML_095	49	F	pAML	NA	NA	46,XX[20]
AML_096	43	F	pAML	NA	NA	46,XX,del (11) (q11q14),inv (16) (p13q22)[16]

AML_097	72	M	pAML	NA	NA	46,XY,t (3;21) (q26;q22),del (12) (p12p13)[20]
AML_098	67	F	pAML	NA	NA	46,XX[25]
AML_099	61	M	pAML	NA	NA	mos47,XXY[2 7]/46,XY[4]
AML_100	65	M	pAML	NA	NA	56~58,XY,+X ,der (5)del (5) (p1?2p1?4)d el (5) (q1?3q15),+8 ,+10,+11,+11 ,+13,+19,+21 ,+22,+mar1,+ mar2[8]/56~5 8,idem,add (2) (q3?6)[16]/46 ,XY[30]
AML_101	63	M	pAML	NA	NA	47,XY,+8[1]/4 6,XY[34]
AML_102	62	M	pAML	NA	NA	5~50,XY,+8,d er (10)t (10;21) (p13;q21),t (11;22;11) (q23;q11.2;q 13),-15,der (17)t (15;17) (q13.3;q25)d el (17) (p11.2)ps,- 21,+der (22)t (11;22;11),+ mar1x2,+mar 2.ish der (10)t (10;21) (AML1+,WC P21+), t (11;22;11) (MLL++,BCR -;MLL-,BCR-, WCP22+;ML L-,BCR+,WC P22+), der (17) (p53-)[cp16]/ 46,XY[4]
AML_103	50	F	pAML	NA	NA	46, XX

AML_104	67	F	pAML	NA	NA	46,XX,t (2;11) (p25;q13),-9,del (9) (p13),add (13) (p11.2),-17,+mar1,+mar2[cp13]/46,XX[2]
AML_105	54	M	pAML	NA	NA	46, XY
AML_106	61	M	pAML	NA	NA	46, XY
AML_108	67	F	pAML	NA	NA	NA
AML_109	18	M	pAML	NA	NA	46, XY
AML_110	49	F	pAML	NA	NA	46, XX
AML_111	28	F	pAML	NA	NA	46, XX
AML_113	23	F	pAML	NA	NA	46, XX
AML_114	56	M	pAML	NA	NA	46, XY
AML_115	42	F	pAML	NA	NA	46,XX, t (5;8) (q31.3;q21)
AML_116	64	M	pAML	NA	NA	47,XY,+4,del (4) (q12q31)[7]/46,XY[13]
AML_119	64	F	pAML	NA	NA	46,XX[12]
AML_121	74	M	pAML	NA	NA	46,XY[25]
AML_123	57	M	pAML	NA	NA	46,XY[18]
AML_124	65	M	pAML	NA	NA	47,XY,+21[8]/46,XY[16]
AML_126	60	M	pAML	NA	NA	46,XY[2] POS FOR del 5q31 10 IN 200 CELLS / FISH FOR CEP 7/del 7q31 NEG IN 200 CELLS
AML_127	66	M	pAML	NA	NA	unknown
AML_128	49	M	pAML	NA	NA	46,XY[1]
AML_133	57	F	pAML	NA	NA	46,XX[27]
AML_135	74	F	pAML	NA	NA	monosomy 7 [7/17]
AML_139	55	F	pAML	NA	NA	46, XX
C_01	84	M	sAML	0	15.3534 2466	46,XY,del (20) (q11.2)[12]
C_02	70	M	MDS/MPN	1	78.0821 9178	46,XY,del (2) (p22),inv (9)

						(p12q13),del (20) (q12)[4].
C_03	62	F	MDS/MPN	0	29.6547 9452	46,XX,del (11) (q23)[3]/46,X X[17]
C_04	68	M	MDS	1	60.9863 0137	46,XY,ins (1;14) (p32;q24q32) [19]/46,XY[1]
C_05	78	M	sAML	0	39.0904 1096	46,XY[13]
C_06	60	M	MDS/MPN	0	35.6054 7945	46,XY (6/2005)
C_07	65	M	MDS	1	23.9013 6986	48,XY,del (5) (q12q33),- 6,del (7)q11),+8,- 13,i (17) (q10),+3mar[4]/46,XY[3]
C_08	79	M	sAML	0	77.6219 1781	46,XY,del (12) (p11.2p13)[c p15]/46,XY,t (4;16) (q12;p13.3),d el (12) (p11.2p13)[5]
C_09	69	M	sAML	1	96	46,XY[20]
C_10	67	M	sAML	0	20.2520 5479	41-44,XY,del (1) (p21)[1],del (3) (q21q26.2)[2] ,inv (3) (q21q26.2),a dd (5) (q22),- 6,add (7) (q22),- 10[19],add (11) (q23)[3],- 12,- 17,+mar1[19] ,+mar2[18][c p20]
C_101	31	F	PNH	0	144.131 5068	46,XX
C_102	85	F	MDS	0	12.4602 7397	46,XX
C_103	20	M	PNH	0	77.3260 274	NA

C_106	77	F	MDS	0	17.5232 8767	45,XX,-7,del (20) (q11.2q13.3)[13]/46,XX[4]
C_107	80	M	MDS	0	49.6109 589	47,XY,+der (1;19) (q10;p10)[20]
C_108	67	F	MDS	1	37.8410 9589	45,XX,del (3) (p14),-5,-7, add (17) (p11.2),+22[4]/46,XX[cp17]
C_11	71	F	sAML	0	13.9068 4932	44-47,XX,del (5) (q12q33)[11], -7[9],del (7) (q22)[2],del (7) (q22q34)x3[1 , -8, -12, -16, - 16, -17, - 18[10], +21[8] , +1- 4r[12], +mar1, +mar2[11], + mar3[10], +m ar4[10][cp12]/ 46,XX[8]
C_113	NA	F	MDS/MPN	NA	NA	NA
C_115	64	F	MPN	1	3.48493 1507	46, XY
C_116	65	F	MPN	1	44.9753 4247	47,XX,+9,der (15)t (1;15) (q21;p11.2)[2]/46,XX,[1].
C_117	31	M	MDS	0	11.6383 5616	45, XY, -7 [20]
C_12	63	M	MDS	1	29.5561 6438	44,X,-Y,-7,del (20) (q11.2)[cp19] /46,XY[1]
C_125	61	M	PNH	0	81	NA
C_127	56	F	sAML	0	0.75616 4384	45,XX,del (5) (q12q33),- 7,+8,del (16) (q22),der (20)t (17;20) (q21;q13.3)[1 6]/46,idem,- 13,- 15,+mar1[4].

C_128	75	F	MDS	0	81.4027 3973	46,XX, del5q
C_129	79	F	MDS	1	35	45,XX,del (5) (q15q33),del (7) (q22),- 22[cp2]/46,X X[18]
C_13	78	F	MDS	0	31.5287 6712	44,XX,-4,del (5) (q12q33),- 17,add (19) (p13)[3]/45,sl, -add (19) (p13),+19,+m ar1[7]/44,sl1,- 6,+inv (6) (p23q25),-7,- 10,+del (10) (q25),- 14,+add (14) (q32),-15,- 22,+der (22)t (15;22) (q11.2;q13),+ mar2[cp6]/46, XX[4]
C_130	64	M	MDS/MPN	0	75.4849 3151	46,XY,del (5) (q12q33)[6]
C_132	70	F	MDS	1	23.5726 0274	47,XX,+8[2]/4 7,idem,del (5) (q22q33)[2]/4 6,XX[17]
C_133	74	M	sAML	0	26.1698 6301	45,XY,del (5) (q13q34),del (7) (q22),+8,- 17,- 18[cp11]/46, XY[9]
C_134	75	M	MDS	0	2.13698 6301	46- 47,XY,ADD (3) (Q11.2),DEL (5) (Q13Q33),+8 , -15,-16,+1- 2MAR[CP20]
C_136	51	M	sAML	0	5.72054 7945	47,XY,+21[16]/47,idem,add (7) (q36)[3]/46,X Y[1]
C_138	73	M	sAML	0	2.79452 0548	45-46,XY,der (3;16) (q10;q10)[19]

						,del (5) (q22q35)[19], add (12) (p11.2)[19],a dd (12) (q13)[19],del (14) (q24q32)[19], -17[9],add (17) (q12)[9],- 19[3],+add (21) (p12)[9],+22[10],+mar1[3][cp19]/46,XY[1]
C_139	56	M	MDS	0	5.78630 137	45,XY,del (5) (q13q33),-7,- 17,- 18,+mar1,+m ar2[20]
C_14	70	M	MDS/MPN	0	20.4164 3836	46,XY[cp20]
C_140	70	M	MDS	0	6.01643 8356	43-44,XY,del (5) (q13q35),- 7,add (9) (p24),psu dic (17;8) (p13;p23),- 18,psu dic (19;14) (q13.4;p13),+ 1-2r[cp20]
C_141	57	F	MDS	0	130.783 5616	46,XX,del (5) (q13q33)[19]/ 46,XX[1]
C_142	80	M	sAML	0	7.23287 6712	46,XY,del (5) (q15q31)[11]/ 46,XY[9].
C_143	72	F	sAML	0	6.93698 6301	44,XX,add (3) (p11),add (3) (q29),del (5) (q13q31),add (9) (q11),der (11) (11pter- >11q1?3::hsr ::14q11.2- >14qter),hsr (11) (q23),- 14,add (14) (p12),-

						19[cp19]/46,XX[1]
C_145	55	F	pAML	1	36.7561 6438	46,XX,del (5)(q31q35)[13]/46,XX[7]
C_15	75	M	MPN	0	12.4602 7397	47,XY,+13,i(17)(q10)[19]/48,idem,+5[1]
C_16	79	F	MDS	0	25.9397 2603	46,XX,del (5)(q15q33)[2]/46,idem,t(2;12)(p13;p11.2)[7]
C_18	75	M	sAML	0	69.6986 3014	45,X,-Y[7]/46,XY[13]
C_19	65	M	MDS	0	15.9452 0548	46,XY[20]
C_20	50	F	MDS	1	28.0767 1233	46,XX,del (9)(q22q34),add(20)(q11.2)[18]/46,idem,del (7)(q21q35)[1]/46,XX[1](4/5/11)
C_21	69	M	MDS/MPN	0	19.7260 274	46,XY[20]
C_23	53	F	MDS/MPN	1	105.008 2192	46,XX[15]
C_24	53	M	MPN	1	28.6684 9315	46,XY[21]
C_26	66	M	MDS/MPN	1	35.4739 726	46,XY[20]
C_27	69	F	MDS	0	3.35342 4658	43,XX,-3,del(5)(q22q35),-7,add(12)(p13),-16[4]/43-44,idem,-2,add(18)(p11.3),+mar1,+mar2[cp15]/46,XX[1]
C_28	63	M	MDS/MPN	1	55.1342 4658	46,XY[20]
C_29	74	M	MDS	0	30.7397 2603	46,XY[20]
C_30	81	F	MDS	0	56.1205 4795	46,XX[20]

C_31	64	M	sAML	0	46.7506 8493	46, XY [20]
C_32	78	M	MDS	0	3.22191 7808	46,XY,del (20) (q11.2)[17]
C_33	65	M	MDS	0	30.8054 7945	46,XY,del (5) (q12q33),del (13) (q12q14)[20]
C_34	83	M	sAML	NA	NA	46,XY[20]
C_35	42	F	PNH	0	138.969 863	NA
C_36	72	F	PNH	0	524.646 5753	46,XX [19]
C_38	64	M	MDS/MPN	1	10	46, XY [20]
C_39	69	M	MDS/MPN	0	27.2219 1781	46,XY[15]
C_40	81	F	MDS	1	64.2082 1918	46,XX,del (20) (q11.2)[20]
C_41	76	F	MDS	0	38.0383 5616	49,XX,- 7,+8,+8,+9,+ 13[6]/46,XX[1 4]
C_42	60	F	MDS	NA	NA	45,XX,- 7[1]/46,XX,de l (11) (q23)[cp2]/47 ,XX,+11[cp26]/46,XX[1]
C_43	59	F	sAML	0	48.0986 3014	46,XX[20]
C_44	76	M	MDS/MPN	0	15.3863 0137	46,XY[20]
C_45	66	M	MDS	0	46.9150 6849	46,XY[20]
C_46	83	F	MDS	0	35.4739 726	46,XX[20].
C_47	71	F	MDS/MPN	0	4.01095 8904	88- 89<4n>,XXX X,ins (4:?) (q12;?)x2,-5,- 5,-7, -7,der (8)t (7;8) (q11.2;q24)x 2,-13,-13,- 19,-19,+3- 5mar[cp5]/46, XX[17].
C_48	62	M	MDS	1	100.8	47,XY,+Y[20]
C_49	65	M	MDS	0	41.5890 411	46,XY

C_50	60	M	MDS/MPN	0	35.1123 2877	45,X,-Y[20]
C_51	64	M	MDS	NA	36	46,XY[20].
C_52	79	F	MDS	1	84.2958 9041	46,XX,del (20) (q11.2q13.1)[18]/46,idem,t (1;15) (p36.3;q11.2) [2]
C_53	75	M	MDS/MPN	0	39.8136 9863	46,XY[20]
C_54	65	F	MDS/MPN	0	31.7917 8082	46,XX[20]
C_55	66	F	MDS	NA	NA	45,X,- X[4]/46,XX[1 6]
C_56	78	F	MDS/MPN	0	17.2273 9726	NA
C_57	67	M	MDS	NA	NA	46, XY [20]
C_58	61	F	MDS	NA	NA	46,XX
C_59	77	M	sAML	0	28.8328 7671	42,XY,add (1) (q32),del (3) (q12),add (4) (q21),del (5)
C_60	77	M	MDS	0	33.8958 9041	46,XY[16]
C_61	72	M	MDS/MPN	NA	NA	46,XY
C_62	42	F	MDS	1	91.5945 2055	46 XX in 19 metaphases and 45, X, -X in one metaphase
C_64	66	M	MDS/MPN	0	11.4739 726	46, XY [20]
C_65	41	F	MDS/MPN	1	19.3643 8356	46,XX,del (13) (q12q22)[18]/ 46,XX[2]
C_66	75	M	MDS/MPN	1	19.5945 2055	46,XY[20]
C_67	3	M	MDS/MPN	0	18.4438 3562	46,XY[20]
C_68	75	M	MDS/MPN	0	9.23835 6164	46,XY[cp30]
C_69	77	M	sAML	0	28.7671 2329	46,XY[20]
C_70	59	M	MDS	0	44.4164 3836	46,XY

C_72	54	M	MDS	0	31.3315 0685	46,XY[20]
C_73	52	M	MDS	0	48.1972 6027	46,XY[12]
C_74	62	F	MDS	1	30.9041 0959	45,XX,- 7[8]/45,idem, del (12) (p12)[9]/46,X X,del (12) (p12)[1]/46,X X[2]
C_75	72	F	MDS	1	54.1150 6849	46,XX [20]
C_76	63	M	MDS	1	90.7726 0274	47,XY,+8[cp1 8]/46,XY[2]
C_77	75	M	MDS/MPN	0	15.3534 2466	no cyto available
C_78	73	F	MDS/MPN	0	4.93150 6849	46,XX[20]
C_79	49	F	MDS	0	19.6931 5068	47, XX, +21[20]
C_80	63	M	MDS/MPN	0	47.4410 9589	46, XY [20]
C_81	56	M	PNH	0	377.654 7945	NA
C_82	46	F	PNH	0	236.778 0822	46, XX [20]
C_83	65	M	MDS	0	19.5945 2055	46,XY,del (5) (q3?1q3?5)[2]/46,XY[cp18
C_84	87	M	MDS	0	40.7342 4658	46,XY[20]
C_85	85	M	sAML	0	22.6191 7808	47,XY,+8[2]/4 6,XY[18]
C_89	71	F	MDS	0	19.1671 2329	45-46,XX,add (4) (q21),- 5[3],psu dic (5;?) (q11.1;?)[16], add (8) (p23),-13[17], -16,add (17) (p11.2),- 18,+r[5],+mar 1,+mar2[cp2 0]
C_90	70	M	MDS	0	66.8712 3288	46,XY,del (20) (q11.2q13.1)[3]/46,XY[19]
C_91	59	F	MDS	0	17.0301 3699	47,XX,del (5) (q22q35)[1]/4 7,sl,+9,add

						(16) (q24)[3]/46,X X[17]
C_92	82	M	MDS	0	95.4082 1918	46,XY [20]
C_93	0	NA	MDS/MPN	NA	NA	NA
C_97	84	F	MDS	0	14.8931 5068	46, XY
C_98	46	M	MDS	0	29.9506 8493	46,XY,+1,der (1;7) (q10;p10)[9]/ 46,XY[3]
C_99	61	M	MDS	1	51.1232 8767	46, XY [20]
CMML-04	NA	F	MDS/MPN	NA	NA	NA
Index13_1 4	NA	M	MDS	NA	NA	46 XY, t (10:18)
Index6_7	62	M	pAML	NA	NA	46, XY
JMML11	13	M	MDS/MPN	NA	NA	46,XY
JMML63	8	F	MDS/MPN	NA	NA	48,XX,+8,+19
JMML72	2	M	MDS/MPN	NA	NA	46,XY
JMML77	1	F	MDS/MPN	NA	NA	47,XX,+8
JMML78	1	M	MDS/MPN	NA	NA	46,XY
JMML82	0	F	MDS/MPN	NA	NA	46,XX
MDS-03	45	M	MDS	NA	NA	NA
MDS-04	72	M	MDS	NA	NA	NA
MDS-06	73	M	MDS	NA	NA	NA
MDS-07	70	M	MDS	NA	NA	NA
MDS-08	75	M	MDS	NA	NA	NA
MDS-09	86	M	MDS	NA	NA	NA
MDS-10	25	F	MDS	1	40.4	NA
MDS-11	64	M	MDS	1	38	NA
MDS1105 20	73	M	MDS	NA	NA	NA
MDS-12	24	F	MDS	NA	NA	NA
MDS-13	40	F	MDS	NA	NA	NA
MDS-14	46	F	MDS	NA	NA	NA
MDS-15	77	M	MDS	1	30.8	NA
MDS-16	86	M	MDS	1	37.6	NA
MDS-17	73	M	MDS	1	19.7	NA
MDS-18	75	F	MDS	NA	NA	NA
MDS-19	78	F	MDS	NA	NA	NA
MDS-20	56	F	MDS	NA	NA	NA
MDS-21	59	M	MDS	0	15.7	NA

NGS1	54	F	sAML	1	17.3917 8082	47,XX,del (5) (q12q33),+?d el (12) (q13)[20]
NGS11	59	F	MDS/MPN	1	105.928 7671	46,XX [20]
NGS13	78	F	MDS/MPN	0	20.0876 7123	47,XX,+8[20]
NGS15	81	M	MDS/MPN	0	5.03013 6986	45,XY,- 7[10]/46,XY,d el (5) (q13q33)[1]/4 6,XY[9]
NGS19	77	F	MDS	0	111.780 8219	46,XX,del (20) (q11.2)[20]
NGS21	72	M	MDS	0	7.56164 3836	46,XY
NGS23	64	F	MPN	1	39.0904 1096	46, XX, del (20) (q11.2)[cp19] /46,XX
NGS25	65	F	MDS/MPN	0	164.383 5616	46,XX
NGS27	NA	M	MDS/MPN	0	18.4438 3562	46,XY[20]
NGS3	77	F	sAML	0	14.7945 2055	46,XX,t (8;180 (q22;q23),t (11;13) (q21;q12)[cp 11]/46,XX[9] on 4/1/09
NGS5	63	F	MDS	1	63.5835 6164	46,XX
NGS7	68	M	MDS/MPN	0	5.26027 3973	46,XY[20]
NGS9	46	M	MDS	1	125.589 0411	46,XY,del (20) (q11.2q13.3)[18]/46,XY[2].
Nick1	74	F	MDS	0	11.0794 5205	46,XX [11]
Nick2	72	M	MDS/MPN	1	52.4054 7945	46,XY[20]
Nick4	68	F	MDS	NA	NA	46,XX
Nick5	68	M	MDS	0	105.534 2466	46,XY[20]
Nick7	68	M	MDS	0	11.0136 9863	46,XY,del (7) (q22),+8,20,a dd (21) (p11.2)[3]/47,

						XY,add (2) (q31),del (7) (q22),+8,+8,d el (11) (p1?),12,20,a dd (21) (p11.2),+mar 1[12]/47,XY,a dd (2) (q31),del (7) (q22),+8,+8,d el (11) (p1?)- 12,- 21,+mar1[2]/ 48,XY,add (2) (q31)del (7) (q22),+8,+8,d el (11) (p1?),- 12,-20,add (21) (p11.2),+mar 1,+mar2[2].
Nick8	43	F	MDS	0	12.7561 6438	46,XX,del (9) (q21q22),del (12) (p12p13)[6]/4 6,XX,del (7) (q22)[4]/46,X X,del (7) (q22),del (9) (q21q22),del (12) (p12p13)[2]/4 6,XX[10]
OT052	NA	F	MDS	NA	NA	NA
OT070	NA	M	MDS	NA	NA	NA
P10	77	F	MDS	1	58.2575 3425	46,XX,del (5) (q13q33)[17]/ 46,XX[3]
P11	83	F	MDS	1	85.3808 2192	46,XX,del (5) (q13q33)[14]/ 46,XX [6]
P12	78	F	MDS	1	71.0465 7534	46,XX,del (5) (q13q33)[6]/4 6,XX[14]
P15	85	F	MDS	1	33.6986 3014	46,XX,del (5) (q13q31)[13]/ 46,XX[7]
P16	80	F	MDS	1	67.1671 2329	46,XX[20] FISH 5q:- 10.5%

P17	82	F	MDS	0	53.3260 274	46,XX,del (5) (q13q33)[19]/ 46,XX[1]
P18	82	F	MDS	0	10.8821 9178	46,XX,del (5) (q13q33)[8]/4 6,XX[14]
P19	62	F	MDS	1	26.0054 7945	46,XX,del (5) (q13q33)[6]/4 6,XX[11]
P2	75	F	MDS	0	60.8219 1781	46,XX,del (5) (q13q33)[7]/4 6,XX[13]
P20	NA		MDS	NA	NA	del (5q) en 11/17 metafasas
P21	83	M	MDS	1	22.7506 8493	46,XY,del (5) (q13q33)[17]/ 46,XY[3]
P22	84	F	MDS	0	32.9095 8904	46,XY,del (5) (q13q33)[1]/4 6,XX,-3,add (5) (p15),del (5) (q13q33),- 7,add (21) (q22)[14]/46, XX[5]
P23	78	F	MDS	0	10.0931 5068	45,XX,del (2) (q33q37),- 5[10]/46,XX,d el (5) (q13q33)[3]4 65,XX,- 7[10]/46,XX,- 14,+der (?)t (1;?) (p13;?),+mar[10][cp13]
P24	73	M	MDS	1	25.2493 1507	46,XY,del (5) (q31q35)[8]/4 6,XY[2]
P25	65	F	MDS	1	25.1506 8493	46,XX,del (5) (q15q33)[20]
P26	50	F	MDS	1	15.3534 2466	46,XX,del (5) (q13q33)[9]/4 6,XX[5]
P27	81	F	MDS	1	20.2520 5479	46,XY,del (5) (q22q35)[20]
P29	70	F	MDS	1	36.3616 4384	46,XX,del (5q) (q13q33)[16]/ 46,XX[4]
P3	55	F	MDS	0	27.9123 2877	46,XX,t (2;11)

						(p21;q23),del (5) (q21q33)[15]
P32	64	M	MDS	1	17.7205 4795	46,XY,del (5) (q13q33)[23]/47,XY,del (5) (q13q33),+21 [1]/46,XY,del (5) (q13q33),del (X) (q22),+add12 (p13)[1]
P33	83	M	MDS	1	12.3945 2055	46,XY,del (5q)[8]/46,XY [12]
P34	63	M	MDS	1	20.7452 0548	46,XY,del (5) (q?q?)[4]/46, XY[16]
P35	76	F	MDS	1	6.93698 6301	SD 35% 5q-FISH
P37	72	F	MDS	1	32.1863 0137	46,XX,del (5) (q13)[19]/46, XX[1]
P38	82	M	MDS	1	11.5068 4932	45,X,-Y,del (5) (q14)[11]/46, XY[3]
P39	35	F	MDS	1	81.4684 9315	46,XX,del (5) (q15q33)[20]
P4	66	F	MDS	1	51.0575 3425	46, XX, del (5) (q14 q33) [16]/ 46, XX [4] (Jan 2011)
P40	NA	F	MDS	1	14.7616 4384	46,XX,del (5) (q12q33)[10]/47,XX,+8[3]/46,XX[6]
P41	61	F	MDS	1	15.8136 9863	46,XY,del (5) (q13q33)[1]/46,XX[4]
P42	72	F	MDS	1	25.3150 6849	46,XX,del (5) (q13q33)[10]/46,XX[13]
P46	65	F	MDS	1	24.7561 6438	46,XX,del (5) (q15q33)[19]/46,XX[1]
P47	71	F	MDS	1	10.6520 5479	46,XX,del (5) (q13q31)[7]/46,XX[13]
P48	NA	F	MDS	1	9.56712 3288	46,XX,del (5q)[4]/46,XX

P5	82	F	MDS	0	18.4438 3562	46,XX,del (5) (q13q33)[9]/4 6,XX[11]
P51	83	M	MDS	1	21.8301 3699	46,XY,del (5) (q15q33)[9]/4 6,XY[11]
P6	85	M	MDS	0	12.7890 411	46,XY,del (5) (q13q31)[17]/ 46,XY[3]
P7	79	M	MDS	1	121.446 5753	46,XY
P8	60	F	MDS	1	50.2684 9315	46,XX
P9	79	F	MDS	1	28.7013 6986	46,XX,del (5) (q13q33)[7]/4 6,XX[13]
PD4060	50	F	MPN	1	0	NA
PD4063	NA	F	MPN	NA	NA	NA
PD4177	NA	F	MPN	NA	NA	NA
PD4771	NA	M	MDS	NA	NA	NA
PD4772	NA	F	MPN	NA	NA	NA
PD4773	NA	F	MPN	NA	NA	NA
PD4775	64	F	MPN	0	NA	NA
PD4776	NA	F	MPN	NA	NA	NA
PD4778	NA	F	MPN	NA	NA	NA
PD4780	NA	F	MPN	NA	NA	NA
PD4943	NA	M	MPN	NA	NA	NA
PD4944	NA	M	MPN	NA	NA	NA
PD4990	NA	F	MPN	NA	NA	NA
PD4996	NA	M	MPN	NA	NA	NA
PD5003	NA	F	MPN	NA	NA	NA
PD5008	NA	F	MPN	NA	NA	NA
PD5010	NA	F	MPN	NA	NA	NA
PD5029	NA	F	MPN	NA	NA	NA
PD5111	NA	F	MPN	NA	NA	NA
PD5115	NA	F	MPN	NA	NA	NA
PD5116	NA	F	MPN	NA	NA	NA
PD5117	64	F	MPN	1	NA	NA
PD5120	NA	M	MPN	NA	NA	NA
PD5121	NA	F	MPN	NA	NA	NA
PD5122	NA	F	MPN	NA	NA	NA
PD5125	NA	F	MPN	NA	NA	NA
PD5133	NA	NA	MPN	NA	NA	NA
PD5134	NA	M	MPN	NA	NA	NA

PD5135	NA	F	MPN	NA	NA	NA
PD5136	NA	NA	MPN	NA	NA	NA
PD5138	NA	F	MPN	NA	NA	NA
PD5141	NA	F	MPN	NA	NA	NA
PD5151	NA	F	MPN	NA	NA	NA
PD5155	NA	F	MPN	NA	NA	NA
PD5158	NA	F	MPN	NA	NA	NA
PD5161	NA	M	MPN	NA	NA	NA
PD5163	NA	F	MPN	NA	NA	NA
PD5164	NA	F	MPN	NA	NA	NA
PD5165	NA	F	MPN	NA	NA	NA
PD5175	NA	F	MPN	NA	NA	NA
PD5182	NA	M	MPN	NA	NA	NA
PD5192	NA	NA	MPN	NA	NA	NA
PD5847	NA	F	MPN	NA	NA	NA
PD5848	NA	F	MPN	NA	NA	NA
PD6060	NA	M	MDS	NA	NA	NA
PD6068	NA	NA	MDS	NA	NA	NA
PD6077	37	F	MDS	0	NA	46,XX
PD6100	NA	NA	MDS	NA	NA	NA
PD6101	NA	M	MDS	NA	NA	NA
PD6110	74	F	MDS	0	NA	NA
PD6116	NA	NA	MDS	NA	NA	NA
PD6122	NA	M	MDS	NA	NA	NA
PD6127	NA	M	MDS	0	NA	NA
PD6193	NA	M	MDS	NA	NA	NA
PD6561	NA	M	MPN	NA	NA	NA
PD6575	NA	NA	MPN	NA	NA	NA
PD6628	NA	F	MPN	NA	NA	NA
PD6637	NA	F	MPN	NA	NA	NA
PD6647	NA	F	MPN	NA	NA	NA
PD6650	NA	NA	MPN	NA	NA	NA
PD6877	NA	M	MDS	NA	NA	NA
PD6964	NA	M	MDS	NA	NA	NA
PD7084	NA	M	MDS	NA	NA	NA
PD7089	NA	NA	MDS	NA	NA	NA
PD7092	NA	M	MDS	NA	NA	NA
PD7103	NA	M	MDS	NA	NA	NA
PD7107	NA	M	MDS	NA	NA	NA
PD7116	64	M	MDS	1	NA	46, XY

PD7117	NA	M	MDS	NA	NA	NA
PD7118	NA	NA	MDS	NA	NA	NA
PD7120	NA	M	MDS	NA	NA	NA
PD7148	NA	M	MDS	NA	NA	NA
PD7151	NA	M	MDS	NA	NA	NA
PD7152	NA	M	MDS	NA	NA	NA
PD7153	NA	NA	MDS	NA	NA	NA
PD7156	NA	M	MDS	NA	NA	NA
PD7157	NA	M	MDS	NA	NA	NA
PD7158	NA	M	MDS	NA	NA	NA
PD7160	NA	M	MDS	NA	NA	NA
PD7161	NA	F	MDS	NA	NA	NA
PD7165	NA	M	MDS	NA	NA	NA
PD7166	NA	M	MDS	NA	NA	NA
PD7168	NA	M	MDS	NA	NA	NA
PD7222	NA	M	MDS	NA	NA	NA
PD7224	NA	M	MDS	NA	NA	NA
PD7225	NA	M	MDS	NA	NA	NA
PD7226	NA	NA	MDS	NA	NA	NA
PD7227	NA	M	MDS	NA	NA	NA
PD7280	NA	M	MPN	NA	NA	NA
PD7281	59	F	MPN	1	NA	NA
PD7395	NA	F	MPN	NA	NA	NA
PD7396	NA	F	MPN	NA	NA	NA
PD7398	NA	NA	MPN	NA	NA	NA
PD7399	NA	M	MPN	NA	NA	NA
PD8624	NA	NA	MPN	NA	NA	NA
PD8625	NA	F	MPN	NA	NA	NA
PD8626	NA	M	MPN	NA	NA	NA
PD8627	NA	F	MPN	NA	NA	NA
PD8628	NA	F	MPN	NA	NA	NA
PD8629	NA	F	MPN	NA	NA	NA
PD8630	NA	NA	MPN	NA	NA	NA
PD8942	NA	F	MPN	NA	NA	NA
PD8943	85	NA	MPN	1	NA	NA
PD8944	NA	F	MPN	NA	NA	NA
PD8945	NA	M	MPN	NA	NA	NA
PD8993	NA	M	MPN	NA	NA	NA
PD8994	NA	M	MPN	NA	NA	NA
PD9409	NA	M	MPN	NA	NA	NA

PD9410	NA	M	MPN	NA	NA	NA
PD9411	NA	NA	MPN	NA	NA	NA
PD9412	NA	M	MPN	NA	NA	NA
PD9415	NA	M	MPN	NA	NA	NA
PD9418	NA	F	MPN	NA	NA	NA
PD9419	NA	M	MPN	NA	NA	NA
PD9420	NA	F	MPN	NA	NA	NA
PD9423	NA	F	MPN	NA	NA	NA
S12_13	86	F	MDS	0	72	46,XX,del (5) (q22q35)[10]/46,XX[10]
S14_15	59	F	pAML	NA	NA	46,XX[17]
S18_19	NA	M	MDS	NA	NA	44,XY,-1,inv (2) (p23q11.2),-5,add (6) (p23),-12,add (12) (q24.1),-14,-16,add (22) (p11.2),+3mar[3]/45,X,-Y[3]/46,XY[16]
S22_23	NA	M	MDS	NA	NA	46,XY[20]
S24_25	78	M	MDS	NA	NA	del5q
S26_27	NA	M	MDS	0	NA	46 XY
S28_29	NA	F	MDS	0	NA	47,XX,+6[7]/46,XX[13]
S32_33	NA	F	MDS/MPN	0	NA	46, XX[20]
S36_37	NA	M	MDS	NA	NA	45-49,XY,del (5) (q22q33),-7,r (7) (p22q36),del (12) (p12),+16,-18,del (20) (q11.2), del (22) (q11.2),1-2mar
S45_46	NA	F	MDS/MPN	NA	NA	46,XX,t (1;11) (p32;q23)[2]/47,idem,+8[3]/46,XX[15]
SJAMLM7010473	0.81	M	pAML	NA	NA	47,XY,+21[9]/46,XY[21]

SJAMLM7 010475	2	M	pAML	NA	NA	47,XY,del (6) (q?16q23),ad d (7) (p22),+21[12] /46,XY[8]
SJAMLM7 010480	1	F	pAML	NA	NA	No metaphase available for analysis
SJAMLM7 010949	1	M	pAML	NA	NA	46, XY, t (9;11) (p22;q23)
SJAMLM7 010951	1	F	pAML	NA	NA	46,XX,t (8;13) (q22;q1?4)[8] /48,idem,+6,+ mar[4]/46,XX[8]
SJAMLM7 010952	1	M	pAML	NA	NA	complex
SJAMLM7 010960	2	M	pAML	NA	NA	NA
SJAMLM7 011234	0	M	pAML	NA	NA	complex (nog navragen Berna)
SJAMLM7 011236	1	F	pAML	NA	NA	38-47,XX,- 7,- 8,+8,+19[cp1 4]/46,XX[7]
SJAMLM7 011238	8	M	pAML	NA	NA	46,XY,del (12) (p11p12) [3]
SJAMLM7 011247	NA	M	pAML	NA	NA	50,XY,del (7) (p13),+8,+19, +21,+21[10]
SJAMLM7 011321	3	M	pAML	NA	NA	54,XY,t (1;22) (p12;q12),+d er (1)t (1;22),+7,+10 ,+14,+19,+21 ,+21,+21[4]/4 6,XY[21]
SJAMLM7 011324	0	M	pAML	NA	NA	47,XY,der (2),der (5),- 7,der (11),+13or14, +mar[5]/46,X Y[20]
SJAMLM7 011326	1	M	pAML	NA	NA	46,XY[15]

SJAMLM7 011327	1	M	pAML	NA	NA	46,XY,der (13) (q14),del (13) (q13q?)[5]/46 ,XY,?t (8;3) (p1?2;q1?4), del (13) (q14q?)[11]/4 6,XY[16]
SJAMLM7 011328	1	F	pAML	NA	NA	49~50,XX,+1, +6,+mar1,+, mar2[cp5]/46, XX[8]
SJAMLM7 015547	NA	M	pAML	NA	NA	NA
SJAMLM7 043	50	M	pAML	NA	NA	46,XY,t (X;1) (q28;q21)
SJAMLM7 044	34	F	pAML	NA	NA	46,XX
SJAMLM7 049	55	M	pAML	NA	NA	47,XY,+8[14]/ 46,XY[7]
SJAMLM7 050	60	M	pAML	NA	NA	46,XY
SJCBF01 6	10	M	pAML	NA	NA	48,XY,+8,inv (16) (p13.1q22),+ 22[12]/46,XY[8]
SJCBF01 7	7	M	pAML	NA	NA	46,XY,inv (16) (p13.1q22)[1 5]/46,XY[5]
SJCBF02 7	7	M	pAML	NA	NA	46,XY[13]/46, XY,inv (16) (p13q22)[7]/4 7,XY,+22,inv (16) (p13q22)[2]
SJCBF02 8	6	F	pAML	NA	NA	46,XX,t (8;21) (q22;q22)[12] /47,XX,+12,t (8;21) (q22;q22)[1]/ 48,XX,+12,t (8;21) (q22;q22),+d er (21)t (8;21) (q22;q22)[1]
SJCBF02 9	10	F	pAML	NA	NA	46,XX[2]/46, XX,t (8;21) (q22;q22),del (9)

						(q13q32)[13]/47,idem,+18[5]
SJCBF030	6	F	pAML	NA	NA	46,XX,t(8;21)(q22;q22),der(9)del(9)(p13)del(9)(q13q22)[22]/47,idem,+?18[2]
SJCBF031	6	M	pAML	NA	NA	45,X,-Y,t(8;21)(q22;q22)[18]/46,XY[2]
SJCBF033	1	M	pAML	NA	NA	Gbanding at outside lab/inv (16) confirmed
SJCBF034	15	F	pAML	NA	NA	Gbanding at outside lab/t(8;21) confirmed
SJCBF035	0	M	pAML	NA	NA	Gbanding at outside lab/inv (16) confirmed
SJCBF037	10	M	pAML	NA	NA	Gbanding at outside lab/inv (16) confirmed
SJCBF038	16	F	pAML	NA	NA	Gbanding at outside lab/inv (16) confirmed
SJCBF039	10	M	pAML	NA	NA	Gbanding at outside lab/t(8;21) confirmed
SJCBF040	12	F	pAML	NA	NA	Gbanding at outside lab/t(8;21) confirmed
SJCBF041	15	F	pAML	NA	NA	Gbanding at outside lab/inv (16) confirmed
SJCBF042	10	M	pAML	NA	NA	Gbanding at outside lab/t(8;21) confirmed
SJCBF043	7	F	pAML	NA	NA	46,XX[2]/45,X,-X,t

						(1;21;8) (p36;q11-q21;q22)[19]
SJCBF04 4	6	F	pAML	NA	NA	46,XX[1]/46,XX,t (8;21)(q22;q22)[10]
SJCBF09 0	1	F	pAML	NA	NA	46,X,t (X;22)(p22;q11.2),i nv (16) (p13.1q22)[23]
SJCBF09 1	12	F	pAML	NA	NA	46,XX,der (2)t (2;11) (p11.2;q21),t (8;21) (q22;q22), der (11)t (2;11)del (11) (p13),i (12) (q10)[20]
SJCBF09 2	9	M	pAML	NA	NA	46,XY,inv (16) (p13.1q22)[17]/47,idem,+2 2[1]/46,XY[2]
SJCBF09 3	4	M	pAML	NA	NA	46,XY,inv (16) (p13.1q22)[20]
SJCBF09 4	4	M	pAML	NA	NA	46,XY,t (8;21) (q22;q22)[19] /46,XY[1]
SJCBF09 5	7	M	pAML	NA	NA	4N,XXYY[12] /46,XY[17]/4 N,XXYY,inv (16) (p13.1q22)[12]/46,XY[17]
SJCBF09 6	3	M	pAML	NA	NA	46,XY,der (16)ins (16) (q22p13.1p13.1)del (16) (q22)[2]/92,id emx2[8]/46,X Y[10]
SJCBF09 7	6	M	pAML	NA	NA	Gbanding at outside lab/t (8;21) confirmed
SJCBF09 8	9	M	pAML	NA	NA	46,X,-Y,t (8;21)

						(q22;q22),+8[20]
SJCBF099	9	M	pAML	NA	NA	Gbanding at outside lab/t (8;21) confirmed
SJCBF100	16	F	pAML	NA	NA	45,X,-X,t (8;21) (q22;q22)[20]
SJCBF101	5	F	pAML	NA	NA	46,XX,t (8;21) (q22;q22)[20]
SJCBF102	15	F	pAML	NA	NA	Gbanding at outside lab/t (8;21) confirmed
SJCBF103	7	M	pAML	NA	NA	Gbanding at outside lab/t (8;21) confirmed
SJCBF104	17	F	pAML	NA	NA	46,XX,t (16;16) (p13.1;q22)[20]
SJCBF105	18	M	pAML	NA	NA	46,XY,inv (16) (p13.1q22)[20]
SJCBF106	1	F	pAML	NA	NA	46,XX,inv (16) (p13.1q22)[18]
SJCBF107	16	M	pAML	NA	NA	46,XY,inv (16) (p13.1q22)[17]/46,XY[3]
SJCBF108	4	M	pAML	NA	NA	45,X,-Y,t (8;21) (q22;q22)[20]
SJCBF109	15	F	pAML	NA	NA	46,XX,inv (16) (p13.1q22)[20]
SJCBF110	7	M	pAML	NA	NA	45,X,-Y,t (8;21) (q22;q22)[17]/46,XY[3]
SJCBF111	15	M	pAML	NA	NA	Gbanding at outside lab/t (8;21) confirmed
SJCBF112	8	M	pAML	NA	NA	46,XY,t (8;21)

						(q22;q22)[19] /46,XY[1]
SJCBF11 3	4	M	pAML	NA	NA	46,XY,inv (16) (p13.1q22)[1 8]/46,XY[2]
SJCBF11 4	13	M	pAML	NA	NA	Gbanding at outside lab/inv (16) confirmed
SJCBF11 6	15	M	pAML	NA	NA	Gbanding at outside lab/inv (16) confirmed
SJCBF12 0	15	F	pAML	NA	NA	46,XX,t (8;21) (q22;q22)[20]
SJCBF12 1	0	F	pAML	NA	NA	Gbanding at outside lab/inv (16) confirmed
SJCBF12 2	18	M	pAML	NA	NA	46,XY,inv (16) (p13.1q22)[1 4]/46,XY[6]
SJCBF12 3	15	M	pAML	NA	NA	Gbanding at outside lab/inv (16) confirmed
SJCBF12 4	5	M	pAML	NA	NA	Gbanding at outside lab/t (8;21) confirmed
SJCBF12 5	10	M	pAML	NA	NA	46,XY,t (8;21) (q22;q22)[4]/ 46,idem,del (2) (q37)[2]/45,id em,- Y[4]/45,idem, -Y,del (2) (q37)[8]
SJCBF13 7	4	F	pAML	NA	NA	46,XX,inv (16) (p13.1q22)[1 0]/46,XX[11]
SJCBF13 8	12	M	pAML	NA	NA	45,X,-Y,ins (8;21) (q22;q22q22) [30]/45,XY,- 7[1]

SJCBF14 0	1	M	pAML	NA	NA	46,XY,inv (16) (p13.1q22)[1 1]/46,XY[9]
SJCBF14 1	4	F	pAML	NA	NA	t (8;21)/-7/- 7Q
SJCBF14 2	15	M	pAML	NA	NA	46,X,-Y,+8,t (8;21) (q22;q22)[19] /46,XY[1]
SJCBF14 3	14	M	pAML	NA	NA	Gbanding at outside lab/t (8;21) confirmed
SJCBF14 4	17	F	pAML	NA	NA	Gbanding at outside lab/inv (16) confirmed
SJCBF14 5	14	F	pAML	NA	NA	46,XX,t (8;21) (q22;q22)[20]
SJCBF14 6	3	M	pAML	NA	NA	46,XY[8]/45, X,-Y,t (8;21) (q22;q22)[4]/ 46,idem,+der (21)t (8;21) (q22;q22)[7]
SJCBF14 7	17	F	pAML	NA	NA	46,XX,t (8;21) (q22;q22)[12] /47,XX,t (8;21) (q22;q22),+m ar[1]
SJCBF14 8	18	F	pAML	NA	NA	46,XX,inv (16) (p13q22)[19]/ 47,XX,+22,in v (16) (p13q22)[1]
SJCBF14 9	4	M	pAML	NA	NA	45,X,-Y,t (3;21;8) (q27;q22;q22)[18]/46,XY[1]
SJCBF15 0	2	F	pAML	NA	NA	Gbanding at outside lab/t (8;21) confirmed
SJCBF15 1	17	M	pAML	NA	NA	46,XY,t (16;16) (p13.1;q22)[1 9]/46,XY[1]

SJCBF15 2	4	M	pAML	NA	NA	Gbanding at outside lab/inv (16) confirmed
tAML-01	NA	M	sAML	NA	NA	NA
tAML-02	NA	F	sAML	NA	NA	NA
tAML-03	NA	M	sAML	NA	NA	NA
tAML-04	NA	M	sAML	NA	NA	NA
tAML-05	NA	M	sAML	NA	NA	NA
tAML-06	NA	M	sAML	NA	NA	NA
tAML-07	NA	F	sAML	NA	NA	NA
TARGET- PAEEYP	1	F	pAML	0	13	46,XX,del (10) (p13)[30]
TARGET- PAERAH	17	M	pAML	0	17	46,XY,inv (2) (p11.2q13)c,t (8;21) (q22;q22)[20]
TARGET- PAKKBK	1	F	pAML	0	12	46,XX,+1,der (1:16) (q10;p10),-2,- 14,+2mar[19] /46,XX[1]
TARGET- PAMYAS	10	M	pAML	1	108	46,XY[20]. nuc ish AML1-ETO positive ~30% of cells consistent with a t (8;21).
TARGET- PANGTF	16	F	pAML	NA	NA	46,XX[20]
TARGET- PANLIZ	8	M	pAML	0	26	47,XY,+8,t (8;21) (q22;q22)[13] /46,XY[7]
TARGET- PANLRE	9	M	pAML	1	61	46,XY,t (8;21) (q22;q22)[20]
TARGET- PANSBH	1.3	M	pAML	NA	NA	46,XY,ider (16) (q10)del (16) (q22)[[30]
TARGET- PANTWV	14	F	pAML	0	47	46,XX,t (6;11) (q27;q23)[20] [cryptic observed by FISH only]
TARGET- PANVGP	13	M	pAML	1	89	46,XY,t (8;20;21)

						(q22;q13.1;q22),del (9)(q22q34)[20]
TARGET-PARGVC	4	M	pAML	0	9	45,X,-Y,t(8;21)(q22;q22),add (13)(p11.2),add (22)(p11.2)
TARGET-PARIEG	13.4	F	pAML	NA	NA	47,XX,t(7;11)(p15;p15),+8[20]
TARGET-PARUCB	11	M	pAML	1	84	47,XY,+21[2]/46,XY[18]
TARGET-PARUNX	6	F	pAML	1	78	48,XX,+der(6)t(1;6)(q21;q27),t(7;12)(q36;p13),+19[18][t(7;12)nuc ish ETV6 sep]
TARGET-PARVUA	15	M	pAML	0	7	47,XY,+8[12]
TARGET-PARWAS	3.6	M	pAML	NA	NA	46,XY,t(9;11)(p22;q23),add (20)(p13)[20]
TARGET-PARWXU	1	M	pAML	1	76	46,XY,del(11)(p11.2p15)[15]/46,XY[5]
TARGET-PASCGC	14	F	pAML	0	13	46,XX,?dup(3)(q24q26.2)[4]/46,XX[12]
TARGET-PASFEW	1	F	pAML	0	10	46,XX,add(22)(q13.3)[18]/46,XX[2]
TARGET-PASRTP	14	F	pAML	1	68	46,XX[21]
TARGET-PATABB	1	M	pAML	1	55	46,XY,der(8)t(8;16)(q13;q22),der(16)inv(16)(p13.1q22)t(8;16)(q13;q22)[4]/47,idem,+22[16]

TARGET-PATELT	12	F	pAML	1	55	46,XX,t (2;11) (q31;p15)[20]
TCGA2802	50	M	pAML	0	12	46, XY[20]
TCGA2803	61	F	pAML	0	26.38356164	Normal
TCGA2804	30	M	pAML	1	84.32876712	46, XY[25]
TCGA2805	77	M	pAML	0	18.93698631	46, XY[20]
TCGA2806	46	M	pAML	0	31.35616438	t (8;21)
TCGA2807	68	F	pAML	0	5.917882192	47,XX,+21[24]
TCGA2808	23	M	pAML	1	94.62739726	47,XXY [17]
TCGA2809	64	F	pAML	0	2.383561644	46,XX[24]
TCGA2810	76	F	pAML	0	1.191788219	ND
TCGA2811	81	M	pAML	0	7.989419589	46, XY[20]
TCGA2812	25	F	pAML	0	12.32876712	46, XX[20]
TCGA2813	78	M	pAML	0	1.191788219	44-45,X,-Y,-5,add (16) (q22),-17,-18,iso (21),+mars[c p5]/82-84,XX,-Y,-3,-4,-11,-12,-19,-21,+21[cp5]
TCGA2814	39	F	pAML	0	26.38356164	46,XX,del (5) (q22q33)[4]/46,XX[16]
TCGA2815	49	M	pAML	0	26.99178822	inv (16)
TCGA2816	57	F	pAML	0	8.547945255	46,XX[20]
TCGA2817	64	M	pAML	0	8.975342466	45,XY,-7, t (9;22) (q34;q11.20) [19]/46,XY[1]
TCGA2818	62	F	pAML	0	9.961643836	46,XX[19]/46,XX,add (7) (p?22)[1]
TCGA2819	52	F	pAML	1	82.62739726	Normal,t (8;21)

TCGA282 0	76	M	pAML	0	NA	53~56,XY,+1, del (2) (q33q34),+8, +10,+11x2,+ 13x1- 2,+14,del (17) (p11.2),+19,a dd (21) (q22),+22[cp 20]
TCGA282 1	64	M	pAML	0	26.9917 8822	47,XY,+8 [10]/46,XY [10]
TCGA282 3	61	F	pAML	0	NA	t (15;17)
TCGA282 4	45	M	pAML	0	0.98631 3699	46, XY[20]
TCGA282 5	31	F	pAML	0	6.93698 6314	46, XX[20]
TCGA282 6	64	F	pAML	0	24.3287 6712	46, XX[20]
TCGA282 7	33	M	pAML	0	8.97534 2466	46,XY[18]/46, XY,del (9) (q21q33)[1]/4 6,XY,del (20) (q?12)[1]
TCGA282 8	55	M	pAML	1	75.9419 5894	NA
TCGA282 9	50	M	pAML	0	8.97534 2466	N.D.; -5q by FISH
TCGA283 0	64	F	pAML	0	9.41958 942	47,XX,i (11) (q10)[18]/46, XX [2]
TCGA283 1	59	M	pAML	0	21.4195 8941	46, XY[20]
TCGA283 2	60	F	pAML	0	12	45,X,- X[21]/46,XX[9]
TCGA283 3	77	M	pAML	0	14.9917 8822	46, XY[18]
TCGA283 4	33	M	pAML	0	8.54794 5255	46,XY,t (11;19) (q23;p13)[17] /46,XY,t (11;19) (q23;p13),inv (12) (p12p13)[3]
TCGA283 5	48	M	pAML	1	55.2739 7263	46, XY[20]
TCGA283 6	35	M	pAML	0	16.9972 6274	46, XY[20]

TCGA283 7	66	F	pAML	0	NA	46, XX[20]
TCGA283 8	67	M	pAML	0	12	46,XY,del (5) (q11.2q31),- 7,+8,+11,add (11) (q23),der (16;17) (q10;q10)[18] /51,idem,+1,+ 5,+8,+10,+21 [2]
TCGA283 9	51	F	pAML	0	15.9788 2192	46, XX[20]
TCGA284 0	74	M	pAML	0	0.92547 9453	t (15;17)
TCGA284 1	51	F	pAML	0	46.6273 9726	t (15;17)
TCGA284 2	65	M	pAML	0	NA	46,XY,del (11) (p12)[2]/46,X Y[18]
TCGA284 3	77	M	pAML	0	7.68493 1568	46,XY,del (20) (q11.2)[23]/9 2,XXYY,del (20) (q11.2)x2[2]/ 46,XY[3]
TCGA284 4	63	M	pAML	0	3.97882 1918	46,XY,t (11;19) (q23;p13)[20]
TCGA284 6	57	F	pAML	0	46.9315 6849	inv (16)
TCGA284 7	53	M	pAML	0	19.9561 6438	46,XY,del (7) (q21q36)[18]/ 46,XY[2]
TCGA284 8	62	M	pAML	0	14.9589 4196	46, XY[20]
TCGA284 9	39	M	pAML	1	72.9863 137	47,XY,del (5) (q22q33),t (10;11) (p13~p15;q2 2~23),i (17) (q10)[3]/46,X Y[17]
TCGA285 0	72	F	pAML	0	1.97262 7397	Incomplete- 46,XX[2]
TCGA285 1	66	F	pAML	0	7.95616 4384	46, XX[20]
TCGA285 2	NA	M	pAML	NA	NA	NA
TCGA285 3	51	M	pAML	0	2.89315 6849	46,XY[20]

TCGA285 4	51	F	pAML	0	12.3287 6712	45,Xc,t (5;21) (q31;q22)[18] /45,idem,t (1;6) (?q21;p23)[1] /45,Xc[1]
TCGA285 5	18	M	pAML	0	6.93698 6314	48,XY,+4,t (10;11) (p13;q21),del (12) (p11.2),+19[6] /48idem,- 17,+i (17) (q10)[14]
TCGA285 6	63	M	pAML	0	4.89863 137	46,XY[20]
TCGA285 7	54	M	pAML	0	1.62739 7263	42,XY,-5,- 7,add (12) (p13),t (14;15) (q10;q10),der (17)t (5;17) (p13;p11.2),- 18[6]/40,idem , -11,-add (12) (p13),der (12)t (?;12) (?;p13),- 19[6]/41,idem , -der (17)[3]/41,ide m,-der (17),+mar1,+ mar[3]/41,ide m,der (1)der (1) (p12)add (1) (p12),+der (1) (q21)add (1) (q21),-3,- 8[2]
TCGA285 8	75	F	pAML	0	18.9369 8631	t (8;21)
TCGA285 9	40	M	pAML	0	9.96164 3836	46,XY[20]
TCGA286 0	60	F	pAML	0	14.5479 4525	44,XX,t (4;11) (q21;q23),-5,- 7,add (12) (q24),add (18) (q23),del (20) (q12)[3]/43,X

						X,del (3) (p12),der (3)t (3;3) (p21;q2?7),p su dic (5;7) (q13;p22),- 10,-15,- 17,add (18) (p11.2),- 21,+22,+mar[cp14]/46,XX[3]
TCGA286 1	76	M	pAML	0	NA	47,XY+8 [15]/48,XY+8 +8[4]/46,XY[1]
TCGA286 2	33	F	pAML	1	47.1369 8631	46,XX[20]
TCGA286 3	63	M	pAML	0	1.19178 8219	48,XY,+8,+8[16]/46,XY[4]
TCGA286 4	54	F	pAML	0	18.9698 6314	47,XX,+8[19]/ 46,XX[2]
TCGA286 5	75	M	pAML	0	2.54794 5255	47,XY,+21[11]/48,XY,+3,+2 1[8]
TCGA286 6	67	M	pAML	0	4.99726 274	46,XY[20]
TCGA286 7	66	F	pAML	0	6.49315 6849	46,XX[20]
TCGA286 8	77	M	pAML	0	4.96438 3562	46,XY,- 5,+8,del (9) (q22),add (10) (q26),der (15;19) (q10;q10),ad d (17) (p11.2),-20,- 21,add (21) (p11),add (22) (q13),+3mar[20]
TCGA286 9	64	F	pAML	1	7.98941 9589	Normal
TCGA287 0	76	M	pAML	0	4.99726 274	Normal,inv (16)
TCGA287 1	51	M	pAML	1	5.31369 8631	Normal
TCGA287 3	51	F	pAML	1	8.97534 2466	Normal
TCGA287 4	59	M	pAML	1	13.1917 8822	45,XY,-7[20]

TCGA287 5	43	M	pAML	1	6.96986 3137	t (8;21)
TCGA287 6	45	F	pAML	1	4.76712 3288	46,XX,1~50d min[12]/46,id em,der (6)t (6;?) (q22;?)[2]/46, XX[6]
TCGA287 7	60	F	pAML	1	2.97534 2466	46,XX,9qh+[2 0]
TCGA287 8	47	F	pAML	0	12	39~47,XX,del (5) (q13q33),- 7,der (8)t (8;?8;8) (p23;?p11.2p 23;q11.2),der (14)t (1;14) (p12;p11.2)d er (1)t (7;16) (p15;q22),+2 mar[cp19]
TCGA287 9	68	F	pAML	1	8.54794 5255	46, XX[20]
TCGA288 0	24	M	pAML	0	13.9397 2627	46, XY[20]
TCGA288 1	48	F	pAML	1	12.9254 7945	Normal,inv (16)
TCGA288 2	73	F	pAML	0	12	45,XX,- 7[12]/46,XX[8 1]
TCGA288 3	60	M	pAML	1	24	52~54,XY,+2, +4,+6,+8,del (11) (q23),+19,+1 9,+21[17]/46, XY[3]
TCGA288 4	44	F	pAML	0	24.3287 6712	46, XX[19]
TCGA288 5	71	M	pAML	0	7.35616 4384	43,XY-3,del (5) (q12q33),- 7,der (10)t (10;11) (q26;q13),- 12,- 18,+2mar[20]
TCGA288 6	26	M	pAML	1	5.95684 9316	t (8;21)
TCGA288 7	60	F	pAML	0	NA	46,XX,del (7) (q11.2)[20]
TCGA288 8	57	M	pAML	1	1.98821 9179	NA
TCGA289 0	69	M	pAML	0	NA	47,XY,+9[10]/ 46,XY[10]

TCGA289 1	42	M	pAML	0	NA	45,XY,der (7) (t:7;12) (p11.1;p11.2) , -12, - 13,+mar[19]/ 46,XY[1]
TCGA289 2	42	F	pAML	1	3.96986 3137	Normal,inv (16)
TCGA289 3	45	M	pAML	0	6.96986 3137	46,XY,t (6;11) (q27;q23)[15]
TCGA289 4	50	F	pAML	0	5.91788 2192	45,XX,-7,t (9;11) (p22;q23)[19] /46,XX[1]
TCGA289 5	41	F	pAML	0	5.31369 8631	N.D.
TCGA289 6	21	F	pAML	0	7.27397 2627	46,XX[15]
TCGA289 7	50	F	pAML	1	7.98941 9589	Normal,t (15;17)
TCGA289 8	69	F	pAML	1	12.9254 7945	47,XX+8 [20]
TCGA289 9	76	F	pAML	0	22.6273 9726	46,XX+13,21[cp17]/46,XX[3]
TCGA290 0	70	M	pAML	0	6.16438 3562	46,XY[20]
TCGA290 1	27	M	pAML	1	1.96849 3157	46,XY,t (9;22) (q34;q11.2[4] /50,idem,+8,+ 10,+21,+der (22) (t (9;22) (q34;q11.2)[1 6]
TCGA290 4	65	M	pAML	0	16.9643 8356	46-50,XY,- 1,del (2),del (5) (q22q33),der (7)t (1;7) (q21;p13),ad d (7) (p11.1),+8,+a dd (11),del (12) (p12),del (13) (q22), 15,add (17) (p11.1),- 20,+22 {cp20}Compl ex Cytogenetics

						, including 5q-,+8,
TCGA290 8	81	M	pAML	0	1.19178 8219	46~49,XY,del (3) (p14),del (5) (p11.2q33),d el (17) (q21q21),add (21) (p11.2),+22, mar[cp20]
TCGA290 9	22	M	pAML	1	0.98631 3699	Normal
TCGA291 0	61	F	pAML	0	NA	47,XX,+der (5)t (2;5) (p11.2;q11.2) ?,t (8;16) (p11.2;p13.3) [19]
TCGA291 1	51	F	pAML	1	38.9917 8822	44~46,XX,del (11) (q23),der (19)?t (11;19) (q23;p13.1)[c p11]/44~45,X X,- 19[cp4]/46,X X [5]
TCGA291 3	61	M	pAML	1	39.9788 2192	Normal,Triso my 8
TCGA291 4	22	F	pAML	1	26.3835 6164	Normal,inv (16)
TCGA291 5	73	F	pAML	0	NA	46,XX,del (3) (q23q26.2),d er (7)t (1:7) (q32;q32),del (10) (q22q25),t (13;16) (q34;p11.2)d up (21) (q22)[cp20]
TCGA291 6	48	F	pAML	1	28.9972 6274	NA
TCGA291 7	41	F	pAML	1	39.9788 2192	46,XX,i (17) (q10)[1]/45,sl - 7[2]/48,sl,+13 ,+19[3]/46,XX [15]
TCGA291 9	54	F	pAML	1	2.54794 5255	Normal
TCGA292 0	44	M	pAML	0	12.3287 6712	45~46,XY,ad d (X)

						(q22)[7],Y[4], der (5)t (5;17) (q13;21)[18],- 7[18],+8[17], del (12) (q23)[16],- 17[18],add (18) (p11.2)[14][c p18]
TCGA292 1	56	F	pAML	0	NA	Normal
TCGA292 2	83	M	pAML	0	NA	Normal
TCGA292 3	78	M	pAML	0	1.19178 8219	Normal
TCGA292 4	59	M	pAML	1	2.95894 1959	Normal
TCGA292 5	57	M	pAML	0	7.95616 4384	Normal
TCGA292 8	43	F	pAML	0	1.93972 6274	45,XX,-7[20]
TCGA292 9	71	F	pAML	0	4.43835 6164	41~44,X,?i (X) (p10),- 7,der (12)t (8;12) (q11.2;p11.2) , -8 [cp11]/46,XX[8]
TCGA293 0	63	F	pAML	0	NA	Normal
TCGA293 1	75	M	pAML	0	NA	NA
TCGA293 2	62	M	pAML	1	NA	Normal
TCGA293 3	58	M	pAML	0	4.19589 4196	Normal
TCGA293 4	65	M	pAML	1	0.92547 9453	Normal
TCGA293 5	66	M	pAML	0	1.97262 7397	44~47,XY,del (5) (q22q35)[20], -7[14],- 8[6],der (12)t (10;12) (p11.2q21)[2] ,add (14) (p12)[11],- 17[13],der (17)t (10;17) (q11.2;p13)[1 4],-18[7],add

						(18) (p11.2)[7],- 21[10],i (21) (q10)[4],- 22[4],+mar[1 0],+mar1x2[6][cp20]
TCGA293 6	61	F	pAML	1	1.93972 6274	Normal
TCGA293 7	36	F	pAML	0	7.68493 1568	t (8;21)
TCGA293 8	76	M	pAML	0	9.96164 3836	Normal
TCGA293 9	72	M	pAML	1	14.9589 4196	37~49,XY,+Y ,der (1)add (1) (p13)del (1) (q21q25),- 5,der (7)inv (7) (p15q11.2)?i nv (7) (q22q32),+17 ,add (17) (p13),+21,+m ar[cp20]
TCGA294 0	35	M	pAML	0	NA	Normal
TCGA294 1	73	M	pAML	0	NA	46,XY,t (9;22) (q34;q11.2)[1 3]/34~37,ide m,-3,del (4),- 4,-5,-7,-9,- 10,t? (11;12),-12,- 14,-14,-16,- 17,- 22[cp6]/46,X Y[1]
TCGA294 2	67	F	pAML	1	21.7397 2627	Normal,inv (16)
TCGA294 3	70	F	pAML	0	NA	46,XX,del (5) (q11.2q33)[1] /48~52,idem, +1,+?del (5) (q15q33),+11 ,+11,?t (12;22) (p13;q12),- 13,-17,+i (22) (q10),+i (22) (q10),+mar[c p19]

TCGA294 4	48	M	pAML	NA	NA	45,XX-7[5]- only 5 metaphases
TCGA294 5	65	F	pAML	0	7.98941 9589	46~47,XX,+i (4) (q10)[12],- 8[5],10[3][cp2 0]
TCGA294 6	41	M	pAML	1	NA	incomplete- 46,XY,del (12) (p11.20[2]/46 ,XY[3]
TCGA294 7	52	M	pAML	1	1.19178 8219	46,XY[20]
TCGA294 8	67	M	pAML	0	19.6849 3157	46,XY[13]
TCGA294 9	58	M	pAML	1	22.9882 1918	47,XY,+22[10]/47,XY,+8[7]/ 45,XY,del (3) (p21),del (4) (p12p15),- 7,?dup (7) (q11.2q36)[3]
TCGA295 0	34	F	pAML	1	1.62739 7263	t (8;21)
TCGA295 2	60	F	pAML	0	1.19178 8219	44~47,XX,t (1;15) (q32;q26)[14] ,del (5) (q13q33)[19], - 7[20],+8[7],d el (12) (p11.2p11.2)[15],del (17) (q21)[8],der (22)t (1;22) (p13;p11.2)[2 0],+mar[13][c p20]
TCGA295 4	55	F	pAML	1	49.5254 7945	Normal
TCGA295 5	56	F	pAML	0	16.7671 2329	Normal
TCGA295 6	61	M	pAML	0	6.16438 3562	Normal,t (9;11)
TCGA295 7	31	M	pAML	1	19.9894 1959	inv (16)
TCGA295 9	71	M	pAML	0	16.7671 2329	47,XY,+13[5]/ 46,XY[15]
TS-MDS-1	NA	M	MDS	NA	NA	NA

TS-MDS-10	NA	F	MDS	NA	NA	NA
TS-MDS-2	70	M	MDS	NA	NA	NA
TS-MDS-3	78	M	MDS	NA	NA	NA
TS-MDS-4	NA	M	MDS	NA	NA	NA
TS-MDS-6	NA	M	MDS	NA	NA	NA
TS-MDS-7	NA	M	MDS	NA	NA	NA
TS-MDS-8	NA	M	MDS	NA	NA	NA
TS-MDS-9	NA	F	MDS	NA	NA	NA
TU007	NA	M	MDS	NA	NA	NA
TW009	NA	F	MDS	NA	NA	NA
TW010	NA	M	MDS	NA	NA	NA
TW011	NA	M	MDS	NA	NA	NA
TW012	NA	F	MDS	NA	NA	NA
TW013	NA	M	MDS	NA	NA	NA
TW014	NA	M	MDS	NA	NA	NA
TW015	NA	M	MDS	NA	NA	NA
TW016	NA	F	MDS	NA	NA	NA
TW018	NA	F	MDS	NA	NA	NA
TW019	NA	F	MDS	NA	NA	NA
UPN10	84	F	MDS/MPN	NA	NA	46, XX (20)
UPN11	83	M	MDS/MPN	NA	NA	normal
UPN12	73	F	MDS/MPN	NA	NA	46 XX (20)
UPN13	86	F	MDS/MPN	NA	NA	46, XX (20)
UPN14	74	F	MDS/MPN	NA	NA	45, XX, t (4; 12) (q13;q12), -7, + mar (20)
UPN15	71	F	MDS/MPN	NA	NA	46, XX, t (7; 9) (q31; q34) [18]/46, XX[5] ish t (7; 9) (wcp7+ ; wcp7+) [5/10]
UPN16	87	M	MDS/MPN	NA	NA	46, XY (20)
UPN18	74	M	MDS/MPN	NA	NA	46,XY (20)
UPN19	89	F	MDS/MPN	NA	NA	46, XY (20)
UPN2	71	M	MDS/MPN	NA	NA	46,XY (20)
UPN20	80	F	MDS/MPN	NA	NA	46,XX[20]
UPN23	67	M	MDS/MPN	NA	NA	46, XY (20)
UPN24	78	M	MDS/MPN	NA	NA	46, XY[20],ish 9 (9pter+, WCP9q+)[30]

UPN25	71	F	MDS/MPN	NA	NA	46, XX (20)
UPN26	63	F	MDS/MPN	NA	NA	46, XX[20], Ish 3q26 (EVI+)[47], Nuc ish (EVI1x2) (5'EC11 sep 3'EVI1)x1 [54/200]
UPN27	70	M	MDS/MPN	NA	NA	46,XY (22)
UPN3	85	M	MDS/MPN	NA	NA	46,XY[20]
UPN31	85	M	MDS/MPN	NA	NA	46, XY (20)
UPN33	80	M	MDS/MPN	NA	NA	45,X- Y[21]/46,XY[1]
UPN34	74	F	MDS/MPN	NA	NA	45, XX, -7, del12p (20)
UPN35	73	M	MDS/MPN	NA	NA	normal
UPN36	48	M	MDS/MPN	NA	NA	normal
UPN38	77	M	MDS/MPN	NA	NA	45,X,-Y [22/26]; 46,XY [4/26]
UPN39	88	F	MDS/MPN	NA	NA	45,X,- X[3]/46,XX[2 0]
UPN4	62	M	MDS/MPN	NA	NA	46XY (20)
UPN40	84	M	MDS/MPN	NA	NA	46,XY[28]
UPN41	66	M	MDS/MPN	NA	NA	46, XY, inv (9) (p12q13) [20/20]
UPN42	83	M	MDS/MPN	NA	NA	47,XY,+14<U +3014>11<U +3015>/46,X Y<U+3014>9 <U+3015>
UPN43	59	M	MDS/MPN	NA	NA	44,XY,del (5) (q21),add (7) (q11,2),add (8) (p21),add (10) (q22),- 13,-18,add (20) (p13),- 21x2,- 22x2,+4mar[22]/43,XY,- 4,-5,add (7) (q11,2),add (9) (p21),- 10,del (12) (p12),add

						(13) (p11,1),- 18x2,add (20) (p13),- 21,- 22x2,+5mar[9]/46,XY[1]
UPN44	77	M	MDS/MPN	NA	NA	46,XY[27]
UPN45	45	F	MDS/MPN	NA	NA	normal
UPN46	85	M	MDS/MPN	NA	NA	45, X, - Y[4]/46, XY[20]
UPN47	65	F	MDS/MPN	NA	NA	46,XX,t (1.3) (p36;q21)[20]
UPN49	71	F	MDS/MPN	NA	NA	46, XX [30]
UPN5	66	M	MDS/MPN	NA	NA	47,XY, +mar (3)/46,XY (17)
UPN6	76	M	MDS/MPN	NA	NA	normal
UPN7	73	M	MDS/MPN	NA	NA	46, XY (20)
UPN8	70	M	MDS/MPN	NA	NA	normal
UPN9	78	M	MDS/MPN	NA	NA	45X,-Y

Chapter 2-2: Raw read processing, variant calling, and quality filtering

All raw read files were first converted to fastq format, then aligned to human genome build hg19 using the Burrows-Wheeler Aligner (BWA) (Li and Durbin 2010). Aligned reads were processed using Samtools (Li, Handsaker et al. 2009), Picard tools (<http://broadinstitute.github.io/picard/>), and Genome Analysis Toolkit (GATK) before variant calling by GATK HaplotypeCaller (McKenna, Hanna et al. 2010). GATK best practices were used in processing reads and variant calling. Variants at positions with read depths below eight were omitted from downstream analysis.

Figure 2: Description of pipeline to call, annotate, and filter variants from fastq files.

Figure 2: Overview of the variant calling pipeline for this studies. Various filters such as population minor allelic frequency, somatic contamination, and etc were applied to ensure the quality of the called variants.

In order to avoid contamination of malignant cells resulting in false positive germline variants, the variant coordinates were cross-checked with the list of somatic mutations in the same patients, and any commonalities were omitted from the germline list. In addition, we discarded variants with statistically low germline variant allele frequencies (VAFs). We performed a binomial test against the null hypothesis that the variant allele count is drawn from a Binomial (n, p) distribution, where n is the read depth at the locus and p is the expected germline VAF of 50%. Variants corresponding to a Bonferroni-corrected P -value below 0.05, under the alternative hypothesis of $p < 0.5$, were discarded.

Figure 3: Filtering suspected somatic contamination

Figure 3: To omit contaminating somatic mutations, we filtered out all variants with allele frequencies (VAFs) that were less than 0.5 at a Bonferroni-corrected $P < 0.05$ significance using a binomial test.

Chapter 2-3: Variant annotation and variant/gene/patient filtering

ANNOVAR{Wang, Li et al. 2010} was used for variant annotation. Rare variants were defined as those with population allele frequencies below 1% across 1000 genomes (all, European, African, and east Asian), ESP (all, European, and African American), non-1000 genomes Haplotype Reference Consortium (HRC), and ExAC (all, African, east Asian, and non-Finnish European) databases, and frequency below 5% in our cohort. Only variants annotated as missense,

nonsense, indel, or splice site were considered in downstream analyses.

Prediction scores from six algorithms – SIFT (Ng and Henikoff 2003), Polyphen-2 (Adzhubei, Schmidt et al. 2010), MutationTaster{Schwarz, Cooper et al. 2014}, MutationAssessor{Reva, Antipin et al. 2011}, PROVEAN{Choi and Chan 2015}, and CADD{Rentzsch, Witten et al. 2018) – were each converted into categories “deleterious”, “benign”, or “uncertain”, using thresholds recommended by the developers of each tool. These six categorizations were converted into a hybrid score for each variant, a value between zero and one indicating the proportion of the tools that classify the variant as deleterious, out of those that are not “uncertain”. Each variant was also assessed according to the American College of Human Genetics (ACMG) criteria, using ClinVar {<https://www.ncbi.nlm.nih.gov/clinvar/>}. We considered a variant to be pathogenic or likely pathogenic if it was annotated as such (or as a risk factor) and with no annotation as benign, likely benign, or “conflicting interpretations”.

Our study focused solely on variants harbored within a curated list of 657 genes (Table 6). This list was assembled from multiple sources, and included genes previously associated with germline susceptibility to cancer{Rahman 2014}, [Zhang, Walsh et al. 2015], {Schrader, Cheng et al. 2016}, {Grobner, Worst et al. 2018}, {Huang, Mashl et al. 2018} (www.omim.org) and myeloid neoplasms{Godley and Shimamura 2017} (www.omim.org), genes known to be recurrently somatically mutated in myeloid malignancies {Jaiswal, Fontanillas et al. 2014}, {Papaemmanuil, Gerstung et al. 2016}, as well as DNA damage repair

and hereditary syndrome-associated genes{Scarpa, Chang et al. 2017}. Finally, to avoid duplicate or closely related patient samples, we tallied rare variant overlap and mutual exclusivity for all pairs of patients. Any pair with Jaccard index greater than 0.4 were considered duplicate or related, and one of them was discarded.

Table 7: Genes under consideration

Gene	Rationale for inclusion (PubMed ID/ website)
<i>ABCB11</i>	24429628, 29489754, 29625052
<i>ABCD1</i>	OMIM Myeloid Leukemia
<i>ABL1</i>	27276561, OMIM Myeloid Leukemia
<i>ABL2</i>	OMIM Myeloid Leukemia
<i>ACD</i>	28600339
<i>ACSL6</i>	OMIM Myeloid Leukemia
<i>ACTR5</i>	27276561
<i>ACVR1B</i>	OMIM Cancer
<i>ADH1B</i>	OMIM Cancer
<i>AIMP1</i>	OMIM Myeloid Leukemia
<i>AKT1</i>	OMIM Cancer, 26556299
<i>AKT2</i>	26556299
<i>AKT3</i>	26556299
<i>ALDH2</i>	OMIM Cancer
<i>ALK</i>	24429628, 29489754, 29625052, 26556299, 28199314, 26580448

<i>ALOX12B</i>	26556299
<i>AMER1</i>	26556299
<i>ANKRD26</i>	28600339
<i>APC</i>	OMIM Cancer, 24429628, 29489754, 29625052, 26556299, 28199314, 2658044
<i>AR</i>	OMIM Cancer, 26556299
<i>ARHGAP26</i>	27276561, OMIM Myeloid Leukemia
<i>ARID1A</i>	25426837, 26556299
<i>ARID1B</i>	26556299
<i>ASCC1</i>	OMIM Cancer
<i>ASXL1</i>	27276561, 25426837, OMIM Myeloid Leukemia, 26556299
<i>ATM</i>	OMIM Cancer, 24429628, 29489754, 29625052, OMIM Myeloid Leukemia, 26556299, 2819931
<i>ATR</i>	OMIM Cancer, 29625052, 26556299, 28199314
<i>ATRX</i>	27276561, OMIM Myeloid Leukemia, 26556299
<i>ATXN7L1</i>	27276561
<i>AURKA</i>	OMIM Cancer
<i>AXIN1</i>	OMIM Cancer, 26556299
<i>AXIN2</i>	OMIM Cancer, 24429628, 29489754, 29625052, 26556299
<i>B2M</i>	26556299
<i>BACH1</i>	28600339
<i>BAP1</i>	OMIM Cancer, 24429628, 29489754, 29625052, 26556299, 28199314, 2658044
<i>BARD1</i>	OMIM Cancer, 26556299, 28199314
<i>BAX</i>	OMIM Cancer, OMIM Myeloid Leukemia
<i>BCAP31</i>	OMIM Myeloid Leukemia

<i>BCL10</i>	OMIM Cancer, 25426837
<i>BCL11B</i>	25426837
<i>BCL2</i>	OMIM Myeloid Leukemia
<i>BCL3</i>	OMIM Myeloid Leukemia
<i>BCL6</i>	25426837
<i>BCOR</i>	27276561, 25426837, 26556299
<i>BCORL1</i>	25426837
<i>BCR</i>	OMIM Myeloid Leukemia
<i>BIRC3</i>	25426837
<i>BLM</i>	24429628, 29489754, 29625052, 26556299, 28199314
<i>BMPR1A</i>	24429628, 29489754, 29625052, 26556299, 28199314, 26580448
<i>BRAF</i>	OMIM Cancer, 27276561, 29489754, 29625052, 25426837, 26556299, 26580448
<i>BRCA1</i>	OMIM Cancer, 24429628, 28600339, 29489754, 29625052, 26556299, 28199314, 2658044
<i>BRCA2</i>	OMIM Cancer, 24429628, 28600339, 29489754, 29625052, 26556299, 28199314, 2658044
<i>BRCA3</i>	OMIM Cancer
<i>BRCC3</i>	25426837
<i>BRIP1</i>	OMIM Cancer, 24429628, 28600339, 29489754, 29625052, 26556299, 2819931
<i>BTG1</i>	25426837
<i>BTG2</i>	25426837
<i>BTK</i>	26556299
<i>BUB1</i>	OMIM Cancer
<i>BUB1B</i>	OMIM Cancer, 24429628, 29489754, 29625052, 28199314

<i>CACNA1S</i>	OMIM Cancer
<i>CALR</i>	OMIM Myeloid Leukemia
<i>CARD11</i>	25426837, 26556299
<i>CASP10</i>	OMIM Cancer
<i>CASP8</i>	OMIM Cancer, 26556299
<i>CBFB</i>	OMIM Myeloid Leukemia
<i>CBL</i>	24429628, 27276561, 29489754, 29625052, 25426837, OMIM Myeloid Leukemia, 26556299, 2658044
<i>CBLB</i>	27276561, 25426837
<i>CBLC</i>	27276561
<i>CBP</i>	29489754
<i>CCND1</i>	OMIM Cancer, OMIM Myeloid Leukemia
<i>CCND2</i>	26556299
<i>CCND3</i>	25426837
<i>CD101</i>	27276561
<i>CD58</i>	25426837
<i>CD70</i>	25426837
<i>CD79A</i>	25426837
<i>CD79B</i>	25426837, 26556299
<i>CD82</i>	OMIM Cancer
<i>CDC73</i>	OMIM Cancer, 24429628, 29489754, 29625052, 26556299, 26580448
<i>CDH1</i>	OMIM Cancer, 24429628, 27276561, 29489754, 29625052, 26556299, 28199314, 2658044
<i>CDK4</i>	OMIM Cancer, 24429628, 29489754, 29625052, 26556299, 28199314, 2658044
<i>CDKN1B</i>	24429628, 27276561, 29489754, 29625052, 26556299, 28199314

<i>CDKN1C</i>	29489754, 29625052, 26580448
<i>CDKN2A</i>	OMIM Cancer, 24429628, 27276561, 29489754, 29625052, 25426837, 26556299, 28199314, 2658044
<i>CDKN2B</i>	27276561, 25426837
<i>CEBPA</i>	24429628, 27276561, 28600339, 29489754, 29625052, 25426837, OMIM Myeloid Leukemia, 2658044
<i>CHD2</i>	25426837
<i>CHEK2</i>	OMIM Cancer, 24429628, 29489754, 29625052, 26556299, 28199314
<i>CHGA</i>	27276561
<i>CHIC2</i>	OMIM Myeloid Leukemia
<i>CHRNA3</i>	OMIM Cancer
<i>CHRNA5</i>	OMIM Cancer
<i>CMM</i>	OMIM Cancer
<i>CNOT3</i>	25426837
<i>COL7A1</i>	24429628, 29489754, 29625052
<i>CREBBP</i>	27276561, 29489754, 25426837, 26556299
<i>CRLF2</i>	25426837
<i>CRTC1</i>	OMIM Cancer
<i>CSF1R</i>	27276561, 25426837, 26556299
<i>CSF2</i>	27276561
<i>CSF3R</i>	25426837
<i>CTC1</i>	28600339
<i>CTCF</i>	25426837, 26556299
<i>CTHRC1</i>	OMIM Cancer
<i>CTLA4</i>	26556299
<i>CTNNA1</i>	27276561, 28199314

<i>CTNNB1</i>	OMIM Cancer, 26556299
<i>CTR9</i>	29625052
<i>CUL3</i>	26556299
<i>CUX1</i>	27276561, 25426837
<i>CXCR4</i>	OMIM Myeloid Leukemia
<i>CYLD</i>	24429628, 29489754, 29625052
<i>CYP2A6</i>	OMIM Cancer
<i>DARS</i>	OMIM Myeloid Leukemia
<i>DCC</i>	OMIM Cancer
<i>DDB2</i>	24429628, 29489754, 29625052
<i>DDR2</i>	26556299
<i>DDX18</i>	27276561
<i>DDX3X</i>	25426837
<i>DDX41</i>	28600339, OMIM Myeloid Leukemia
<i>DEC1</i>	OMIM Cancer
<i>DEK</i>	OMIM Myeloid Leukemia
<i>DHCR7</i>	29489754
<i>DICER1</i>	OMIM Cancer, 24429628, 29489754, 29625052, 26556299, 28199314, 26580448
<i>DIRC2</i>	OMIM Cancer
<i>DIS3</i>	25426837
<i>DIS3L2</i>	24429628, 29489754, 29625052, 28199314
<i>DKC1</i>	24429628, 28600339, 29489754, 29625052
<i>DLC1</i>	OMIM Cancer
<i>DLEC1</i>	OMIM Cancer
<i>DNMT1</i>	27276561, 26556299

<i>DNMT3A</i>	27276561, 25426837, 26556299
<i>DNMT3B</i>	26556299
<i>DOCK8</i>	24429628, 29489754, 29625052
<i>DROSHA</i>	29625052
<i>EBF1</i>	25426837
<i>EED</i>	25426837
<i>EGFR</i>	OMIM Cancer, 24429628, 27276561, 29489754, 29625052, 26556299, 2819931
<i>EGR2</i>	OMIM Myeloid Leukemia
<i>EHBP1</i>	OMIM Cancer
<i>ELAC2</i>	OMIM Cancer
<i>ELANE</i>	24429628, 28600339, 29489754, 29625052
<i>ELF1</i>	27276561
<i>EP300</i>	OMIM Cancer, 27276561, 29489754, 25426837, 26556299
<i>EPCAM</i>	OMIM Cancer, 29489754, 29625052, 26556299, 28199314, 26580448
<i>EPHB2</i>	OMIM Cancer
<i>ERBB2</i>	OMIM Cancer
<i>ERBB3</i>	26556299
<i>ERBB4</i>	26556299
<i>ERCC1</i>	29489754, 29625052
<i>ERCC2</i>	24429628, 29489754, 29625052, 26556299, 28199314
<i>ERCC3</i>	24429628, 29489754, 29625052, 26556299, 28199314
<i>ERCC4</i>	24429628, 28600339, 29489754, 29625052, 26556299, 28199314
<i>ERCC5</i>	24429628, 29489754, 29625052, 26556299, 28199314
<i>ERCC6</i>	OMIM Cancer
<i>ERG</i>	27276561

<i>ESR1</i>	OMIM Cancer, 26556299
<i>ETV6</i>	27276561, 28600339, 29625052, 25426837, OMIM Myeloid Leukemia, 26556299
<i>EXT1</i>	24429628, 29489754, 29625052, 28199314
<i>EXT2</i>	24429628, 29489754, 29625052, 28199314
<i>EZH2</i>	27276561, 25426837, 26556299
<i>EZR</i>	25426837
<i>FAH</i>	24429628, 29489754, 29625052
<i>FAM126A</i>	OMIM Myeloid Leukemia
<i>FAM175A</i>	26556299, 28199314
<i>FAM175B</i>	27276561
<i>FAM46C</i>	25426837
<i>FANCA</i>	24429628, 28600339, 29489754, 29625052, 26556299, 28199314
<i>FANCB</i>	28600339, 28199314
<i>FANCC</i>	24429628, 28600339, 29489754, 29625052, 26556299, 28199314
<i>FANCD1</i>	28600339
<i>FANCD2</i>	28600339, 29489754, 29625052, 28199314
<i>FANCDE</i>	28600339
<i>FANCE</i>	29489754, 29625052, 28199314
<i>FANCF</i>	28600339, 29489754, 29625052, 28199314
<i>FANCG</i>	24429628, 28600339, 29489754, 29625052, 28199314
<i>FANCI</i>	28600339, 29489754, 29625052, 28199314
<i>FANCJ</i>	28600339
<i>FANCL</i>	28600339, 29489754, 29625052, 28199314
<i>FANCM</i>	28600339, 29489754, 29625052, 28199314
<i>FANCN</i>	28600339

<i>FANCO</i>	28600339
<i>FANCP</i>	28600339
<i>FANCQ</i>	28600339
<i>FANCR</i>	28600339
<i>FANCS</i>	28600339
<i>FANCT</i>	28600339
<i>FANCU</i>	28600339
<i>FANCV</i>	28600339
<i>FAND2</i>	29489754
<i>FAS</i>	OMIM Cancer, 24429628, 25426837
<i>FASLG</i>	OMIM Cancer
<i>FBXO11</i>	25426837
<i>FBXW7</i>	27276561, 25426837
<i>FGF23</i>	OMIM Cancer
<i>FGF3</i>	26556299
<i>FGFR1</i>	26556299
<i>FGFR1OP</i>	OMIM Myeloid Leukemia
<i>FGFR2</i>	OMIM Cancer, 29489754, 26556299
<i>FGFR3</i>	OMIM Cancer, 29489754, 26556299
<i>FGFR4</i>	OMIM Cancer
<i>FH</i>	OMIM Cancer, 24429628, 29489754, 29625052, 26556299, 28199314, 2658044
<i>FLCN</i>	OMIM Cancer, 24429628, 29489754, 29625052, 26556299
<i>FLT3</i>	27276561, 25426837, OMIM Myeloid Leukemia
<i>FLT4</i>	26556299
<i>FMR1</i>	29489754

<i>FOXE1</i>	OMIM Cancer
<i>FOXL2</i>	26556299
<i>FOXP1</i>	25426837, 26556299
<i>FYN</i>	25426837
<i>GALNT12</i>	OMIM Cancer
<i>GALNT3</i>	OMIM Cancer
<i>GATA1</i>	27276561, 28600339, 25426837, OMIM Myeloid Leukemia, 26556299
<i>GATA2</i>	24429628, 27276561, 28600339, 29489754, 29625052, 25426837, OMIM Myeloid Leukemia, 26556299, 28199314, 2658044
<i>GATA3</i>	25426837, 26556299
<i>GBA</i>	24429628, 29489754, 29625052
<i>GJB2</i>	24429628, 29489754, 29625052
<i>GJC2</i>	OMIM Myeloid Leukemia
<i>GLI3</i>	29489754
<i>GNA11</i>	26556299
<i>GNA13</i>	25426837
<i>GNAQ</i>	26556299
<i>GNAS</i>	27276561, 25426837, 26556299
<i>GNAS1</i>	29489754
<i>GNB1</i>	25426837, OMIM Myeloid Leukemia
<i>GPC3</i>	OMIM Cancer, 24429628, 29489754, 29625052
<i>GREM1</i>	26556299
<i>GRIN2A</i>	26556299
<i>H19</i>	OMIM Cancer, 29489754
<i>HABP2</i>	OMIM Cancer
<i>HAX1</i>	28600339

<i>HFE</i>	24429628, 29489754, 29625052
<i>HGF</i>	26556299
<i>HIKESHI</i>	OMIM Myeloid Leukemia
<i>HIP1</i>	OMIM Cancer
<i>HIPK2</i>	27276561
<i>HIST1H1B</i>	25426837
<i>HIST1H1C</i>	25426837
<i>HIST1H1D</i>	25426837
<i>HIST1H1E</i>	25426837
<i>HIST1H3B</i>	25426837
<i>HLAVA</i>	25426837
<i>HMBS</i>	24429628, 29489754, 29625052
<i>HMGA2</i>	27276561
<i>HMMR</i>	OMIM Cancer
<i>HNF1A</i>	OMIM Cancer, 29625052, 26556299
<i>HNF1B</i>	OMIM Cancer
<i>HOXB13</i>	28199314
<i>HPCX</i>	OMIM Cancer
<i>HRAS</i>	OMIM Cancer, 24429628, 27276561, 29489754, 29625052, 26556299, 2658044
<i>HSPD1</i>	OMIM Myeloid Leukemia
<i>ID3</i>	25426837
<i>IDH1</i>	27276561, 25426837, 26556299
<i>IDH2</i>	27276561, 25426837, 26556299
<i>IFNGR1</i>	26556299
<i>IGF1</i>	26556299

<i>IGF1R</i>	26556299
<i>IGF2R</i>	OMIM Cancer
<i>IKBKB</i>	25426837
<i>IKBKG</i>	OMIM Cancer
<i>IKZF1</i>	27276561, 25426837
<i>IKZF2</i>	25426837
<i>IKZF3</i>	25426837
<i>IL1B</i>	OMIM Cancer
<i>IL1RN</i>	OMIM Cancer
<i>IL7R</i>	25426837, 26556299
<i>ING1</i>	OMIM Cancer
<i>INSR</i>	26556299
<i>INTS12</i>	25426837
<i>INVS</i>	27276561
<i>IRAK4</i>	OMIM Cancer
<i>IRF1</i>	OMIM Cancer, 27276561, OMIM Myeloid Leukemia
<i>IRF4</i>	25426837
<i>IRF8</i>	25426837
<i>ITK</i>	24429628, 29489754, 29625052
<i>JAK1</i>	25426837
<i>JAK2</i>	27276561, 25426837, OMIM Myeloid Leukemia, 26556299
<i>JAK3</i>	27276561, 25426837, 26556299
<i>JARID2</i>	25426837
<i>JMJD1C</i>	29625052
<i>KCNQ1OT1</i>	29489754
<i>KDM2B</i>	27276561

<i>KDM5A</i>	27276561
<i>KDM5C</i>	26556299
<i>KDM6A</i>	27276561, 25426837, 26556299
<i>KDR</i>	26556299
<i>KDSR</i>	OMIM Myeloid Leukemia
<i>KIT</i>	OMIM Cancer, 24429628, 27276561, 29489754, 29625052, 25426837, OMIM Myeloid Leukemia, 26556299, 2819931
<i>KL</i>	OMIM Cancer
<i>KLF6</i>	OMIM Cancer
<i>KLHL6</i>	25426837
<i>KLLN</i>	28199314
<i>KMT2A</i>	27276561, OMIM Myeloid Leukemia, 26556299
<i>KMT2C</i>	27276561
<i>KMT2D</i>	27276561, 26556299
<i>KMT2E</i>	27276561
<i>KRAS</i>	OMIM Cancer, 27276561, 29489754, 29625052, 25426837, OMIM Myeloid Leukemia, 26556299, 2658044
<i>L2HGDH</i>	29489754
<i>LCORL</i>	27276561
<i>LEF1</i>	OMIM Cancer, 25426837
<i>LIG4</i>	OMIM Myeloid Leukemia
<i>LILRA3</i>	27276561
<i>LMO1</i>	29625052, OMIM Myeloid Leukemia
<i>LMO2</i>	OMIM Myeloid Leukemia
<i>LPP</i>	OMIM Myeloid Leukemia
<i>LRRK2</i>	25426837

<i>LTB</i>	25426837
<i>LUC7L2</i>	25426837
<i>LYL1</i>	OMIM Myeloid Leukemia
<i>LZTR1</i>	29489754
<i>LZTS1</i>	OMIM Cancer
<i>MAD1L1</i>	OMIM Cancer
<i>MALT1</i>	25426837
<i>MAML2</i>	OMIM Cancer
<i>MAP2K1</i>	29489754, 29625052, 25426837, 26556299, 26580448
<i>MAP2K2</i>	29489754, 29625052, 26556299, 26580448
<i>MAP2K5</i>	27276561
<i>MAP3K1</i>	26556299
<i>MAP3K14</i>	25426837
<i>MAP3K8</i>	OMIM Cancer
<i>MAX</i>	24429628, 29489754, 29625052, 26556299, 28199314, 26580448
<i>MC1R</i>	OMIM Cancer
<i>MCC</i>	OMIM Cancer
<i>MDM2</i>	OMIM Cancer
<i>MECOM</i>	27276561
<i>MED12</i>	25426837, 26556299
<i>MEF2B</i>	25426837
<i>MEN1</i>	OMIM Cancer, 24429628, 29489754, 29625052, 26556299, 28199314, 2658044
<i>MET</i>	OMIM Cancer, 24429628, 27276561, 29489754, 29625052, 26556299, 2819931
<i>MFHAS1</i>	OMIM Cancer

<i>MHS2</i>	OMIM Cancer
<i>MHS4</i>	OMIM Cancer
<i>MHS6</i>	OMIM Cancer
<i>MINPP1</i>	OMIM Cancer
<i>MITF</i>	OMIM Cancer, 29489754, 29625052, 26556299, 28199314
<i>MKL1</i>	OMIM Myeloid Leukemia
<i>MLF1</i>	OMIM Myeloid Leukemia
<i>MLH1</i>	OMIM Cancer, 24429628, 28600339, 29489754, 29625052, 26556299, 28199314, 2658044
<i>MLH3</i>	OMIM Cancer
<i>MLL</i>	25426837
<i>MLL2</i>	25426837
<i>MLLT10</i>	OMIM Myeloid Leukemia
<i>MMD2</i>	27276561
<i>MMR</i>	29489754
<i>MN1</i>	27276561
<i>MPL</i>	27276561, 29625052, 25426837, OMIM Myeloid Leukemia, 26556299, 28199314
<i>MPO</i>	OMIM Cancer, OMIM Myeloid Leukemia
<i>MPZ</i>	OMIM Myeloid Leukemia
<i>MRE11A</i>	29489754, 26556299, 28199314
<i>MSH2</i>	OMIM Cancer, 24429628, 28600339, 29489754, 29625052, 26556299, 28199314, 2658044
<i>MSH3</i>	OMIM Cancer
<i>MSH6</i>	OMIM Cancer, 24429628, 28600339, 29489754, 29625052, 26556299, 28199314, 2658044

<i>MSMB</i>	OMIM Cancer
<i>MSR1</i>	OMIM Cancer
<i>MST1R</i>	OMIM Cancer
<i>MTAP</i>	OMIM Cancer, 24429628, 27276561, 29489754, 29625052
<i>MUTYH</i>	OMIM Cancer, 24429628, 29489754, 29625052, 26556299, 28199314
<i>MXI1</i>	OMIM Cancer
<i>MXRA5</i>	25426837
<i>MYB</i>	OMIM Myeloid Leukemia
<i>MYC</i>	27276561
<i>MYCN</i>	26556299
<i>MYD88</i>	25426837, 26556299
<i>NAF1</i>	28600339
<i>NBN</i>	24429628, 29489754, 29625052, OMIM Myeloid Leukemia, 26556299, 28199314
<i>NBS1</i>	29489754
<i>NDUFA13</i>	OMIM Cancer
<i>NEK1</i>	28199314
<i>NF1</i>	24429628, 27276561, 29489754, 29625052, OMIM Myeloid Leukemia, 26556299, 28199314, 2658044
<i>NF2</i>	24429628, 29489754, 29625052, 26556299, 28199314, 26580448
<i>NFIX1</i>	29489754
<i>NHP2</i>	28600339, 29489754, 29625052
<i>NKX2-1</i>	OMIM Cancer, 26556299
<i>NLRP1</i>	27276561
<i>NOLA2</i>	28600339
<i>NOLA3</i>	28600339

<i>NOP10</i>	28600339, 29489754, 29625052
<i>NOTCH1</i>	27276561, 25426837, 26556299
<i>NOTCH2</i>	25426837, 26556299
<i>NOTCH3</i>	26556299
<i>NPM1</i>	27276561, 25426837, OMIM Myeloid Leukemia
<i>NQO1</i>	OMIM Cancer, OMIM Myeloid Leukemia
<i>NQO2</i>	OMIM Cancer
<i>NR5A1</i>	27276561
<i>NRAS</i>	OMIM Cancer, 27276561, 29489754, 29625052, 25426837, 26556299, 2658044
<i>NRDC</i>	27276561
<i>NSD1</i>	27276561, 29489754, OMIM Myeloid Leukemia, 26556299
<i>NTHL1</i>	29625052
<i>NTRK1</i>	OMIM Cancer, 29489754, 26556299, 28199314
<i>NTRK2</i>	26556299
<i>NUMA1</i>	OMIM Myeloid Leukemia
<i>NUP214</i>	OMIM Myeloid Leukemia
<i>NUP98</i>	27276561
<i>OCA2</i>	27276561
<i>OGG1</i>	OMIM Cancer
<i>OPCML</i>	OMIM Cancer
<i>P2RY8</i>	25426837
<i>PALB2</i>	OMIM Cancer, 24429628, 28600339, 29489754, 29625052, 26556299, 28199314, 2658044
<i>PALLD</i>	OMIM Cancer
<i>PAPD5</i>	25426837

<i>PARK2</i>	OMIM Cancer, 26556299
<i>PARN</i>	28600339
<i>PAX5</i>	29489754, 29625052, 25426837, OMIM Myeloid Leukemia, 26556299, 26580448
<i>PAX5</i>	29489754, 29625052, 25426837, OMIM Myeloid Leukemia, 26556299, 26580448
<i>PBX1</i>	OMIM Myeloid Leukemia
<i>PCAP</i>	OMIM Cancer
<i>PDCD1</i>	26556299
<i>PDGFRA</i>	OMIM Cancer, 24429628, 27276561, 29489754, 29625052, 26556299
<i>PDGFRB</i>	OMIM Myeloid Leukemia, 26556299
<i>PDGFRL</i>	OMIM Cancer
<i>PDS5B</i>	25426837
<i>PDSS2</i>	25426837
<i>PHB</i>	OMIM Cancer
<i>PHF12</i>	27276561
<i>PHF6</i>	27276561, 25426837
<i>PHOX2B</i>	24429628, 29489754, 29625052, 26556299, 28199314, 26580448
<i>PICALM</i>	OMIM Myeloid Leukemia
<i>PIK3CA</i>	OMIM Cancer, 25426837, 26556299
<i>PIK3CD</i>	26556299
<i>PIK3R1</i>	26556299
<i>PIK3R2</i>	26556299
<i>PKP3</i>	27276561
<i>PLA2G2A</i>	OMIM Cancer
<i>PML</i>	OMIM Myeloid Leukemia

<i>PMP22</i>	OMIM Myeloid Leukemia
<i>PMS1</i>	29625052, 26556299, 28199314
<i>PMS2</i>	OMIM Cancer, 24429628, 29489754, 29625052, 26556299, 28199314, 2658044
<i>POLD1</i>	OMIM Cancer, 24429628, 29489754, 29625052, 28199314
<i>POLE</i>	OMIM Cancer, 24429628, 29489754, 29625052, 26556299, 2819931
<i>POLH</i>	24429628, 29489754, 29625052
<i>POLR1C</i>	OMIM Myeloid Leukemia
<i>POLR3A</i>	OMIM Myeloid Leukemia
<i>POLR3B</i>	OMIM Myeloid Leukemia
<i>POT1</i>	OMIM Cancer, 29625052, 25426837
<i>POU2AF1</i>	25426837
<i>POU2F2</i>	25426837
<i>POU6F2</i>	OMIM Cancer
<i>PPM1D</i>	OMIM Cancer
<i>PPP2R1B</i>	OMIM Cancer
<i>PRCC</i>	OMIM Cancer
<i>PRDM1</i>	25426837
<i>PRDM9</i>	29625052
<i>PRDX2</i>	27276561
<i>PRF1</i>	29625052, 28199314
<i>PRKAR1A</i>	OMIM Cancer, 24429628, 29489754, 29625052, 26556299, 28199314, 26580448
<i>PRKCA</i>	OMIM Cancer
<i>PRPF40B</i>	27276561, 25426837
<i>PRPF8</i>	25426837

<i>PRSS1</i>	24429628, 29489754, 29625052
<i>PTCH1</i>	OMIM Cancer, 24429628, 29489754, 29625052, 26556299, 28199314, 2658044
<i>PTCH2</i>	OMIM Cancer
<i>PTEN</i>	OMIM Cancer, 24429628, 27276561, 29489754, 29625052, 25426837, 26556299, 2658044
<i>PTHLH</i>	OMIM Cancer
<i>PTPN1</i>	25426837
<i>PTPN11</i>	24429628, 27276561, 29489754, 29625052, 25426837, OMIM Myeloid Leukemia, 26556299, 2658044
<i>PTPN12</i>	OMIM Cancer
<i>PTPRJ</i>	OMIM Cancer
<i>PYCR2</i>	OMIM Myeloid Leukemia
<i>RAD21</i>	27276561, 25426837
<i>RAD50</i>	27276561, 26556299, 28199314
<i>RAD51</i>	OMIM Cancer, 28600339, 29489754, 26556299
<i>RAD51B</i>	26556299
<i>RAD51C</i>	OMIM Cancer, 24429628, 28600339, 29489754, 29625052, 26556299, 2819931
<i>RAD51D</i>	OMIM Cancer, 24429628, 29489754, 29625052, 26556299, 28199314
<i>RAD54B</i>	OMIM Cancer
<i>RAD54L</i>	OMIM Cancer, 28199314
<i>RAF1</i>	29489754, 29625052, 26556299, 26580448
<i>RAG1</i>	OMIM Cancer
<i>RAG2</i>	OMIM Cancer
<i>RAP1GDS1</i>	OMIM Myeloid Leukemia

<i>RARA</i>	OMIM Myeloid Leukemia
<i>RARS</i>	OMIM Myeloid Leukemia
<i>RASA1</i>	OMIM Cancer, 26556299
<i>RASSF1</i>	OMIM Cancer
<i>RB1</i>	OMIM Cancer, 24429628, 27276561, 29489754, 29625052, 26556299, 28199314, 2658044
<i>RB1CC1</i>	OMIM Cancer
<i>RBBP4</i>	25426837
<i>RBBP8</i>	OMIM Cancer
<i>RBM10</i>	26556299
<i>RBM15</i>	OMIM Myeloid Leukemia
<i>RECQL</i>	29625052
<i>RECQL3</i>	29489754
<i>RECQL4</i>	24429628, 29489754, 29625052, 26556299, 28199314
<i>REST</i>	OMIM Cancer
<i>RET</i>	OMIM Cancer, 24429628, 29489754, 29625052, 26556299, 28199314, 2658044
<i>REV7</i>	28600339
<i>RHBDF2</i>	OMIM Cancer, 24429628, 29489754, 29625052
<i>RHOA</i>	25426837
<i>RINT1</i>	27276561
<i>RIT1</i>	25426837, 26556299
<i>RMRP</i>	24429628, 29489754, 29625052
<i>RNASEL</i>	OMIM Cancer
<i>RNF139</i>	OMIM Cancer
<i>RNF43</i>	OMIM Cancer

<i>RNF6</i>	OMIM Cancer
<i>RORC</i>	27276561
<i>RPL10</i>	25426837
<i>RPL11</i>	28600339
<i>RPL15</i>	28600339
<i>RPL23</i>	28600339
<i>RPL26</i>	28600339
<i>RPL27</i>	28600339
<i>RPL31</i>	28600339
<i>RPL35A</i>	28600339
<i>RPL36</i>	28600339
<i>RPL5</i>	28600339, 25426837
<i>RPS10</i>	28600339
<i>RPS15</i>	28600339, 25426837
<i>RPS17</i>	28600339
<i>RPS19</i>	28600339
<i>RPS2</i>	25426837
<i>RPS24</i>	28600339
<i>RPS26</i>	28600339
<i>RPS27</i>	28600339
<i>RPS27A</i>	28600339
<i>RPS28</i>	28600339
<i>RPS29</i>	28600339
<i>RPS7</i>	28600339
<i>RRAS2</i>	OMIM Cancer
<i>RSPO1</i>	OMIM Cancer

<i>RTEL1</i>	28600339, 28199314
<i>RUNX1</i>	OMIM Cancer, 24429628, 27276561, 28600339, 29489754, 29625052, 25426837, OMIM Myeloid Leukemia, 26556299, 2658044
<i>RUNX1T1</i>	27276561
<i>RYR1</i>	OMIM Cancer
<i>SAMD9</i>	OMIM Cancer, 28600339
<i>SAMD9L</i>	28600339
<i>SBDS</i>	24429628, 28600339, 29489754, 29625052, 28199314
<i>SDHA</i>	24429628, 29489754, 29625052, 26556299, 28199314, 26580448
<i>SDHAF2</i>	24429628, 29489754, 29625052, 26556299, 26580448
<i>SDHB</i>	OMIM Cancer, 24429628, 29489754, 29625052, 26556299, 28199314, 2658044
<i>SDHC</i>	OMIM Cancer, 24429628, 29489754, 29625052, 26556299, 28199314, 2658044
<i>SDHD</i>	OMIM Cancer, 24429628, 29489754, 29625052, 26556299, 28199314, 2658044
<i>SEPT9</i>	OMIM Myeloid Leukemia, OMIM Cancer
<i>SERPINA1</i>	24429628, 29489754, 29625052
<i>SETBP1</i>	29489754, 29625052
<i>SF1</i>	27276561
<i>SF3A1</i>	27276561, 25426837
<i>SF3B1</i>	27276561, 25426837, OMIM Myeloid Leukemia
<i>SFRS2</i>	25426837
<i>SGK1</i>	25426837
<i>SH2B3</i>	27276561, 29489754, 29625052, OMIM Myeloid Leukemia
<i>SH2D1A</i>	24429628, 29489754, 29625052, 26556299

<i>SH3GL1</i>	OMIM Myeloid Leukemia
<i>SHOC2</i>	29489754, 29625052, 26580448
<i>SLC22A18</i>	OMIM Cancer
<i>SLC25A13</i>	24429628, 29489754, 29625052
<i>SLX4</i>	28600339, 29489754, 28199314
<i>SMAD3</i>	26556299
<i>SMAD4</i>	OMIM Cancer, 24429628, 29489754, 29625052, 26556299, 28199314, 2658044
<i>SMAD7</i>	OMIM Cancer
<i>SMARCA2</i>	29489754
<i>SMARCA4</i>	OMIM Cancer, 24429628, 29489754, 29625052, 26556299, 26580448
<i>SMARCB1</i>	OMIM Cancer, 24429628, 29489754, 29625052, 26556299, 26580448
<i>SMARCE1</i>	24429628, 29489754, 29625052
<i>SMC1A</i>	25426837
<i>SMC3</i>	25426837
<i>SMO</i>	OMIM Cancer
<i>SOCS1</i>	27276561, 25426837
<i>SOS1</i>	24429628, 29489754, 29625052, 26580448
<i>SOX17</i>	26556299
<i>SOX2</i>	26556299
<i>SOX9</i>	26556299
<i>SPI1</i>	27276561
<i>SPRTN</i>	29625052
<i>SPRY4</i>	25426837
<i>SRC</i>	OMIM Cancer
<i>SRGAP1</i>	OMIM Cancer

<i>SRP72</i>	28600339
<i>SRPK2</i>	27276561
<i>SRSF2</i>	27276561
<i>SRY</i>	24429628, 29489754, 29625052
<i>ST11</i>	OMIM Cancer
<i>ST3</i>	OMIM Cancer
<i>STAG1</i>	25426837
<i>STAG2</i>	27276561, 25426837
<i>STAT3</i>	24429628, 29489754, 29625052, 25426837
<i>STAT5A</i>	25426837
<i>STAT5B</i>	25426837, OMIM Myeloid Leukemia
<i>STAT6</i>	25426837
<i>STK11</i>	OMIM Cancer, 24429628, 29489754, 29625052, 26556299, 28199314, 2658044
<i>STK17B</i>	27276561
<i>STN1</i>	28600339
<i>SUFU</i>	24429628, 29489754, 29625052, 26556299, 28199314, 26580448
<i>SUZ12</i>	25426837
<i>SWAP70</i>	25426837
<i>TAL1</i>	OMIM Myeloid Leukemia
<i>TAL2</i>	OMIM Myeloid Leukemia
<i>TBL1XR1</i>	25426837
<i>TBX3</i>	26556299
<i>TCAB1</i>	28600339
<i>TCF3</i>	25426837
<i>TCF4</i>	27276561

<i>TCL1A</i>	OMIM Myeloid Leukemia
<i>TCL1B</i>	OMIM Myeloid Leukemia
<i>TCL4</i>	OMIM Myeloid Leukemia
<i>TERC</i>	28600339
<i>TERT</i>	OMIM Cancer, 24429628, 28600339, 29489754, 29625052, OMIM Myeloid Leukemia, 26556299, 2819931
<i>TET1</i>	27276561, 25426837
<i>TET2</i>	27276561, 25426837, OMIM Myeloid Leukemia
<i>TFAP2A</i>	29489754
<i>TFE3</i>	OMIM Cancer
<i>TGCT1</i>	OMIM Cancer
<i>TGFBR1</i>	24429628, 29489754, 29625052, 26556299
<i>TGFBR2</i>	OMIM Cancer, 26556299
<i>TINF2</i>	28600339
<i>TIRAP</i>	OMIM Cancer
<i>TLR2</i>	OMIM Cancer
<i>TMEM127</i>	24429628, 29489754, 29625052, 26556299, 26580448
<i>TMEM30A</i>	25426837
<i>TNF</i>	25426837
<i>TNFAIP3</i>	25426837
<i>TNFRSF10B</i>	OMIM Cancer
<i>TNFRSF14</i>	25426837
<i>TNFRSF6</i>	29489754, 29625052
<i>TP53</i>	OMIM Cancer, 24429628, 27276561, 28600339, 29489754, 29625052, 25426837, 26556299, 28199314, 2658044

<i>TP63</i>	26556299
<i>TPMS2</i>	28600339
<i>TPP1</i>	28600339
<i>TRAF3</i>	25426837
<i>TRIM37</i>	24429628, 29489754
<i>TRIM37</i>	24429628, 29489754
<i>TSC1</i>	24429628, 29489754, 29625052, 26556299, 28199314, 26580448
<i>TSC2</i>	24429628, 29489754, 29625052, 26556299, 28199314, 26580448
<i>TSG101</i>	OMIM Cancer
<i>TSHR</i>	OMIM Cancer, 29625052, 26556299, 28199314
<i>TSR2</i>	28600339
<i>TUBB4A</i>	OMIM Myeloid Leukemia
<i>TWIST1</i>	OMIM Myeloid Leukemia
<i>TYR</i>	OMIM Cancer
<i>TYW1</i>	25426837
<i>U2AF1</i>	27276561, 25426837
<i>U2AF2</i>	27276561, 25426837
<i>UBE2T</i>	28600339
<i>UBR5</i>	25426837
<i>UFSP2</i>	OMIM Myeloid Leukemia
<i>UROD</i>	24429628, 29489754, 29625052
<i>VHL</i>	OMIM Cancer, 24429628, 29489754, 29625052, 26556299, 28199314
<i>VHL1</i>	26580448
<i>VPS11</i>	OMIM Myeloid Leukemia
<i>WAS</i>	24429628, 29489754, 29625052, 28199314
<i>WHSC1L1</i>	OMIM Myeloid Leukemia

<i>WRAP53</i>	28600339
<i>WRN</i>	24429628, 29489754, 29625052, 28199314
<i>WT1</i>	OMIM Cancer, 24429628, 27276561, 29489754, 29625052, 25426837, 26556299, 2658044
<i>WWOX</i>	OMIM Cancer
<i>XBP1</i>	25426837
<i>XIAP</i>	26556299
<i>XPA</i>	24429628, 29489754, 29625052, 28199314
<i>XPC</i>	24429628, 29489754, 29625052, 28199314
<i>XPO1</i>	25426837
<i>XRCC2</i>	28600339, 28199314
<i>XRCC3</i>	OMIM Cancer, 29489754
<i>YAP1</i>	26556299
<i>ZBTB16</i>	OMIM Myeloid Leukemia
<i>ZEB2</i>	27276561
<i>ZFHX3</i>	OMIM Cancer
<i>ZNF471</i>	25426837
<i>ZRSR2</i>	27276561, 25426837

After these filters were applied, we were left with 5,880 rare variants (3,934 unique) in the cohort, among which the vast majority (93.4%) were missense, with 2.6% in-frame indels, 2.6% truncating, and 0.9% splice site variants (Figure 5).

Figure 4: Overview of patient and variant characteristics.

Figure 4: (A) Distribution of rare variant effects on proteins. (B) Distribution of rare germline variant burden by patient. (C) Distribution of rare germline variant burden by gene.

Chapter 2-4: Filtering putative compound heterozygotes and rare homozygotes

For compound heterozygotes, we first extracted all variant pairs that appeared in the same gene and patient. We next computed the linkage disequilibrium measure r for each pair, using LDlink (Machiela and Chanock 2018), removing any pair with $r > 0$ and $r^2 > 0.1$ as likely being *in cis*. Finally, we manually examined reads using IGV (Robinson, Thorvaldsdottir et al. 2011). Any pairs of variants appearing on the same fragment were removed as being *in cis*. Since standard variant-calling algorithms often have difficulty accurately determining

the alleles in long insertions and deletions polymorphisms, we omitted long (> 9 bp) indels from consideration when querying for rare allele homozygotes and compound heterozygotes.

Chapter 2-5: Statistical testing

All statistical testing was performed using the R software. To determine the null frequency of compound heterozygotes and rare allele homozygotes in a gene with n rare variants across the cohort, we implemented a permutation scheme. For each permutation, we first assigned rare variant-carrying status to n of the $2 \times 690 = 1380$ chromosomes. Chromosomes were then randomly paired, and the number of pairs where both chromosomes were rare variant-carrying was recorded (these pairs represent the compound heterozygote/rare allele homozygote patients for that permutation). To generate null values for a collection of 21 Fanconi anemia genes, the permutation was performed for the observed numbers of rare variants ($n_1, n_2, \dots, n_{21}$) in each of these genes and the result was summed. The null distribution was generated from 100,000 such permutations. The P -value was assigned by comparing observed value to this distribution, which also yielded the confidence interval.

All differences in proportions were assessed using a test of equal proportions (the function `prop.test` in R). Differences in rare-variant burdens between different classes of genes were tested using a Wilcoxon test. Difference in overall survival was tested using the Cox proportional hazards model, adjusting for patient age. Concordance between *MPO* mutational status and cytogenetic lesions, and

between mutation status in pairs of genes, was tested using Fisher's exact test with the R package `exact2x2`.

Chapter 3: Results

Chapter 3-1: Rare variants in genes with known roles in germline myeloid malignancy susceptibility

Introduction

The World Health Organization (WHO), the Society for Hematopathology and the European Association for Haematopathology, published the third edition of the *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues* in 2001 (Swerdlow, 2008). Since then, the classification has been constantly updated as researchers and clinicians gain more information about myeloid malignancies. Although most cases of myeloid malignancies are sporadic diseases, there is a subgroup of cases which clearly have germline predisposition corresponding to certain genes which cause the diseases (Baptista, 2017). With the advancement in sequencing technologies, researchers and clinicians can now incorporate genetic changes in diagnosing myeloid malignancies. As a result, the World Health Organization (WHO) revised their classification of myeloid neoplasms and acute leukemia to include a new category, termed "myeloid neoplasms with germline predisposition" in 2016 (Arber, Orazi et al. 2016). The category is summarized in Table 9.

Table 8: Classification of myeloid neoplasms with germ line predisposition

Myeloid neoplasm classification
Myeloid neoplasms with germ line predisposition without a pre-existing disorder or organ dysfunction
AML with germ line <i>CEBPA</i> mutation
Myeloid neoplasms with germ line <i>DDX41</i> mutation
Myeloid neoplasms with germ line predisposition and pre-existing platelet disorders
Myeloid neoplasms with germ line <i>RUNX1</i> mutation
Myeloid neoplasms with germ line <i>ANKRD26</i> mutation
Myeloid neoplasms with germ line <i>ETV6</i> mutation
Myeloid neoplasms with germ line predisposition and other organ dysfunction
Myeloid neoplasms with germ line <i>GATA2</i> mutation
Myeloid neoplasms associated with BM failure syndromes
Myeloid neoplasms associated with telomere biology disorders
JMML associated with neurofibromatosis, Noonan syndrome or Noonan syndrome-like disorders
Myeloid neoplasms associated with Down syndrome

Result

We identified 11 patients in our cohort who would fit the criteria owing to mutations in *DDX41* (Figure 5), four of whom harbored previously-described (Polprasert, Schulze et al. 2015, Cheah, Hahn et al. 2017, Guidugli, Johnson et al. 2017, Makishima, Yoshizato et al. 2018) *DDX41* variants with strong evidence for disease roles. One of the four patients also harbored a somatic *DDX41* mutation (p.R525H) *in trans* with his germline variant (p.I396T) (Polprasert, Schulze et al. 2015).

Also specified in the 2016 WHO criteria are germline mutations in *ANKRD26*, *CEBPA*, *ETV6*, *GATA2*, and *RUNX1*. Although 31 patients had rare *ANKRD26* variants, none had variants in the 5' untranslated region, which has been the site of nearly all causal *ANKRD26* variants identified to date (Noris, Perrotta et al.

2011). No *CEBPA* mutations were observed, but five patients were found to have *ETV6* mutations (Figure 5). One such patient carried a p.R353Q mutation affecting the highly-conserved ETS domain, a well-known hotspot for both germline and somatic mutations in *ETV6* among myeloid malignancy patients (Zhang, Churpek et al. 2015). The p.R353Q variant is exceedingly rare, present at allele frequency 2.83×10^{-5} in the gnomAD database (Karczewski, 2019), and has a hybrid score of 0.83. Three patients with *GATA2* mutations and six with *RUNX1* mutations were found (Figure 5). The *RUNX1* variants in four of the six patients were novel (neither present in any of the population databases nor assigned rsIDs), and three were unanimously deemed deleterious by the six scoring algorithms, including two alleles (p.R166Q and p.Y287X) that were previously found (Song, Sullivan et al. 1999, Michaud, Wu et al. 2002) through pedigree analyses of families with familial platelet disorder with predisposition to acute myeloid leukemia (FPD/AML). In our cohort, both individuals carrying these latter alleles were pediatric cases, with ages-of-onset 1 and 12 years.

Figure 5: Germline mutations in genes implicated in myeloid neoplasms with germline predisposition.

Figure 5: Color indicates patient diagnosis and shape indicates impact on amino acid sequence. Specific variants previously reported as susceptibility alleles are indicated with a dagger (†).

Other genes associated with familial MDS are *SAMD9/SAMD9L*, *SRP72*, *MECOM*, and *TERT*. *SAMD9/SAMD9L* are adjacent homologs on chromosome 7q21.2 that have recently been implicated by several groups in a substantial portion of pediatric bone marrow failure patients with monosomy 7 (Schwartz, Ma et al. 2017, Bluteau, Sebert et al. 2018). We identified 42 patients with rare *SAMD9/SAMD9L* variants, though none are pediatric cases with monosomy 7. No clearly deleterious variants were found in the other genes. Overall, therefore, our cohort included 25 patients (3.6%) with variants that would qualify them as having germline predisposition, including six (0.9%) harboring previously identified, likely causal variants.

Discussion

Here we show that our cohort has 25 patients (3.6%) that would qualify as having germline predisposition, including 6 harboring previously identified, likely causal variants. Those variants are found in *DDX41*, *ETV6*, *GATA2* and *RUNX1*. Recent studies have shown that there are numerous pathogenic or risk factor variants on those genes associated with myeloid malignancies. (Polprasert, 2015) (Walker, 2002) (Lewinsohn, 2016) (Li, 2016) (Cardoso, 2016) (Zhang, 2015) (Landrum, 2018) (Hahn, 2011) (Luo, 2019). The fact that our study can capture these germline variants strengthens the fact that myeloid malignancy has heritable components. However, since most of those variants were discovered in familial settings, there is a possibility that there are variants which remain

undiscovered in the population. *DDX41*, *ETV6*, *GATA2* and *RUNX1* are well known to be mutated somatically in myeloid malignancy (Polprasert, 2015) (Fisher, 2017) (Harada, 2004) (Wang, 2014) (Barjesteh van Waalwijk van Doorn-Khosrovani, 2005) (Sood, 2017). At the same time, patients who have these germline variants are known to have an increased risk of developing myeloid neoplasms, including MDS, AML, and chronic myeloid leukemia later in their lives (Lewinsohn, 2016) (Gao, 2019). These observations support the idea that the somatic aspects of human malignancies can offer clues to guide the search for inherited predisposition variants. Genes that are important for malignancy onset and progression tend to be those that acquire mutations somatically, or that are subject to somatic duplication or deletion events. Therefore, these same genes make attractive candidates in the search for important germline mutations. Understanding the interaction between germline variants and somatic mutation in myeloid malignancies will give a better picture of the gene and pathway disruptions that lead to disease phenotypes, eventually yielding new therapeutics.

Chapter 3-2: Pathogenic rare variant burden and *MPO* as a candidate predisposition gene

Introduction

Next generation sequencing is becoming more popular in both research and clinical diagnosis. As a result, researchers are able to discover new variants. This creates challenges in determining whether the newly found variants are

associated with the disease and how the variants affect disease phenotypes. In order to solve these problems, there is a need to have a standard guideline in variant classification for both researchers and clinicians. Therefore, a workgroup consisting of American College of Medical Genetics (ACMG), Association for Molecular Pathology (AMP), and College of American Pathologists, representing clinical laboratory directors and clinicians, was formed in 2013 to create a recommendation for the use of standard terminology for classifying sequence variants. Using the recommendation, variants that are found can be classified based on various evidence weighted according to an established system (Richards, 2015).

The ACMG standards and guidelines for the interpretation of sequence variants is a five-tier terminology system using the terms “pathogenic,” “likely pathogenic,” “uncertain significance,” “likely benign,” and “benign”. Pathogenic variants refer to variants which are proven to be disease-causing whereas likely pathogenic variants are variants which are very possible, more than 90% of chance, to be disease causing. We first examine both pathogenic and likely pathogenic variants because these variants can have large effects on both protein production and formation. Our analysis of these variants show *MPO* as a candidate predisposition gene.

Result

A total of 146 variants were classified as pathogenic or likely pathogenic (Figure 6A), spread across 65 genes and 137 patients (19.9%). The gene with the

largest pathogenic/likely pathogenic variant burden is *MPO*, which harbors such a variant in 19 of the patients. *MPO* (myeloperoxidase) codes for a heme protein produced during myeloid differentiation. Neutrophil azurophilic granules have MPO as their major component, and MPO is expressed mostly in blood cells. The pathogenic variants found in our cohort are dominated by two substitutions: a splice site variant (c.2031-2A>C; eleven patients), and a missense variant (c.C1705T, p.R569W; seven patients) (Figure 6B). Multi-species alignment shows that both of the corresponding wild-type alleles are highly conserved. An earlier report associated the c.2031-2A>C variant with myeloperoxidase deficiency, and transfection of a construct harboring the variant resulted in mRNA with 109 nucleotides inserted between exons 11 and 12, yielding a protein that should lack enzymatic activity (Marchetti, Patriarca et al. 2004). The variant was also identified as a being associated with protein abundance, both in cis (*MPO*) and in trans (*RAB26*), in human plasma (Sun, Maranville et al. 2018).

To examine the prevalence of these pathogenic *MPO* alleles in control and other case cohorts, we queried the gnomAD database (Karczewski, 2019) of 60,144 control exomes, as well as 531 AML patient exomes from the recently-published Beat AML study (Tyner, Tognon et al. 2018). Frequencies of both alleles are significantly higher in our cohort (0.8% and 0.51% for c.2031-2A>C and p.R569W, respectively) than in the gnomAD database (0.38% and 0.13%; $P = 0.012$ and 4.0×10^{-4} , respectively). The allele frequencies in the Beat AML study (0.8% and 0.6%) are similar to those in our cohort. As in gnomAD, both variants

clarity, significance is only shown for comparisons between our cohort and gnomAD. * $P < 0.05$; *** $P < 0.0005$. (D, E) Associations between the presence of cytogenetic lesions and the presence of a rare *MPO* variant (left) or pathogenic rare *MPO* variant (right). OR = odds ratio. Filled circles indicate OR estimate, with whiskers indicating a 95% confidence interval for the OR.

Discussion

Here, we show that *MPO* has the highest pathogenic rare germline variant burden. In fact, *MPO* is an appealing target for myeloid malignancies as there is growing evidence showing that there are relationships among *MPO*, inflammation, and cancer (Hanahan, 2011) (Al-Salihi, 2015) (Grivennikov, 2010). In fact, *MPO* polymorphism is linked to abnormal *MPO* expression and greater risks of various diseases (Ding, 2013) (Zhang, 2001). A previous study showed that the p.R569W variant, one of the driving signals, can cause defects in *MPO* (Nauseef, 1994). It is also known that *MPO* can produce oxidants which can lead to DNA damage, and thus promote mutagenesis (VanderVeen, 2001).

Myeloperoxidase is a hallmark enzyme of acute myeloid lineage. Khan *et al.* reported that in AML patients, there are higher plasma *MPO* levels (range 1.0–9514 ng/mL) as compared to healthy subjects (range 3.5–20.6 ng/mL) (Khan, 2018). Furthermore, patients with high endogenous levels of *MPO* are found to have higher chance of developing cancer (He, 2009)..

Chapter 3-3: Exploring the overall germline rare variant burden and investigating two-hit hypothesis among germline rare variants and with somatic mutations.

Introduction

In 1971 Knudson proposed that retinoblastoma was initiated by inactivation of a putative tumor suppressor, the *Rb1* gene (Knudson, 1971). According to a "two-hit" model, dominantly inherited predisposition to cancer starts with a germline mutation. In order to initiate tumorigenesis, a second hit is required. The second hit often comes as somatic mutation which patients acquire later in their lives. On the other hand, non-hereditary cancer also requires the same two hits, but both are somatic.

Knudson's two-hit hypothesis can also apply to solely germline variants when those variants are either homozygous or compound heterozygous. Homozygosity is a situation in which there are two identical alleles at one specific site, one inherited from each parent.

On the other hand, compound heterozygosity occurs when two mutations affect one gene in two different positions. The two mutations may exist either in the same copy (*in cis* heterozygosity) or in both paternal and maternal alleles, each at a different position (*in trans* compound heterozygosity). In the *in trans* case, both alleles may behave abnormally and cause a disease.

In order to assess whether the variant pair are an *in cis* or *in trans* arrangement, one can look at the linkage disequilibrium (LD) between the variants. LD refers

to the non-random association of alleles at linked loci. Variants which are in linkage disequilibrium are considered to be correlated with each other and this also indicates that the variant pair is *in cis* arrangement. The degree of linkage disequilibrium can be measured by D' , an indicator of allelic segregation for two genetic variants, and R^2 , a measure of correlation of alleles for two genetic variants (Machiela, 2018).

Result

Examination of rare variant burden across all 657 genes under consideration (104 of which have no rare variants in our cohort; Figure 7A), irrespective of pathogenicity, shows that genes associated with Fanconi anemia (FA) and, in general, those associated with autosomal recessive (AR) disorders, have higher rare variant burdens than do the other genes under consideration (Wilcoxon test $P = 0.002$ and 0.027 , respectively; Figure 7B). We also observe an enrichment of truncating variants (nonsense, frameshift, and splice site) in FA and AR genes (Figure 7C,D). The 205 truncating variants are spread across 167 patients and 119 genes. Of these variants, 97 (47.3%) were found in AR genes and 18 (8.9%) were found in FA genes, proportions higher than the proportions of all variants (29.0% in AR genes, $P = 1.3 \times 10^{-8}$ and 6.6% in FA genes, $P = 0.14$) and proportions of all genes under consideration (25.0% AR genes, $P = 1.0 \times 10^{-9}$ and 3.2% FA genes, 7.7×10^{-4}). Moreover, the proportion of rare variants that are truncating is significantly higher in AR genes (5.7%) and FA genes (4.6%) than in other genes (2.5%; $P = 1.5 \times 10^{-9}$ and 0.013 , respectively).

Figure 7: Autosomal recessive genes and Fanconi anemia genes have higher overall and truncating rare germline variant burden.

Figure 7: (A) Each gene (with at least one germline variant) is represented by a small bar, whose height indicates the number of rare germline variants observed in that gene. A bar is colored if its corresponding gene is autosomal recessive (red), a FA gene (blue), or both (purple). (B) Rare germline variant burden for genes, classified by Fanconi anemia and autosomal recessive status. $*P < 0.05$; $**P < 0.005$. (C) Bars indicate (from left to right) proportions of all truncating rare germline variants that are in AR/FA genes, proportions of all variants that are in FA/AR genes, and proportion of all genes under consideration that are FA/AR genes. $**P < 0.001$; $***P < 10^{-7}$; *N.S.*: not significant at 0.05 level.

An AR gene harboring a rare disruptive variant may have the other parental copy disrupted through several possible mechanisms, one of which is inheritance of a rare variant on the other parental allele. This biallelic scenario may either take the form of a rare allele homozygote (RAH), or of a compound heterozygote (CH). Unsurprisingly, RAHs were exceedingly rare in our data set, with only 17 present. Interestingly, the only gene harboring a RAH in more than one patient was *BRCA2* (also known as *FANCD1*). Among the three patients carrying *BRCA2* RAHs was one diagnosed at 15 years, and a 57-year-old who died eight months after diagnosis (the third patient did not have age or survival information available). In general, patients with rare germline *BRCA2* variants, whether in a heterozygous or homozygous constellation, have significantly worse outcomes with regard to overall survival (Figure 8B; age-adjusted hazard ratio 2.62, 95% CI 1.26-5.44, $P = 0.0072$).

Patients with CHs were also identified. Compound heterozygosity here is sometimes putative, because *in silico* it is only possible to conclusively determine whether the two variants are *in trans* (thereby affecting both parental homologs) or *in cis* (affecting one homolog at two loci) if read pairs cover both loci (see Patients and Methods). We observed a total of 95 putative CHs, across 85 different patients and 71 unique genes. Among the 95 CHs, 30 were in AR genes (Figure 8C), with two in *BRCA2* (Figure 8D). A larger proportion of CH and RAH are in Fanconi anemia genes, compared to the proportion of all rare variants in FA genes (10.7% vs. 6.6%; $P = 0.06$) and compared to the proportion of FA

genes among all genes under consideration (10.7% vs. 3.2%; $P = 6.4 \times 10^{-5}$), though the former does not attain statistical significance.

Under the null hypothesis of random assortment of affected chromosomes, we could calculate the number of RAH/CH that would be expected in a gene given the total number of rare variants present in that gene across the cohort. A higher-than-expected number of RAH/CH events is indicative of a disease predisposition for individuals with biparental inheritance of rare variants in the gene. Using this principle, we tested for enrichment of RAH/CH across all FA genes (Figure 8E) given the total number of rare alleles present in these genes across patient chromosomes (see Patients and Methods). Assuming random assortment, we would expect 4.6 (95% confidence interval 1 - 9) patients to have RAH/CH in FA genes, but we observe 12 ($P = 0.002$).

Figure 8: Genes with rare variants in both copies in the same patient.

Figure 8: (A) Both parental homologs may be impacted by rare germline variants either through homozygosity (RAH; left) or compound heterozygosity (CH; right). (B) Presence of rare germline *BRCA2* variants are associated with poor overall survival. (C) Counts for instances of RAH/CH in autosomal recessive genes. These categories combined are enriched for Fanconi anemia genes. (D) *BRCA2* has five such RAH/CH pairings in four different patients. Individual patients are indicated by distinct numbers 1-4 adjacent to their variants. Patient diagnosis is indicated by color.

Figure 9: Global and patient-specific concordance of rare germline variants and somatic mutations in each gene.

Figure 9: (A) The genes with high overall germline variant burden (horizontal axis) do not tend to be those with high overall somatic mutation frequency (vertical axis). *TET2* is one of the few genes with high overall frequencies of both somatic and rare germline variants. (B) Rare germline variant-somatic mutation pairs in the same patient are shown. Individual patients are indicated by distinct numbers 1-4 adjacent to their variants. Patient diagnosis is indicated by color and effect on amino acid sequence is indicated by shape.

Discussion

The FA pathway is known to be an essential DNA repair pathway, and deficiency of FA genes result in Fanconi anemia, and hence increase the chance of having myeloid malignancies. In line with our finding, Maung et al. also show that there is an enrichment of deleterious germline rare variants on FA genes in AML patients (Maung, 2018).

Previous studies have demonstrated that patients with FA will have an earlier onset of childhood cancers if they carry biallelic *BRCA2* mutations (Offit, 2003) (Howlett, 2002) (Hirsch, 2004). It is also known that biallelic *BRCA2* mutations would increase the risk of AML, acute T-cell lymphoma, medulloblastoma and Wilms tumor (Wagner, 2004) (Hirsch, 2004) (Loizidou, 2016), and it is associated with shortened lifespan (Alter, 2007) (Wang, 2007).

Chapter 4: Discussion & Future Directions

The first population-based germline rare variants study of myeloid malignancy

Here we have presented results from the analysis of the largest collection, to our knowledge, of myeloid malignancy germline exomes reported to date. Most publications of large myeloid neoplasm patient exome cohorts have focused on the characterization of somatic mutations (Cancer Genome Atlas Research 2013) (Makishima, Yoshizato et al. 2017) (Tyner, Tognon et al. 2018). The dearth of germline genome-wide analysis studies published to date in these diseases may partially be the result of publication bias, as most efforts have likely focused on common variants represented on genotyping arrays, and probably yielded negative results. One exception is a recent GWAS in myeloproliferative neoplasms (Tapper, Jones et al. 2015), which identified common risk variants from a large meta-analysis of array data.

In our cohort of 690 patients, we were able to identify previously-reported, likely-casual variants in approximately 1% of patients. Overall, we identified 25 (3.6%) patients with a disease that could be classified as myeloid neoplasms with germline predisposition, according to the 2016 WHO category. We lacked family history information for most of these patients, so we could not determine whether there was evidence for familial disease tracking with these variants. There were no statistical differences in age-of-onset between these 25 patients and the rest of the cohort, although there were three under 13 years of age.

Whole genome sequencing instead of whole exome sequencing

Technology advancement enables researchers to explore the contribution of low-frequency and rare genetic variants in disease phenotypes, for example this project. By focusing the protein coding region in the human genome, which is about 2% of the whole human, researchers are already able to identify new genes implicated in various human diseases. Since the cost of next generation sequencing is lower, attention has turned to exploring the remaining 98% of the human genome. Here we show how germline rare exonic variants contributes to myeloid malignancies. However, the contribution from germline rare non-coding variants is still an unknown. Understanding the contributions from those variants can explain the rest of the missing heritability of myeloid malignancies, and hence discover novel targets for treatments.

However, there are still challenges ahead in understanding rare variants, one of which is that variant frequency can be different from one population to another population as there are isolates or different ethnic groups. To deal with this challenge, studies on different populations are necessary in order to determine truly rare variants. Also, although whole genome sequencing in low-depth coverage is available, whole genome sequencing with high-depth coverage will be beneficial in understanding the contribution of germline rare non-coding variant. The third problem is to validate the *in silico* finding using either *in-vitro* or *in-vivo* experiments in order to know impacts of these rare variants. Despite

these challenges, studying the contributions of germline rare variants in the human genome will be worthwhile (Zuk et al. 2014).

MPO as a novel myeloid malignancy predisposition gene

Our analysis revealed a novel candidate risk gene, myeloperoxidase (*MPO*), with two pathogenic variants in particular showing elevated frequencies both in our cohort and in a recently-published independent cohort of AML patients (Tyner, Tognon et al. 2018). *MPO* is an appealing candidate as a myeloid malignancy susceptibility gene for a number of reasons. It is expressed, on both the mRNA and protein levels, most prominently in blood cells (Consortium 2015) (Schmidt, Samaras et al. 2018). High *MPO* expression is characteristic of acute promyelocytic leukemia (APL), though none of the carriers of the *MPO* rare variants here have the t (15;17) translocation causing APL. Germline variants in *MPO* (including one of those with elevated frequency in our cohort) have been associated with high counts of leukocytes, monocytes, neutrophils, and other blood cell types (Astle, Elding et al. 2016).

The importance of FA genes in myeloid malignancies

Fanconi anemia (FA) genes were a category that emerged recurrently in our analysis. We found that, compared to other genes under consideration, FA genes were enriched for overall rare germline variant burden, truncating mutations, and compound heterozygotes/rare allele homozygotes. Even after accounting for the total FA rare germline variant content in patient chromosomes, the number of patients for which affected FA genes are paired is significantly higher than would

be expected by chance. Given that nearly all FA genes show an autosomal recessive pattern in hematologic malignancies, this implies that observed FA enrichment is not random, and suggest biparental inheritance of disruptive variants in an FA gene as a myeloid malignancy risk factor. Of all genes under consideration, the FA gene *BRCA2* had the highest incidence of compound heterozygosity/rare allele homozygosity in the patient cohort. In general, patients with rare *BRCA2* germline variants had worse outcomes. Although *BRCA2* is associated with autosomal recessive inheritance in FA, it is considered an autosomal dominant gene in cancer. In the current setting, therefore, germline variants here may be acting in a dominant manner in myeloid malignancy severity, or the other *BRCA2* copy may be compromised *via* other mechanisms. The latter may also be true for the abundance of heterozygous truncating mutations observed in autosomal recessive genes in general.

Crosstalk between germline rare variants and somatic mutations in the same patient

We queried the data for synergy between germline and somatic contributors to disease, but were unable to detect anything statistically significant. We observed no evidence of germline and somatic variants cooperating, with no pairs (either within the same gene or in separate genes) occurring more than would be expected by chance. However, querying all possible pairs entails an enormous number of tests, and we were likely severely under-powered for any such test to withstand the necessary multiple-hypothesis corrections.

Improvement in sample size

This study can be further expanded by analyzing more patient sequencing data. Our data set comprised in total 690 myeloid malignancy patient whole exomes. It is suggested that a discovery sample of at least 25,000 subjects and a substantial replication set is needed for a well-powered study that aims to identify rare variants (Zuk et al. 2014). However, over time, there will be more myeloid malignancy patients sequencing available throughout different databases such as dbGaP, The European Genome-phenome Archive (EGA), St. Judes Cloud PeCan, Genomic Data Commons Data Portal, etc. All these databases hold large sequencing data sets that researchers can use to discover rare variant contributions to various human diseases. Also, collaboration with different research teams and forming large consortiums can effectively solve the sample size problem.

Table 9: Number of whole-genome and whole-exome patient samples available from public sources

<u>Resource</u>	<u>Website URL</u>	<u>Acute myeloid leukemia</u>		<u>Myelodysplastic syndrome</u>	
		<u>WGS</u>	<u>WXS</u>	<u>WGS</u>	<u>WXS</u>
dbGaP	dbgap.ncbi.nlm.nih.gov	1336	178	0	16
EGA	ebi.ac.uk/ega/home	157	78	5	529
GDC	gdc.cancer.gov	229	138	0	0
ICGC	icgc.org	32	254	0	0
Pecan	pecan.stjude.cloud	106	360	0	4

Limitations of our study

Our study has a number of limitations. First, our use of control data was limited, since the patient-level variant data is not provided on public websites such as gnomAD's. As such, it is not possible to determine, for example, the number of patients harboring variants in a particular collection of genes, or to identify compound heterozygotes. Second, although the collection of myeloid malignancy exomes analyzed here is the largest to date, we are substantially underpowered to detect case/control differences in rare-allele burden, even after restricting to our list of 657 genes. It follows that validation studies are needed for many of the results presented here. Third, determining whether pairs of variants in the same gene and patient were *in cis* or *in trans* was sometimes not possible.

Nonetheless, our permutation approach (see Patients and Methods) to testing for elevated levels of homozygosity/compound heterozygosity in a gene provides a framework for performing such tests using whole exome cohorts in general.

Conclusion

Overall, this study sheds light on some candidate mechanisms by which inherited alleles may confer risk of myeloid malignancy. The identification of such alleles has several practical benefits. Individuals carrying susceptibility alleles may benefit from increased surveillance, facilitating early preventative treatment.

These individuals may also benefit from genetic counseling. Asymptomatic family members who are carriers of the deleterious allele may be excluded as candidate bone marrow donors. Finally, understanding the mechanisms-of-action of these

alleles will help elucidate disease biology, potentially leading to improved patient treatment.

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