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Germline variants in severe hematological disorders: Prevalence and impact on hematopoietic stem cell transplantation and fertility preservation

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ACADEMIC DISSERTATION

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And once the storm is over, you won't remember how you made it through, how you managed to survive. You won't even be sure, whether the storm is really over. But one thing is certain. When you come out of the storm, you won't be the same person who walked in. That's what this storm's all about.

Haruki Murakami

Abstract

Germline variants are increasingly recognized as contributors to hematological malignancies, influencing disease risk, treatment outcomes, and long-term complications, particularly in the context of allogeneic hematopoietic stem cell transplantation (HSCT). Despite their clinical relevance, routine analysis of germline predisposition has remained underutilized in many hematologic patient populations. As prognosis in hematological disorders has improved, fertility concerns are increasingly recognized as an important component of comprehensive care for pediatric and adolescent patients. However, the impact of the disease itself on reproductive capacity remains poorly understood.

This thesis aimed to investigate the prevalence of cancer-predisposing germline variants in pediatric and adult patients with severe hematological diseases, assess the impact of these variants on HSCT, and evaluate fertility preservation potential in prepubertal boys with these diseases.

The work is based on four sub-studies using exome sequencing, clinical data, and histological evaluation. Study populations included patients with acute lymphoblastic leukemia (n = 148), HSCT recipients and donors (n = 877 and 669, respectively), and pediatric patients undergoing testicular tissue biopsy for fertility preservation (n = 43). Variant analyses focused on genes predisposing to hematological disorders and solid cancers and clinically significant secondary findings.

Pathogenic or likely pathogenic germline variants were identified in 10% of patients with acute lymphoblastic leukemia. Enrichment was most notable in hematological malignancy genes in children and solid tumor predisposition genes in adults, suggesting age-related patterns of inherited risk.

Among allogeneic HSCT recipients, 17.8% carried clinically relevant germline variants. *CHEK2* variants were the most prevalent and significantly enriched compared to control population but not associated with short-term transplant outcomes. A notable proportion of patients with related donors had not undergone germline testing prior to HSCT, indicating a missed opportunity for informed donor selection.

In the fertility preservation cohort, testicular biopsies from prepubertal boys with severe hematological diseases revealed that 49% had reduced spermatogonial counts, and 19% showed severely depleted germ cell numbers before receiving any cytotoxic treatment. The most pronounced depletion was observed in patients with Fanconi anemia and other inherited bone marrow failure syndromes, indicating a disease-related impairment of spermatogenesis.

This thesis highlights the prevalence and clinical impact of germline predisposition in hematological patients. Furthermore, the results emphasize the importance of genetic analysis and early fertility preservation measures as part of developing more individualized and long-term treatment strategies in hematology.

Tiivistelmä

Iturata variantit tunnetaan yhä laajemmin hematologisten maligniteettien riskitekijöinä. Ne voivat vaikuttaa paitsi sairastumisriskiin, myös hoitovasteeseen ja pitkäaikaiskomplikaatioihin, erityisesti allogeenisen hematopoieettisen kantasolusiirron (KSS) yhteydessä. Kliinisestä merkityksestään huolimatta iturataan liittyvän alttiuden tutkiminen ei ole vakiintunut käytäntö useissa hematologisissa potilasryhmissä. Hematologisten sairauksien ennusteen parantuessa hedelmällisyyteen liittyvät kysymykset tunnistetaan yhä paremmin tärkeäksi osaksi kokonaisvaltaista hoitoa. Itse sairauksien vaikutuksista hedelmällisyyteen tiedetään kuitenkin vielä vähän.

Tässä väitöskirjassa tutkittiin syöpäriskiä lisäävien iturata varianttien esiintyvyyttä lapsi- ja aikuispotilailla, joilla on vakava hematologinen sairaus, sekä arvioitiin näiden varianttien vaikutusta kantasolusiirtoon. Lisäksi selvitettiin hedelmällisyyden säilyttämisen mahdollisuuksia näitä sairauksia sairastavilla pojilla ennen murrosikää.

Väitöskirja perustuu neljään osatyöhön, joissa hyödynnettiin eksomisekvensointidataa, kliinisiä tietoja ja histologista näytteitä. Tutkimusaineistoon sisältyi akuuttiin lymfoblastileukemiaan sairastuneita potilaita (n = 148), kantasolusiirtopotilaita ja heidän luovuttajiaan (n = 877 ja 669) sekä esimurrosikäisiä poikia, joilta oli otettu kivesbiopsia hedelmällisyyden säilyttämiseksi (n = 43). Varianttianalyysit kohdistuivat hematologisille ja kiinteille syöville altistaviin geeneihin sekä kliinisesti merkittäviin sivulöydöksiin.

Akuuttia lymfoblastileukemiaa sairastavista potilaista 10 %:lla todettiin patogeeninen tai todennäköisesti patogeeninen iturata variantti. Varianttien rikastumista havaittiin erityisesti lapsipotilailla hematologisiin maligniteetteihin ja aikuispotilailla kiinteisiin kasvaimiin liittyvissä geeneissä, mikä viittaa erilaisiin perinnöllisen alttiuden muotoihin.

Allogeenisen KSS:n saaneista potilaista 17,8 %:lla todettiin kliinisesti merkittävä iturata variantti. *CHEK2*-variantit olivat näistä yleisimpiä ja väestökontrolleihin verrattuna rikastuneita, mutta ne eivät vaikuttaneet lyhyen aikavälin siirron tuloksiin. Merkittävä osa sukulaisluovuttajalta kantasolusiirteen saaneista potilaista ei ollut saanut geneettistä diagnoosia ennen kantasolusiirtoa, mikä paljastaa hukatun mahdollisuuden huomioida perinnöllinen alttius luovuttajavalinnassa.

Vaikeaa hematologista sairautta sairastavista esimurrosikäisistä pojista 49 %:lla oli vähentynyt siittiöiden kantasolujen määrä jo ennen sytotoksista hoitoa. 19 %:lla havaittiin vaikea-asteista sukusolujen katoa. Merkittävimmät löydökset koskivat Fanconin anemiaa ja muita perinnöllisiä luuytimen vajaatoimintaa sairastavia potilaita, mikä viittaa itse sairauteen liittyvään häiriöön siittiöiden tuotannossa.

Tämä väitöskirja tuo esiin iturata varianttien yleisyyden ja kliinisen merkityksen hematologisilla potilailla. Tämän lisäksi tulokset korostavat geneettisen analyysin ja varhaisien toimien hedelmällisyyden säilyttämiseksi tärkeyttä osana yksilöllisempien ja pitkäjänteisempien hoitolinjojen kehittämistä hematologiassa.

List of abbreviations

AA	Aplastic anemia
ACMG	American College of Medical Genetics and Genomics
ALL	Acute lymphoblastic leukemia
AML	Acute myeloid leukemia
B-ALL	B-cell acute lymphoblastic leukemia
BM	Bone marrow
BMF	Bone marrow failure
BMFS	Bone marrow failure syndrome
CAR	Chimeric antigen receptors
CB	Cord blood
CD	Cluster of differentiation
CH	Clonal hematopoiesis
CHIP	Clonal hematopoiesis of indeterminate potential
CI	Confidence interval
CLL	Chronic lymphocytic leukemia
CLP	Common lymphoid progenitor
CML	Chronic myeloid leukemia
CMML	Chronic myelomonocytic leukemia
CMP	Common myeloid progenitor
COSMIC	Catalogue of Somatic Mutations in Cancer
DBA	Diamond-Blackfan anemia
DC	Dyskeratosis congenita
DNA	Deoxyribonucleic acid
EBMT	European Society for Blood and Marrow Transplantation
ECD	Erdheim-Chester disease
ET	Essential thrombocythemia
FA	Fanconi anemia
FA/BRCA	Fanconi anemia/Breast Cancer susceptibility
FHRB	Finnish Hematological Registry and Biobank
FI	Fertility index
FIMM	Institute for Molecular Medicine Finland
FuGU	Functional Genomics Unit
GATK	Genome Analysis Toolkit
GMP	Granulocyte-macrophage progenitor
GnomAD	Genome Aggregation Database
GVHD	Graft-versus-host disease
HLA	Human leukocyte antigen
HLH	Hemophagocytic lymphohistiocytosis

HSC	Hematopoietic stem cell
HSCT	Hematopoietic stem cell transplantation
HUH	Helsinki University Hospital
ICC	International Consensus Classification
IEI	Inborn error of immunity
ITD	Internal tandem duplication
JMML	Juvenile myelomonocytic leukemia
LCH	Langerhans cell histiocytosis
LFS	Li-Fraumeni syndrome
LP	Likely pathogenic
MAC	Myeloablative conditioning
MAF	Minor allele frequency
MDS	Myelodysplastic syndrome
MDS/MPN	Myelodysplastic/myeloproliferative neoplasm
MEP	Megakaryocyte-erythroid progenitor
MFD	Matched family donor
MM	Multiple myeloma
MPN	Myeloproliferative neoplasm
MUD	Matched unrelated donor
NA	Not applicable
NF1	Neurofibromatosis type 1
NHGRI	National Human Genome Research Institute
NK	Natural killer
NMA	Non-myeloablative conditioning
NOS	Not otherwise specified
NRM	Non-relapse mortality
OR	Odds ratio
OS	Overall survival
P	Pathogenic
PB	Peripheral blood
PCR	Polymerase chain reaction
Ph+ ALL	Philadelphia-positive acute lymphoblastic leukemia
PMF	Primary myelofibrosis
PV	Polycythemia vera
RIC	Reduced-intensity conditioning
RR	Relapse rate
SCID	Severe combined immunodeficiency
SCN	Severe congenital neutropenia
SD	Standard deviation
SDS	Shwachman-Diamond syndrome
SF	Secondary Findings
SM	Systemic mastocytosis
SNP	Single nucleotide polymorphism
SPRI	Solid-phase reversible immobilization
S/T	Spermatogonia per round tubular cross-section
T-ALL	T-cell acute lymphoblastic leukemia
TBD	Telomere biology disorder

TBI	Total body irradiation
TUH	Turku University Hospital
VAF	Variant allele fraction
VUS	Variant of uncertain significance
WES	Whole exome sequencing
WHO	World Health Organization

Table of contents

List of original publications

This thesis is based on the following publications, which are referred to in the text by their roman numerals:

I Douglas SPM*, **Lahtinen AK***, Koski JR, Leimi L, Keränen MAI, Koskenvuo M, Heckman CA, Jahnukainen K, Pitkänen E, Wartiovaara-Kautto U**, Kilpivaara O**. Enrichment of cancer-predisposing germline variants in adult and pediatric patients with acute lymphoblastic leukemia. *Scientific Reports* 2022, 12(1):10670

II **Lahtinen AK**, Koski J, Ritari J, Hyvärinen K, Koskela S, Partanen J, Vettenranta K, Koskenvuo M, Niittyvuopio R, Salmenniemi U, Itälä-Remes M, Jahnukainen K**, Kilpivaara O**, Wartiovaara-Kautto U**. Clinically relevant germline variants in allogeneic hematopoietic stem cell transplant recipients. *Bone Marrow Transplantation* 2023, 58(1):39-45

III **Lahtinen AK**, Funke M, Krallmann C, Wyrwoll MJ, Jarisch A, Yang Y, Bjarnason R, Romerius P, Sundin M, Norén-Nyström U, Langenskiöld C, Cremers JF, Kliesch S, Stukenborg JB, Neuhaus N, Jahnukainen K. Decreased spermatogonial numbers in boys with severe haematological diseases. *British Journal of Haematology* 2024, 205(1):229-235

IV **Lahtinen AK***, Karu M*, Koski J, Ritari J, Hyvärinen K, Koskela S, Nihtilä J, Partanen J, Vettenranta K, Koskenvuo M, Niittyvuopio R, Salmenniemi U, Itälä-Remes M, Jahnukainen K**, Kilpivaara O**, Wartiovaara-Kautto U**. Impact of *CHEK2* germline variants on hematologic malignancy risk and outcomes of allogeneic HSCT. Submitted to *British Journal of Haematology*

* Equal contribution
** Jointly supervised

1 Introduction

The history of hematological diseases is, in many ways, a story of medical perseverance. From the early 19th century descriptions of leukemia and lymphoma to the development of radiation and chemotherapy, the journey to understand and treat blood cancers has been long and complex. These diseases gradually became better defined, first through clinical observation and later through advances in pathology, cytogenetics, and molecular biology.

One of the most transformative steps in hematology was the development of hematopoietic stem cell transplantation (HSCT). Once an experimental and highly risky procedure, allogeneic hematopoietic stem cell transplantation has evolved into a potentially curative option for many malignant and non-malignant hematological disorders. The early decades of HSCT were marked by numerous setbacks as graft failures, infections, and severe graft-versus-host disease were common. Despite these challenges, determined researchers continued to push the field forward, refining conditioning regimens, improving donor matching, and developing effective strategies for immune suppression. Over the past six decades, improvements in donor matching, conditioning regimens, and supportive care have transformed HSCT from an experimental therapy into a cornerstone of modern hematology.

At the same time, our understanding of inherited susceptibility to hematological malignancies has grown rapidly. What was once thought to be limited to rare familial syndromes is now recognized as a more widespread phenomenon. Germline predisposition can exist even in patients without a known family history or syndromic features, and these inherited variants may influence disease development, treatment response, and long-term complications. For patients undergoing HSCT, these germline findings can have important clinical implications, including donor selection, conditioning intensity, and long-term monitoring.

The Finnish population represents a well-characterized genetic isolate, shaped by multiple historical population bottlenecks, founder effects, and relative geographic and cultural isolation. These events have led to a unique population structure with reduced genetic diversity and enrichment of certain rare variants and mutations that are either very uncommon or absent in other populations. Beyond the well-known conditions, also known as Finnish Disease Heritage, the genetic

isolate status of the Finnish population also enables the identification of novel disease-associated variants.

In the context of hematological diseases, the Finnish population offers a particularly valuable opportunity to explore the role of rare variants and founder mutations that may be overlooked in more genetically heterogeneous populations. The enrichment of certain variants, combined with comprehensive nationwide health records, high-quality biobank data, and centralized diagnostic practices, enables powerful genotype-phenotype correlation studies. This unique genetic landscape enhances our ability to uncover disease mechanisms, improve risk stratification, and potentially identify targets for personalized therapies.

The first HSCT in Finland was performed in 1974. Since then, the number of HSCT procedures has increased rapidly. In Finland, approximately 150 allogeneic HSCTs are performed annually, with 20–30 involving pediatric patients. Pediatric patients are treated exclusively at the New Children's Hospital in Helsinki, while allogeneic stem cell transplantations for adults are conducted in Helsinki and Turku. While leukemia remains the predominant indication for adult transplantations, pediatric HSCTs increasingly address congenital disorders of hematopoiesis and the immune system. In Finland, haploidentical transplantations were introduced in 2014 for adult patients and extended to pediatric patients in 2017, expanding the donor pool to half-matched family members and improving treatment options when a fully matched donor is unavailable.

Another emerging area of interest is fertility preservation. Especially in pediatric and adolescent patients, concerns about long-term quality of life, including reproductive health, are gaining more attention. While it is well known that chemotherapy and radiation impair fertility, recent research suggests that reduced fertility may also be associated with the disease itself or with inherited predisposition, even before treatment begins.

This thesis focuses on exploring the prevalence of germline variants in patients with severe hematological disorders, their implications in the setting of HSCT and fertility preservation. Drawing data from both pediatric and adult patient cohorts, and combining clinical, genetic, and histological analyses, the goal is to provide a deeper understanding of how inherited factors shape not only disease risk but also patient care.

2 Review of the literature

2.1 Hematopoiesis

Blood cell production, known as hematopoiesis, is a tightly regulated process essential for maintaining homeostasis and immune function. It occurs primarily in the bone marrow, where hematopoietic stem cells (HSCs) differentiate into mature blood cells with specialized functions. The balance between proliferation, differentiation, and apoptosis ensures the continuous renewal of blood components while preventing uncontrolled growth or dysfunction. Disruptions in this process can lead to hematological malignancies, highlighting the importance of understanding normal hematopoiesis in disease pathogenesis and treatment strategies.

2.1.1 Hematopoietic stem cells

Hematopoiesis originates from stem cells, which reside in specialized bone marrow niches and possess both self-renewal capacity and multipotency. Classically, these stem cells have been thought to differentiate through a hierarchical process, giving rise to two primary progenitor lineages: common myeloid progenitors (CMPs) and common lymphoid progenitors (CLPs). Myeloid progenitors further differentiate into erythrocytes, megakaryocytes, granulocytes (neutrophils, eosinophils, and basophils), and monocytes, while lymphoid progenitors give rise to T cells, B cells, and natural killer (NK) cells. Hematopoiesis and the differentiation process is tightly regulated by transcription factors and influenced by a complex network of cytokines and signaling pathways that ensure proper lineage specification and cellular homeostasis. Dysregulation at any stage of this process can lead to ineffective hematopoiesis, immune deficiencies, or malignant transformation. Although this classical hierarchical model has been central for understanding hematopoiesis, recent studies have revealed greater complexity, including early lineage priming already at the level of hematopoietic stem cells and the existence of multiple differentiation routes leading to the same lineage outcome. [1–4]

2.1.2 Roles of differentiated blood cells

The differentiation of hematopoietic stem cells follows a hierarchical pathway to generate functionally distinct blood cell types (Figure 1). The common myeloid progenitor gives rise to two major progenitor populations: megakaryocyte-erythroid progenitors (MEPs) and granulocyte-macrophage progenitors (GMP).

The MEP lineage is responsible for producing erythrocytes and platelets. Under the influence of erythropoietin, MEPs differentiate into erythroblasts, which mature into erythrocytes capable of oxygen transport via hemoglobin. These cells maintain systemic oxygenation and carbon dioxide removal, with their production finely tuned to oxygen availability. Alternatively, MEPs can differentiate into megakaryocytes, which produce platelets. The differentiation of megakaryocytes is driven by thrombopoietin, and the mature platelets play an essential role in hemostasis by aggregating at sites of vascular injury.

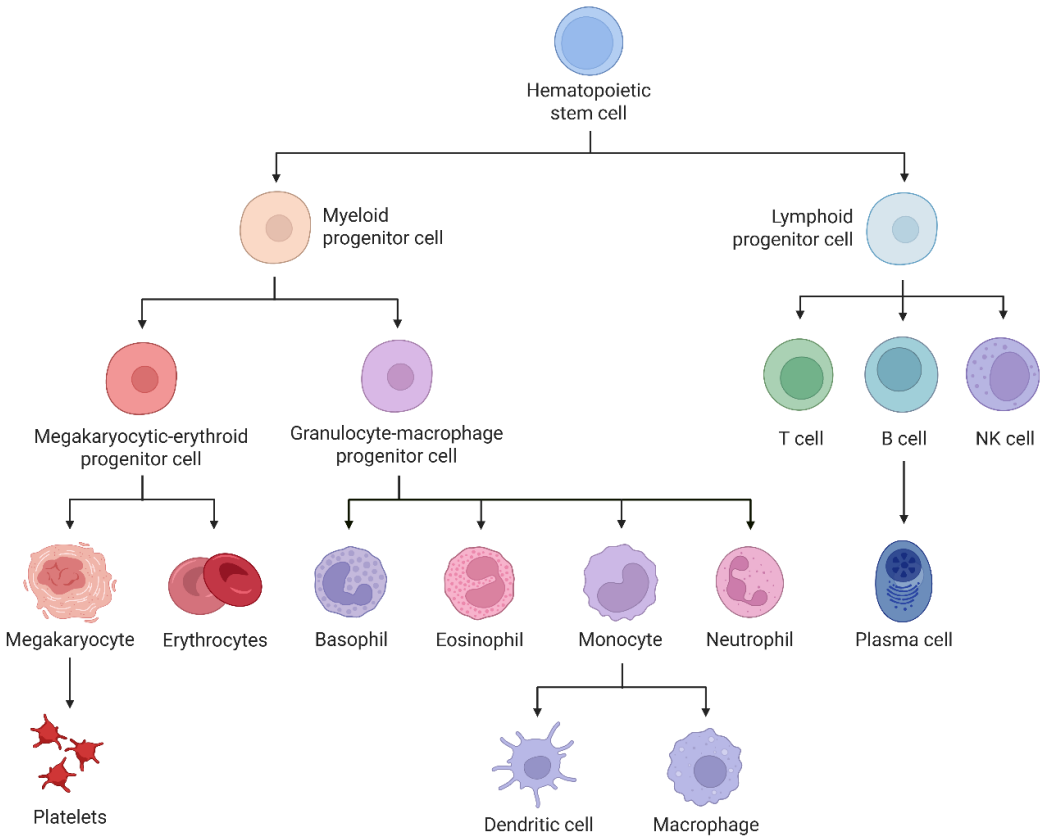


Figure 1. Differentiation of hematopoietic stem cells into myeloid and lymphoid lineages. Created with BioRender.com

The GMP lineage gives rise to granulocytes, including neutrophils, eosinophils, and basophils, and monocytes. Neutrophils are the most abundant type of white blood cell and serve as the first responders to infections, utilizing phagocytosis and the release of antimicrobial substances to eliminate pathogens. Eosinophils are specialized in combating parasitic infections and regulating allergic responses, while basophils, along with mast cells, play a role in inflammation by releasing histamine and other mediators upon activation. Monocytes circulate in the bloodstream and, upon entering tissues, differentiate into macrophages or dendritic cells, both of which are crucial for antigen presentation and immune modulation.

In parallel to myeloid differentiation, the common lymphoid progenitor gives rise to T cells, B cells, and natural killer cells, which coordinate adaptive and innate immune responses. While myeloid cells are primarily involved in immediate immune defense and tissue homeostasis, lymphoid cells regulate long-term immunity, antigen-specific responses, and immune memory.

The intricate differentiation of hematopoietic progenitors into distinct cell types ensures a dynamic and adaptable hematopoietic system. Any disruptions in this tightly regulated process due to genetic mutations, epigenetic alterations, or external stressors can contribute to hematological malignancies, emphasizing the need for a comprehensive understanding of normal hematopoiesis.

2.2 Clonal hematopoiesis and malignant transformation

2.2.1 Clonal hematopoiesis

Clonal hematopoiesis (CH) refers to the expansion of hematopoietic stem and progenitor cells that acquire somatic mutations conferring a selective advantage.[5] CH is strongly an age-related process, with prevalence rising sharply after the fifth decade of life and up to 10–20% of individuals over 70 harbor detectable CH clones.[5] Beyond age, environmental factors such as smoking, chemotherapy, and chronic inflammation contribute to CH development by inducing deoxyribonucleic acid (DNA) damage and promoting the selection for resistant clones.[6] This phenomenon is increasingly recognized as a key intermediary state between normal hematopoiesis and hematological malignancies.[7] Emerging evidence suggests that inherited genetic factors play a crucial role in influencing the susceptibility to CH and its progression.[8–10]

CH arises from mutations in key regulatory genes, most commonly *DNMT3A*, *TET2*, and *ASXL1*, which are involved in epigenetic modification and transcriptional regulation.[5] However, inherited mutations in genes regulating DNA repair, chromatin remodeling, and telomere maintenance may predispose individuals to the acquisition and expansion of such clones.[9–11] Defects in DNA

damage response genes, such as those involved in homologous recombination or mismatch repair pathways, can create a cellular environment where mutations accumulate more readily, facilitating clonal selection.[10] Similarly, germline mutations in telomere biology disorder (TBD) genes can lead to premature hematopoietic stem cell exhaustion, favoring the outgrowth of clones with increased proliferative potential.[12]

A subset of CH, termed clonal hematopoiesis of indeterminate potential (CHIP), is defined by the presence of somatic mutations in leukemia-associated genes at a variant allele fraction (VAF) ≥2% without overt hematological malignancy.[13] CHIP is generally asymptomatic and the transformation of CH into malignancy is relatively rare, 0.5–1% per year.[5] Individuals with inherited predisposition syndromes, including those with mutations in hematopoietic transcription factors or tumor suppressor genes, may experience accelerated clonal expansion due to a reduced capacity for normal hematopoietic regeneration.[8–10] Moreover, CH is emerging as a factor influencing outcomes in hematopoietic stem cell transplantation, as donor-derived CHIP increases the risk of graft failure and secondary malignancies.[12]

In conclusion, CH represents a dynamic state of hematopoiesis that bridges normal hematopoiesis and malignant transformation. Recognizing its clinical significance is essential for the advancement of personalized medicine, particularly in hematological malignancy surveillance and risk management. Furthermore, acknowledging the influence of inherited genetic factors will be crucial in refining risk prediction and developing targeted interventions for individuals.

2.2.2 Malignant transformation in hematopoietic cells

Cancer arises from the progressive accumulation of genetic and epigenetic alterations that disrupt normal cellular homeostasis, leading to uncontrolled proliferation, resistance to cell death, and immune evasion.[14,15] While the fundamental mechanisms of oncogenesis are shared across different cancer types, the manifestation of these processes varies between solid tumors and hematological malignancies.[16,17] Unlike solid tumors, which form localized masses and invade surrounding tissues, hematological cancers originate from cells of the blood and immune system, inherently circulating within the body and exploiting specialized niches like the bone marrow and lymphoid organs.

The Hallmarks of Cancer, a conceptual framework proposed by Hanahan and Weinberg, provides a comprehensive model to understand the biological traits that enable malignant transformation.[14,15,18] Initially described as six core features, later expanded to eight, and further refined in 2022, these hallmarks encompass the ability of cancer cells to sustain proliferative signaling, evade growth suppressors, resist cell death, enable replicative immortality, induce or access vasculature,

activate invasion and metastasis, reprogram metabolism, and evade immune destruction.[14,15] In addition to these core hallmarks, enabling characteristics, genomic instability and tumor-promoting inflammation further drive oncogenesis.[15,19,20]

Recently, the Hallmarks of Cancer framework have been proposed to include new hallmarks (unlocking phenotypic plasticity and senescent cells) and enabling characteristics (nonmutational epigenetic reprogramming and polymorphic microbiomes).[15] While these emerging capabilities are primarily studied in solid tumors, their implications in hematological malignancies are increasingly recognized, particularly in the context of differentiation blockades and lineage switching, which contribute to disease progression and therapeutic resistance.[21,22] The role of epigenetic reprogramming is particularly relevant in hematopoietic malignancies, where such changes contribute to altered stem cell function and clonal dominance.[5]

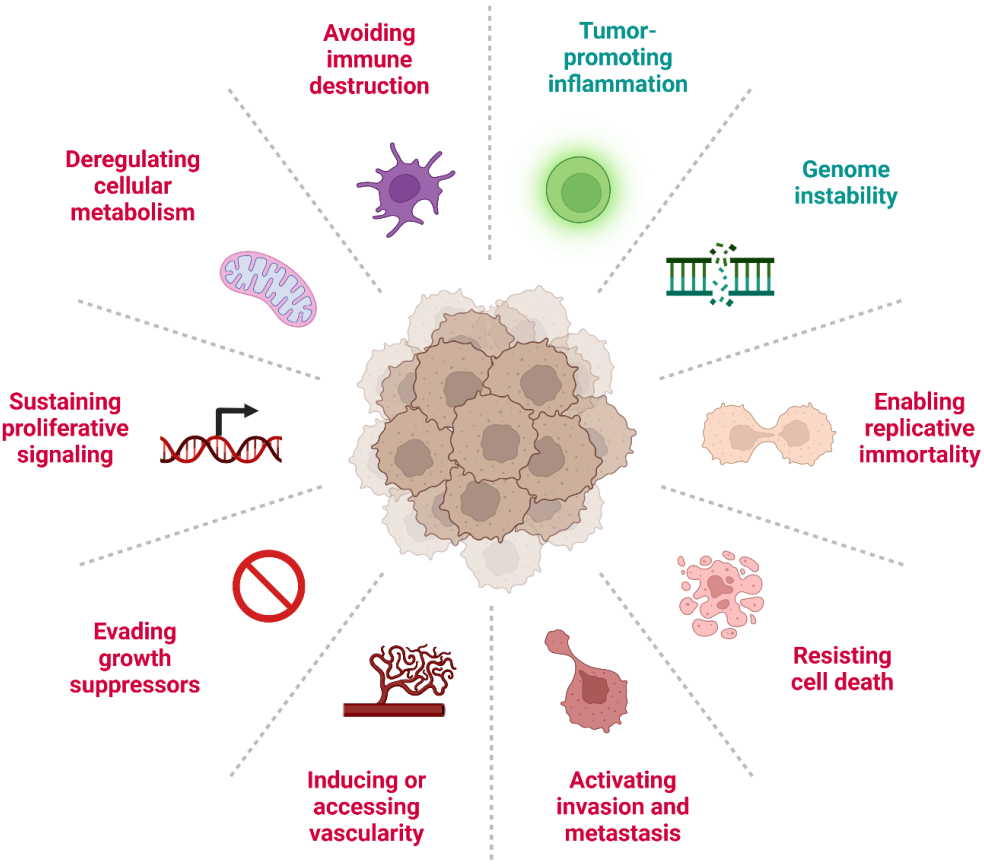


Figure 2. The Hallmarks of Cancer including eight hallmark capabilities (red) and two enabling characteristics (turquoise). Created with BioRender.com

Clonal hematopoiesis represents an early evolutionary step in malignant transformation, where genetic and epigenetic alterations accumulate in hematopoietic stem cells, conferring a selective advantage.[5,23] This process exemplifies several hallmarks of cancer at a pre-malignant stage, including sustained proliferative signaling and genome instability. Understanding these distinct aspects of hematological malignancies is essential for advancing personalized treatment and monitoring strategies, ultimately improving patient outcomes.[24]

2.3 Clinical characteristics of hematological malignancies and disorders

Hematological malignancies are a diverse group of cancers affecting the blood, bone marrow, and hematopoietic system. Unlike solid tumors, these cancers originate from blood-forming cells and typically involve widespread systemic disease from the outset.

Hematological malignancies are broadly classified into leukemias, myelodysplastic syndromes (MDS), myeloproliferative neoplasms (MPNs), and plasma cell disorders.[25,26] Leukemias are further divided into acute and chronic forms based on disease progression, as well as into lymphoid and myeloid leukemias according to the lineage of the affected blood cells. Acute lymphoblastic leukemia (ALL) arises from immature lymphoid progenitor cells, while acute myeloid leukemia (AML) originates from myeloid precursors. Similarly, chronic lymphocytic leukemia (CLL) develops from mature lymphoid cells, whereas chronic myeloid leukemia (CML) affects myeloid cells.

MDS is characterized by ineffective blood cell production and a risk of progression to acute leukemia, whereas MPNs involve excessive blood cell production due to mutations in key regulatory genes. Plasma cell disorders, such as multiple myeloma (MM), result from the uncontrolled proliferation of plasma cells, leading to abnormal antibody production and associated organ damage. Other hematological malignancies include myelodysplastic/myeloproliferative neoplasms (MDS/MPNs), histiocytic neoplasms, and other rare bone marrow disorders.

Although lymphomas also arise from hematopoietic cells, they are generally considered separate from blood cancers due to their primary involvement of lymphoid tissues.

The incidence of hematological malignancies has increased in recent years, partly due to improved diagnostics and an aging population. Advances in molecular profiling and targeted therapies have significantly improved outcomes, but challenges remain in disease monitoring, treatment resistance, and complications related to hematopoietic stem cell transplantation. As survival rates improve,

aspects such as fertility preservation and long-term health effects are gaining more attention in patient care.

In addition to hematological malignancies, non-malignant hematological disorders comprise a heterogeneous group of diseases affecting blood cell development and function. Although their clinical manifestations and prognoses vary widely, the most severe forms rely on allogeneic hematopoietic stem cell transplantation (HSCT) as a curative treatment.

2.3.1 Acute lymphoblastic leukemia (ALL)

ALL is an aggressive malignancy of lymphoid progenitor cells, leading to the uncontrolled proliferation of immature lymphoblasts in the bone marrow and peripheral blood. It is the most common leukemia in children but also occurs in adults, where it generally has a poorer prognosis.[27,28] The incidence of ALL follows a bimodal distribution, with the highest peak in children at the of age 2–5 years and a second, smaller peak in older adults.

ALL is classified into B-cell ALL (B-ALL) and T-cell ALL (T-ALL) based on the cell of origin.[29,30] B-ALL accounts for the majority of cases, while T-ALL is less common and often presents with a high white blood cell count or mediastinal involvement.[26,30] Genetic abnormalities play a key role in the classification and prognosis of ALL, with the Philadelphia chromosome (Ph+ ALL) being the most common cytogenetic abnormality in adult patients.[29,30] The Philadelphia chromosome results from a t(9;22) translocation, which creates the *BCR-ABL1* fusion gene, leading to uncontrolled tyrosine kinase activity and increased cell proliferation.[29] Other genetic alterations, such as *KMT2A* rearrangements, *ETV6-RUNX1* fusion, and hyperdiploidy, also influence disease progression and treatment.[26,29,30]

The clinical presentation is often nonspecific, with symptoms related to bone marrow failure, such as anemia, thrombocytopenia, and neutropenia, as well as bone and joint pain, fever, organomegaly, bruising and lymphadenopathy. In some cases, leukemic infiltration of the central nervous system may occur. Diagnosis is based on bone marrow examination, immunophenotyping, and genetic testing to guide risk stratification.[30]

Treatment consists of multi-phase chemotherapy with central nervous system prophylaxis, and in high-risk cases refractory to intensive chemotherapy, hematopoietic stem cell transplantation. In children, treatment outcomes have improved significantly, with a 5-year survival rate exceeding 90%, as a result of intensive chemotherapy regimens and risk-adapted treatment strategies.[27] In contrast, adult patients continue to face worse outcomes, with a 5-year survival rate below 50%, particularly in older populations.[28] Targeted therapies, such as tyrosine kinase inhibitors for Ph+ ALL, and immunotherapies like blinatumomab and

chimeric antigen receptor (CAR) T-cell therapy, have improved survival in relapsed and refractory cases.[28,30] However, challenges remain, particularly in adult patients, where treatment-related toxicities and long-term complications continue to impact survival and quality of life.[28,30]

While most cases of acute lymphoblastic leukemia occur sporadically, inherited genetic predisposition plays a role in a subset of patients.[26,29] Germline mutations in genes such as *PAX5*, *IKZF1*, and *TP53* have been linked to familial ALL and increased leukemia risk.[29,30]

2.3.2 Myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML)

MDS and AML are hematological malignancies that arise from myeloid progenitor cells and share overlapping genetic and clinical features.[29] MDS is a heterogeneous group of clonal hematopoietic disorders characterized by ineffective hematopoiesis, cytopenias, and dysplastic bone marrow morphology.[25,29] The disease course ranges from indolent cases requiring only supportive care to high-risk forms that progress to AML.[25,29]

AML, in contrast, is a rapidly progressing malignancy marked by the uncontrolled proliferation of immature myeloid blasts in the bone marrow and peripheral blood.[25,29] Traditionally, AML was defined by a blast threshold of 20% in the bone marrow, but modern classifications recognize that cases with 10–19% blasts exist in a transitional state between high-risk MDS and overt AML.[25,29,31] This distinction reflects the evolving understanding that MDS and AML are part of a single disease spectrum, rather than completely separate entities.

MDS primarily affects elderly individuals, with incidence significantly increasing after the age of 70. AML is also more common in older adults, with a median age at diagnosis of approximately 70 years, and its incidence increases significantly with advancing age. Risk factors for both diseases include genetic predisposition, environmental exposures, and clonal hematopoiesis.[5,25,29]

Recent classifications emphasize the molecular landscape of MDS and AML, moving beyond purely morphological criteria. Key genetic mutations include *TP53*, *SF3B1*, *RUNX1*, and *ASXL1* in MDS, with *TP53* alterations conferring a particularly poor prognosis.[25,29,31] In AML, genetic markers like *FLT3* internal tandem duplication (*FLT3*-ITD), *NPM1*, and *CEBPA* mutations guide risk stratification and targeted therapy selection.[25,29]

Patients with MDS often present with fatigue, anemia, recurrent infections, and bleeding tendency due to bone marrow failure, whereas AML symptoms develop more acutely, with rapidly progressing cytopenias, leukocytosis, and systemic complications. Diagnosis is based on bone marrow biopsy, cytogenetics, and molecular analysis.[25,29]

Treatment strategies depend on disease severity and patient characteristics.[32,33] Low-risk MDS is often managed with supportive therapy, erythropoiesis-stimulating agents, or hypomethylating agents, while high-risk MDS and AML require intensive chemotherapy, targeted therapies, or hematopoietic stem cell transplantation in eligible patients.[32,33] Newer targeted treatments have improved outcomes in AML, but prognosis remains poor in older adults, where survival rates remain below 30% at five years.[34]

While myelodysplastic syndromes and acute myeloid leukemia are typically acquired, they can also develop in individuals with an inherited genetic predisposition. Germline mutations in *RUNX1*, *GATA2*, *CEBPA*, and *DDX41*, for example, increase susceptibility to these malignancies, often manifesting as early-onset or therapy-related disease.[25,29,35] Familial MDS/AML syndromes may present with bone marrow failure, immune dysregulation, or other systemic features before leukemic transformation, highlighting the importance of genetic screening in high-risk individuals.[25,29,35]

2.3.3 Myeloproliferative neoplasms (MPNs)

MPNs are a heterogeneous group of hematological malignancies characterized by clonal proliferation of myeloid lineage cells. The latest edition of the World Health Organization (WHO) Classification of Haematolymphoid Tumours and the International Consensus Classification (ICC) have refined the classification of MPNs by integrating morphologic, molecular, and clinical attributes to improve diagnostic accuracy and risk stratification.[25,36–38] The major entities in this category include chronic myeloid leukemia, polycythemia vera (PV), essential thrombocythemia (ET), primary myelofibrosis (PMF), chronic neutrophilic leukemia, chronic eosinophilic leukemia, juvenile myelomonocytic leukemia (JMML), and myeloproliferative neoplasm, not otherwise specified (MPN-NOS).[25,36,37] These disorders are largely distinguished by clinical presentation, peripheral blood counts, and the presence of key driver mutations, particularly in the *JAK2*, *CALR*, and *MPL* genes.[25,36] The *JAK2* V617F mutation is the most common molecular aberration in MPNs, detected in almost all cases of PV and in a significant proportion of ET and PMF patients, while *CALR* and *MPL* mutations are more specific to ET and PMF but are absent in PV.[25,36]

In addition to classical MPNs, there is a hybrid category of MDS/MPNs, which includes disorders that exhibit both dysplastic and proliferative hematologic features.[25,38] This category includes chronic myelomonocytic leukemia (CMML), MDS/MPN with neutrophilia, MDS/MPN with *SF3B1* mutation and thrombocytosis (MDS/MPN-*SF3B1*-T), as well as MDS/MPN NOS.[25,37,38] These entities are characterized by both ineffective hematopoiesis and proliferative

activity, often posing diagnostic challenges and requiring integration of morphologic, cytogenetic, and molecular findings for accurate classification.[38,39]

Clinically, MPNs present with chronic proliferation of myeloid cells, leading to variable manifestations and complications.[36] PV is characterized by excessive erythrocyte production, often accompanied by leukocytosis, thrombocytosis, and an increased risk of thrombotic events.[36] ET is distinguished by sustained thrombocytosis, which predisposes patients to both thrombotic and hemorrhagic complications.[36] PMF, in contrast, follows a more aggressive course, leading to progressive bone marrow fibrosis, extramedullary hematopoiesis, cytopenias, and systemic symptoms, often culminating in secondary acute myeloid leukemia.[36] Unlike these Philadelphia chromosome-negative neoplasms, chronic myeloid leukemia is defined by the presence of the *BCR-ABL1* fusion gene, which drives uncontrolled proliferation of granulocytic precursors and, without treatment, leads to disease progression.[25]

Risk stratification in MPNs is essential for prognosis and treatment decisions. Scoring systems incorporate clinical variables and high-risk genetic mutations to predict disease progression and patient survival.[37,40,41] Myelofibrosis, in particular, is associated with a higher burden of high-risk mutations, which correlate with poor overall survival (OS) and an increased likelihood of leukemic transformation.[25,38,41]

The treatment of MPNs is guided by disease type, risk stratification, and symptom burden.[36,42] For high-risk PV and ET, cytoreductive therapy, typically with hydroxyurea or interferon-α, is recommended to reduce thrombotic complications.[36,42] In PMF, *JAK* inhibitors have significantly improved symptom control and splenomegaly reduction, particularly in intermediate- and high-risk cases.[36,42] However, for patients with advanced myelofibrosis or those with poor prognostic features, allogeneic hematopoietic stem cell transplantation remains the only curative approach, though its applicability is often limited by age and comorbidities.[41,43] Advances in genetic profiling have refined the classification and prognostication of MPNs, allowing for more personalized treatment approaches.[36,40]

2.3.4 Lymphoproliferative disorders

Lymphoproliferative disorders or lymphoid neoplasms, encompass a diverse group of hematological malignancies arising from the clonal proliferation of B cells, T cells, or natural killer cells. These disorders range from indolent conditions with slow progression to highly aggressive malignancies. According to the 5th edition of the WHO classification of hematolymphoid tumors, lymphoid neoplasms are classified based on morphology, immunophenotype, genetic alterations, and clinical behavior.[26]

This category includes acute leukemias, which have been addressed in an earlier section, as well as lymphomas, which are also part of the lymphoid neoplasms but are not covered in this discussion.[26] Instead, the focus here is on chronic lymphoproliferative disorders and plasma cell neoplasms.

Chronic lymphocytic leukemia is the most prevalent leukemia in adults, with an incidence increasing with age; the median age at diagnosis is 70 years, and it is rare in individuals under 45.[44] CLL is characterized by the clonal expansion of CD5-positive (cluster of differentiation 5) B lymphocytes, accumulating in the blood, bone marrow, and lymphoid tissues.[26,44] The disease course is highly variable, with some patients remaining asymptomatic for years, while others experience rapid disease progression.[44] While pharmacological therapies improve survival, CLL remains incurable without allogeneic hematopoietic stem cell transplantation, which is considered for younger patients with high-risk or refractory disease.[44]

Multiple myeloma is a malignancy of terminally differentiated plasma cells, primarily affecting individuals over 65 years old, with only 2% of cases occurring in patients younger than 40.[45] It typically evolves from monoclonal gammopathy of undetermined significance, which progresses to symptomatic MM at a rate of approximately 1% per year.[45,46] The disease is characterized by clonal plasma cell expansion in the bone marrow, overproduction of monoclonal immunoglobulins, osteolytic bone lesions, anemia, and renal dysfunction.[45,46]

Risk stratification in MM is based on cytogenetic abnormalities and biochemical markers.[45,47] Treatment typically includes immunomodulatory drugs, proteasome inhibitors, and monoclonal antibodies.[45] Autologous hematopoietic stem cell transplantation is the standard approach for eligible patients, whereas allogeneic HSCT offers a potential cure for selected high-risk patients, though its use is limited by high toxicity and complications.[45] The development of CAR T-cell therapy and bispecific antibodies has improved outcomes in relapsed disease.[48] Plasma cell leukemia is a rare and aggressive form of myeloma, characterized by circulating plasma cells in peripheral blood and a poor prognosis, requiring intensive therapy.[25,45]

2.3.5 Bone marrow failure (BMF)

BMF is a broad term encompassing a spectrum of disorders characterized by the inability of hematopoietic stem cells to produce adequate numbers of blood cells.[49,50] It can be classified into inherited and acquired forms, with varying implications for disease progression, treatment, and prognosis.[49,50] Inherited BMF syndromes, such as Fanconi anemia (FA), dyskeratosis congenita (DC), Diamond-Blackfan anemia (DBA), and Shwachman-Diamond syndrome (SDS), are associated with increased risks of hematological malignancies and other systemic complications.[50,51] Acquired BMF, most notably aplastic anemia (AA), is frequently

immune-mediated and can be secondary to various environmental, infectious, or idiopathic causes.[50,52]

The pathogenesis of inherited BMF syndromes is typically linked to genetic mutations affecting DNA repair, ribosomal biogenesis, or telomere maintenance.[49–51] For instance, FA results from mutations in genes involved in the FA/BRCA (Fanconi anemia/Breast Cancer susceptibility) pathway, leading to genomic instability and increased cancer susceptibility.[49,51] DC is associated with defects in telomere maintenance, which predisposes patients to premature cellular senescence and hematopoietic failure.[49,50] DBA is caused by mutations in ribosomal protein genes, impairing erythropoiesis.[49,50]

In contrast, acquired aplastic anemia is largely mediated by autoimmune mechanisms. Autoreactive cytotoxic T lymphocytes attack hematopoietic progenitors, leading to bone marrow hypocellularity and pancytopenia.[49,50] Environmental triggers such as viral infections, toxins, drugs, or radiation exposure have been implicated in acquired BMF, though the precise mechanisms remain incompletely understood.[53]

Patients with BMF typically present with signs of cytopenias, including anemia-related fatigue, thrombocytopenia-associated bleeding, and neutropenia-driven infections.[49,50] Inherited forms often have syndromic features, such as congenital anomalies in Fanconi anemia or skin and nail abnormalities in dyskeratosis congenita.[49,50] Diagnosis involves a combination of clinical assessment, bone marrow biopsy, and genetic or molecular testing to differentiate between inherited and acquired etiologies.[49,50]

The treatment of BMF varies based on etiology, severity, and patient-specific factors. Hematopoietic stem cell transplantation is the preferred curative option for many inherited and severe acquired cases, particularly in younger patients with matched donors.[49] In acquired aplastic anemia, first-line treatment consists of immunosuppressive therapy with cyclosporine and anti-thymocyte globulin, combined with a thrombopoietin receptor agonist in severe cases without a suitable HSCT donor.[49,52,54]

The prognosis of BMF has improved significantly with advances in supportive care, transplantation techniques, and targeted therapies.[49,51,55] However, long-term risks such as clonal evolution to myelodysplastic syndromes or acute myeloid leukemia remain concerns, particularly in inherited forms.[51,55]

2.3.6 Other hematological disorders

In addition to the previously discussed hematological malignancies and bone marrow failure, rare and severe hematological disorders encompass a diverse group of conditions affecting blood cells and hematopoietic function. These diseases can lead to severe cytopenias, systemic inflammation, or progressive organ damage.

They can be categorized by the primary cell type or system involved, including histiocytic and dendritic cell disorders, mast cell disorders, erythroid disorders, bone disorders, and metabolic disorders with hematologic involvement. While many rare disorders exist, this section focuses on five conditions with representation in our patient cohorts: histiocytosis, mastocytosis, hemoglobinopathies, osteopetrosis, and adrenoleukodystrophy.

Histiocytosis comprises a heterogeneous group of disorders characterized by the proliferation of histiocytes, which can lead to systemic inflammation and organ dysfunction.[25,56–58] Key subtypes include Langerhans cell histiocytosis (LCH), Erdheim-Chester disease (ECD), and hemophagocytic lymphohistiocytosis (HLH).[25,57,58] LCH is a clonal neoplasm driven by MAPK pathway mutations, often affecting bones, skin, and the central nervous system.[25,56] ECD is a non-Langerhans histiocytosis frequently associated with *BRAF* V600E mutations and characterized by multi-organ involvement, including cardiovascular and skeletal manifestations .[25,58] HLH is a hyperinflammatory syndrome, which results from immune dysregulation and is associated with cytokine storm and multi-organ failure.[25,57] HSCT is considered in refractory cases of HLH and select patients with severe LCH or ECD.[25,56–58]

Mastocytosis is a clonal disorder characterized by the abnormal proliferation and accumulation of mast cells in one or more organs.[25] It can be classified into cutaneous mastocytosis, which primarily affects the skin, and systemic mastocytosis (SM), which involves the bone marrow and other organs.[25,59,60] SM is further divided into bone marrow, indolent, smoldering, and aggressive mastocytosis, and mast cell leukemia subtypes.[25,61] Mast cell proliferation is typically driven by constitutive activation of the KIT receptor, and the majority of cases are associated with *KIT* D816V mutations.[25,59] Clinical manifestations vary from mild mediator-related symptoms (flushing, anaphylaxis) to severe organ dysfunction.[25,61,62] Treatment is based on disease severity, ranging from symptomatic management in indolent cases to cytoreductive therapies and hematopoietic stem cell transplantation in advanced SM.[25,59,63]

Hemoglobinopathies are inherited disorders affecting hemoglobin structure or production, with sickle cell disease and thalassemia being the most clinically significant.[64–67] Sickle cell disease is caused by biallelic point mutations in the β-globin gene, causing hemoglobin polymerization leading to hemolysis, vaso-occlusive crises, and progressive organ damage.[67] Thalassemia results from impaired globin chain synthesis leading to an imbalance with globin chains.[65,68] α-thalassemia results from deletions or mutations affecting one to four α-globin gene copies, reducing α-globin chain production.[68] Disease severity depends on the number of affected genes, ranging from an asymptomatic carrier state and mild anemia to chronic hemolytic anemia and lethal hydrops fetalis.[64,69] β-thalassemia results from mutations or deletions in β-globin gene, leading to ineffective

erythropoiesis.[65] The disease spectrum ranges from the asymptomatic carrier state to moderate (β-thalassemia intermedia) or severe transfusion-dependent anemia (β-thalassemia major).[65,70] Management of hemoglobinopathies includes regular blood transfusions and iron chelation therapy to prevent iron overload and associated complications.[67,69,70] For severe cases, hematopoietic stem cell transplantation remains the only curative option, while emerging gene therapies offer promising alternatives.[67,69,70]

Osteopetrosis is a rare genetic disorder caused by defective osteoclast-mediated bone resorption, leading to increased bone density and skeletal fragility.[71] The disease varies in severity, with autosomal recessive osteopetrosis presenting in infancy with bone marrow failure, cranial nerve compression, and recurrent fractures, while autosomal dominant osteopetrosis is typically milder and diagnosed in adolescence or adulthood.[71] HSCT is the only curative option for severe forms as it restores osteoclast function.[71]

X-linked adrenoleukodystrophy is a metabolic disorder caused by mutations in *ABCD1*, leading to the accumulation of very-long-chain fatty acids and progressive demyelination of the central nervous system.[72,73] The disease manifests in childhood as cerebral adrenoleukodystrophy, adolescent/adult adrenomyeloneuropathy, or adrenal insufficiency.[72] HSCT is the only established therapy for early-stage cerebral adrenoleukodystrophy, preventing further neurological decline, but it does not reverse existing damage.[72,73]

2.3.7 Inborn errors of immunity (IEIs)

Inborn errors of immunity, previously known as primary immunodeficiencies, encompass a broad and expanding spectrum of genetic disorders affecting immune system function.[74,75] These conditions can manifest as increased susceptibility to infections, immune dysregulation, autoimmunity, autoinflammation, allergy, and predisposition to malignancy.[74,75] Recent advances in genetic and molecular technologies have significantly enhanced the identification, classification, and treatment of these disorders, with a total of approximately 500 genetic defects now recognized in relation to IEIs.[74–77]

The early identification of IEIs is crucial, as it enables timely interventions that can significantly improve clinical outcomes.[75] Newborn screening programs, particularly for severe combined immunodeficiency (SCID), have revolutionized early detection and management.[78,79] These programs facilitate early hematopoietic stem cell transplantation before severe infections develop, leading to improved survival rates.[78,79] The role of next-generation sequencing in diagnosing IEIs has grown substantially, improving the ability to detect both common and rare immune defects as many IEIs present with variable clinical phenotypes, complicating early recognition.[75,76,80] Comprehensive genomic evaluations have demonstrated that

many previously undiagnosed immune disorders are linked to genetic mutations.[76,77]

Hematopoietic stem cell transplantation remains the definitive curative treatment for many IEIs, particularly for SCID.[79] Advances in gene therapy also hold promise for correcting specific monogenic defects, providing a potential alternative for patients without suitable donors.[75,80]

2.4 Germline predisposition to hematological malignancies

Inherited genetic predisposition significantly contributes to the risk of developing hematological malignancies, affecting both children and adults. Approximately 5–15 % of hematological malignancies are now recognized as arising from germline predisposition mutations, with growing awareness due to advances in genetic diagnostics.[81–83] These inherited factors typically follow autosomal dominant inheritance patterns, although penetrance and phenotypic expressivity can vary widely, even within affected families.[82,84,85] Germline mutations may lead directly to hematological malignancies or predispose carriers through syndromes characterized by pre-existing organ dysfunctions, platelet disorders, or bone marrow failure.[25,29,86]

The expanding identification of germline predisposition syndromes has emphasized the clinical importance of early genetic diagnosis, personalized monitoring, and informed treatment decisions, particularly regarding donor selection for hematopoietic stem cell transplantation.[29,85,87] The complexity of these syndromes necessitates comprehensive genetic counseling and careful clinical management to optimize patient outcomes and reduce secondary malignancy risks.[81,83,86] This chapter provides an overview of key genes and syndromes involved in hereditary predisposition to hematological malignancies, aiming to clarify clinical implications, diagnostic approaches, and management strategies informed by the latest genetic research.

In addition, inherited cancer predisposition often arises from germline mutations in genes that govern fundamental cellular processes, such as DNA repair, cell cycle regulation, apoptosis, and chromatin remodeling.[88] While many predisposition genes are associated with specific cancer types, there is substantial overlap in cancer risk across different tissues due to the broad functional impact of these pathways.[89] For example, alterations in DNA damage response genes like *BRCA1/2*, *ATM*, and *CHEK2* are linked to a spectrum of malignancies, including both solid tumors and hematological cancers.[88,89] Although these genes are predominantly associated with solid tumors, they have also been reported in hematological malignancies, typically as rare findings and often considered low- to moderate-risk genes in this context.[90,91] Their exact role in the pathogenesis of

hematological malignancies remains unclear, highlighting the need for further research.[91] This shared vulnerability underscores the importance of considering broad genetic panels in cancer predisposition testing, as well as the clinical relevance of detecting germline variants even in unexpected cancer types.

2.4.1 Genes with an inherited risk of hematological malignancies

Germline mutations in the *CEBPA* (CCAAT/enhancer-binding protein alpha) gene significantly predispose individuals to AML, characterized by autosomal dominant inheritance and high penetrance.[85,92–94] These mutations typically occur at the N-terminal region, resulting in disrupted protein translation and an increased expression of the truncated p30 isoform, which impairs normal myeloid differentiation.[92,94] Leukemic transformation frequently involves acquiring an additional somatic mutation in the C-terminal domain of *CEBPA*, further compromising its function.[92,93] AML linked to germline *CEBPA* mutations typically presents at a younger age and has a favorable initial prognosis, but often recurs as independent leukemic episodes, necessitating careful monitoring and tailored treatment strategies.[93,94]

Germline mutations in the *DDX41* (DEAD-box helicase 41) gene represent one of the most common inherited factors predisposing adults to myeloid malignancies, notably MDS and AML.[95,96] These mutations follow an autosomal dominant inheritance pattern and typically consist of loss-of-function variants, primarily truncating or frameshift mutations, manifesting clinically later in adulthood (median onset around 65–70 years).[95–97] Affected individuals frequently acquire a secondary somatic mutation — most commonly the recurrent p.Arg525His variant — leading to biallelic impairment and subsequent disease development.[96,97] Clinically, these conditions often exhibit bone marrow hypocellularity and preceding cytopenias, and typically follow an indolent course with a comparatively favorable prognosis.[95,96] Given the hereditary nature and clinical significance of *DDX41* mutations, regular surveillance, and cautious selection of donors for hematopoietic stem cell transplantation are essential components of management.[95,97]

Biallelic germline mutations in the *ERCC6L2* (ERCC excision repair 6 like 2) gene predispose individuals to severe hematological disorders, primarily characterized by BMF and a significantly elevated risk of developing myeloid malignancies, particularly MDS and AML.[98–100] *ERCC6L2* functions crucially in DNA replication, DNA repair pathways including non-homologous end joining, and maintaining genomic stability by alleviating replication stress at complex chromosomal regions, such as centromeres.[100–102] Clinically, *ERCC6L2*-related disease typically presents as early-onset BMF that often progresses into MDS and AML, frequently characterized by somatic *TP53* mutations and erythroid

predominance.[99,100] The hematological phenotype is severe despite relatively mild peripheral blood abnormalities, underscoring the necessity for timely diagnosis and regular surveillance.[100] Due to the aggressive nature of *ERCC6L2*-related malignancies and poor prognosis once leukemic transformation occurs, early intervention with hematopoietic stem cell transplantation is recommended.

Germline mutations in *RUNX1* (RUNX family transcription factor 1), a transcription factor crucial for hematopoiesis, cause an autosomal dominantly inherited condition known as familial platelet disorder with predisposition to myeloid malignancies.[103,104] Individuals carrying these mutations typically exhibit mild-to-moderate thrombocytopenia and platelet dysfunction, with a significantly increased risk of developing MDS and AML, among other hematological malignancies.[103,105,106] Leukemic transformation in *RUNX1*-associated familial disease involves acquiring secondary somatic mutations, frequently affecting genes such as *TET2*, *DNMT3A*, *ASXL1*, and *BCOR*, leading to clonal hematopoiesis and eventual malignant progression.[106,107] Although penetrance is incomplete, approximately 35-45% of mutation carriers develop hematological malignancies over their lifetime, underscoring tailored clinical management including consideration for hematopoietic stem cell transplantation.[103,104,108]

Germline mutations in *ANKRD26* (Ankyrin Repeat Domain Containing 26) cause an autosomal dominant disorder known as *ANKRD26*-related thrombocytopenia, characterized by moderate thrombocytopenia, normal platelet size, and increased predisposition to hematological malignancies, especially myeloid neoplasms such AML, MDS, and CMML.[109,110] Pathogenic mutations occur predominantly in the 5' untranslated region of the *ANKRD26* gene, disrupting the inhibitory binding of transcription factors *RUNX1* and *FLI1*, leading to persistent *ANKRD26* overexpression in megakaryocytes.[110,111] This aberrant overexpression causes hyperactivation of the thrombopoietin/MPL signaling pathway, particularly via MAPK/ERK1/2 signaling, which impairs normal proplatelet formation and contributes to leukemic predisposition.[109,111] The variable penetrance and risk for malignancy necessitate ongoing surveillance and individualized management strategies for affected individuals and their families.[94,109]

Germline mutations in *ETV6* (ETS variant transcription factor 6), encoding an essential transcription factor involved in hematopoiesis, cause an autosomal dominant disorder characterized primarily by mild to moderate thrombocytopenia and significantly increased risk of hematological malignancies, particularly ALL and less commonly, AML and MDS.[112–115] These mutations typically involve missense, nonsense, or frameshift variants affecting primarily the ETS domain responsible for sequence specific DNA-binding and protein-protein interaction and lead to impaired transcriptional repression, altered megakaryocyte differentiation, and defective platelet formation.[113,114] Although platelet counts are generally low, platelet size remains normal, distinguishing *ETV6*-related thrombocytopenia from

many other inherited platelet disorders.[113] The predisposition to malignancy, particularly childhood B-cell ALL, underscores the importance of early genetic screening, vigilant clinical surveillance, and tailored management strategies in affected families.[94,112,115]

Germline mutations in *TP53* (Tumor Protein P53), a tumor suppressor gene, cause Li-Fraumeni syndrome (LFS), an autosomal dominant condition characterized by a high risk of various malignancies, including hematological cancers such as AML, MDS, ALL, and therapy-related myeloid neoplasms.[84,94,116,117] *TP53* mutations often result in loss-of-function, severely impairing DNA repair, cell cycle regulation, and apoptosis, thus predisposing individuals to early cancer development and secondary malignancies following therapy.[116,118] LFS-associated hematological malignancies frequently exhibit complex karyotypes, aggressive disease courses, resistance to conventional therapies, and poor clinical outcomes.[116–118] Importantly, germline *TP53* mutations substantially increase the risk of therapy-related leukemias and secondary malignancies, underscoring the need for personalized treatment and surveillance strategies, and cautious consideration of treatment-related risks.[94,116,117]

Germline mutations in *PAX5* (Paired Box 5), a critical transcription factor essential for B-cell lineage differentiation, predispose individuals primarily to familial B-cell precursor acute lymphoblastic leukemia in an autosomal dominant inheritance pattern with incomplete penetrance.[119–121] Mutations commonly involve highly conserved DNA-binding domains, notably the paired domain or the octapeptide domain, leading to impaired DNA-binding affinity, disruption of normal transcriptional regulation, and compromised B-cell differentiation.[119–121] Leukemic progression requires additional somatic genetic alterations, often involving loss of the wild-type *PAX5* allele, resulting in biallelic dysfunction and leukemogenesis.[121,122] Germline *PAX5*-associated leukemia generally manifests at various ages, ranging from early childhood to adulthood, with diverse cytogenetic abnormalities and clinical outcomes, emphasizing the necessity for tailored surveillance and family screening in affected individuals.[119,120,122]

Germline mutations in *IKZF1* (IKAROS Family Zinc Finger 1), encoding the transcription factor essential for lymphoid differentiation and hematopoiesis, predispose individuals primarily to B-ALL, typically following an autosomal dominant inheritance pattern with incomplete penetrance.[123–125] Mutations predominantly affect DNA-binding zinc-finger domains, impairing IKAROS function through loss of DNA binding, abnormal nuclear localization, altered transcriptional regulation, and disturbed hematopoietic differentiation.[123,124] Germline *IKZF1* variants contribute to aberrant cell adhesion, altered bone marrow niche interactions, and reduced sensitivity to antileukemic therapies, emphasizing their clinical impact in leukemogenesis and disease prognosis.[123,125] Recognition of

germline *IKZF1* mutations is vital for targeted surveillance and tailored therapeutic management in affected individuals and families.[124,125]

2.4.2 Syndromes with a risk of hematological malignancies

GATA2 deficiency, caused by germline mutations in *GATA2*, encoding a critical hematopoietic transcription factor, predispose individuals to a spectrum of myeloid malignancies, including MDS, AML, and CMML, with an autosomal dominant inheritance and incomplete penetrance.[85,126–129] Patients typically present during adolescence or early adulthood, manifesting with cytopenias, immunodeficiency (characterized by monocytopenia, B and NK cell lymphopenia), and a heightened susceptibility to infections, particularly mycobacterial and viral.[126,128] Pathogenic *GATA2* mutations include truncating variants, missense mutations affecting the zinc finger 2 domain, and noncoding alterations in regulatory enhancer regions, all leading predominantly to haploinsufficiency.[127–129] Progression to advanced myeloid malignancies frequently involves secondary somatic events, notably monosomy 7, trisomy 8, and mutations in genes such as *ASXL1* and *STAG2*.[126,129] Given the high risk of malignancy and severe infections, early identification, close monitoring, and timely consideration of hematopoietic stem cell transplantation are essential components in managing GATA2 deficiency.[126,128]

Germline mutations in *SAMD9* and *SAMD9L*, located on chromosome 7q21, predispose individuals to pediatric MDS, BMF, and AML, frequently associated with monosomy 7.[130–132] These gain-of-function mutations typically exhibit autosomal dominant inheritance with variable penetrance and diverse clinical outcomes, ranging from transient cytopenias and spontaneous recovery through hematopoietic revertant mosaicism, to rapid progression toward severe hematological malignancies.[132–134] *SAMD9* mutations are specifically linked to MIRAGE syndrome, characterized by multisystem involvement including growth restriction, immunodeficiency, adrenal insufficiency, and genital anomalies, alongside significant hematological risks.[130,132] Conversely, *SAMD9L* mutations are associated with ataxia-pancytopenia syndrome, presenting neurological abnormalities such as cerebellar dysfunction and significant predisposition to myeloid malignancies, particularly with monosomy 7.[131,134] Clinical management involves careful monitoring and individualized treatment strategies, including early consideration of hematopoietic stem cell transplantation, given the high risk of leukemic transformation.[94,132,133]

Fanconi anemia is a rare, predominantly autosomal recessive syndrome caused by germline mutations in at least 22 genes involved in the FA/BRCA DNA repair pathway, leading to genomic instability and hypersensitivity to DNA crosslinking agents.[135–137] FA is characterized by congenital malformations, progressive bone marrow failure, endocrine dysfunction, and a markedly elevated risk for

hematological malignancies, primarily MDS and AML.[138–140] Individuals with FA exhibit extreme chromosome fragility, which correlates with disease severity, onset of bone marrow failure, and increased cancer risk.[140] Hematopoietic stem cell transplantation remains the primary curative treatment for hematological complications; however, HSCT itself does not eliminate the increased risk of solid malignancies, such as squamous cell carcinomas, which persist lifelong.[139,141] The prognosis of FA strongly depends on the genetic subtype and mutation type, with varying clinical outcomes and malignancy risks across different genotypes.[135,137,140]

Shwachman-Diamond syndrome is an autosomal recessive inherited bone marrow failure syndrome (BMFS), characterized by exocrine pancreatic insufficiency, neutropenia, skeletal abnormalities, and significantly increased risk of hematological malignancies, predominantly MDS and AML.[142–144] Most patients (~90%) carry biallelic mutations in the *SBDS* gene, crucial for ribosome maturation and cellular proliferation, resulting in impaired ribosomal function and severe translational stress.[142,145,146] Hematological malignancies occur in 10–30% of SDS patients and are associated with poor prognosis due to therapy-resistant disease and significant treatment-related toxicities.[144,147] Somatic clonal evolution, including recurrent mutations in genes such as *TP53* and *EIF6*, significantly influences disease progression and malignant transformation.[146,148] Early diagnosis, rigorous hematological surveillance, and timely consideration of hematopoietic stem cell transplantation remain essential in managing patients with SDS to mitigate malignant progression and improve outcomes.[144,147]

Telomere biology disorders are a heterogeneous group of inherited conditions characterized by critically short telomeres, resulting from germline mutations in genes essential for telomere maintenance.[149–151] These disorders include Dyskeratosis congenita as the prototypic syndrome, along with more severe pediatric variants such as Hoyeraal-Hreidarsson, Revesz, and Coats plus syndromes.[149–151] DC and related TBDs exhibit multisystem clinical manifestations including mucocutaneous features, progressive bone marrow failure, pulmonary fibrosis, liver abnormalities, and notably a high risk of hematological malignancies, particularly MDS and AML.[149,152,153] Inheritance patterns can be autosomal dominant, autosomal recessive, X-linked recessive, or arise de novo, with autosomal recessive and X-linked forms often associated with more severe phenotypes and earlier disease onset, leading to worse clinical outcomes and higher malignancy risk.[150,152] Due to significant cancer predisposition and progressive nature of TBDs, vigilant clinical surveillance and individualized management approaches are essential in patient care.[149,152,153]

Severe congenital neutropenia (SCN) encompasses a group of inherited bone marrow disorders characterized by profound neutropenia, impaired granulocyte maturation, and heightened susceptibility to life-threatening bacterial infections from early infancy.[154–156] Germline mutations in SCN involve multiple genes,

notably *ELANE*, *HAX1*, and *G6PC3*, among others, with inheritance patterns ranging from autosomal dominant to autosomal recessive.[154,157] Individuals with SCN have an increased lifelong risk of developing MDS and AML.[154,158] Granulocyte-colony-stimulating factor therapy has improved survival significantly but does not eliminate the risk of malignant transformation; thus, hematopoietic stem cell transplantation remains the only curative treatment for selected high-risk patients.[155,158]

Diamond-Blackfan anemia is a rare inherited bone marrow failure syndrome primarily characterized by congenital erythroid aplasia, various congenital anomalies, and a significantly elevated risk of hematological malignancies, mainly MDS and AML.[159,160] DBA is predominantly caused by heterozygous mutations in ribosomal protein genes, disrupting ribosomal RNA processing and causing nucleolar stress, p53 activation, cell-cycle arrest, and apoptosis, which collectively impair erythroid differentiation and increase the risk of malignant transformation.[160] Approximately 20-30% of DBA patients progress to hematological malignancies by adulthood, with cancer risks further exacerbated by prolonged steroid use and chronic iron overload due to repeated transfusions.[159,160] Optimal clinical management involves regular hematologic and cancer surveillance, early iron chelation therapy, and timely consideration of hematopoietic stem cell transplantation.[159,160]

RASopathies are a heterogeneous group of genetic syndromes caused by germline mutations in genes encoding components of the RAS/MAPK signaling pathway, leading to dysregulated cellular proliferation, differentiation, and survival.[161] Syndromes within this group include neurofibromatosis type 1 (NF1), Noonan syndrome, *CBL* syndrome (Noonan syndrome-like disorder), cardiofaciocutaneous syndrome, and Costello syndrome, each characterized by distinct yet overlapping clinical features and increased predisposition to malignancies, especially JMML.[29,161,162] JMML associated with NF1 occurs through germline heterozygous NF1 mutations and a subsequent somatic second-hit event, causing loss of neurofibromin function and hyperactivation of RAS signaling, with frequent somatic loss of heterozygosity via uniparental disomy.[161,163] Similarly, JMML linked to Noonan syndrome and *CBL* syndrome involves germline mutations in *PTPN11* or *CBL*, respectively, accompanied by acquired somatic alterations — often monosomy 7 or loss-of-heterozygosity.[164–166] Understanding the molecular basis of these RASopathies and their unique mutation patterns is essential for accurate diagnosis, risk assessment, and personalized clinical management.[29,161,166]

Several other constitutional syndromes are associated with significant risks for hematological malignancies. Down syndrome (trisomy 21) markedly elevates the risk for hematological cancers, especially ALL and AML, often presenting in childhood.[167] Bloom syndrome, an autosomal recessive chromosomal instability

disorder characterized by severe growth restriction, immunodeficiency, and early onset of cancers, frequently presents hematological malignancies.[168] Constitutional mismatch repair deficiency, resulting from biallelic mutations in DNA mismatch repair genes (*MLH1*, *MSH2*, *MSH6*, and *PMS2*), manifests predominantly in childhood with aggressive and early-onset malignancies, including hematological cancers.[169] Nijmegen breakage syndrome, caused by biallelic mutations in the *NBN* gene, is associated with immunodeficiency, radiosensitivity, and high risk of hematological malignancies, especially lymphoid neoplasms, due to impaired DNA repair and chromosomal instability.[170,171] Ataxia-telangiectasia is an autosomal recessive disorder resulting from mutations in the *ATM* gene characterized by progressive neurological decline, immunodeficiency, and markedly elevated risk for lymphoid malignancies, reflecting defective DNA damage response and genomic instability.[172,173]

2.4.3 Clinical significance of inherited mutations

Inherited mutations significantly influence clinical outcomes in hematological malignancies, impacting prognosis, treatment decisions, and surveillance strategies. Germline predisposition is associated with earlier disease onset, unique disease phenotypes, and distinctive clinical courses compared to sporadic cases.[83,85,174] Inherited mutations can be categorized based on their underlying molecular pathways, highlighting specific risks and management challenges. For example, mutations in DNA repair genes (e.g., mutations in *TP53* associated with Li-Fraumeni syndrome and Fanconi anemia-related genes) confer a heightened risk not only for hematological malignancies but also for solid tumors and therapy-related cancers.[117,135,137] Mutations affecting hematopoietic transcription factors are typically associated with distinctive hematological manifestations, including cytopenias or immunodeficiency, and predispose primarily to myeloid malignancies.[104,107,126] Additionally, inherited defects may concurrently predispose individuals to hematological malignancies and reproductive disorders such as azoospermia.[175,176] Defects in biological pathways regulating genomic integrity, specifically in DNA repair genes, have been implicated in both impaired spermatogenesis and elevated cancer risk due to their essential role in maintaining genomic stability during germ cell development.[176,177] For example, biallelic *FANCA* and *FANCM* mutations have been identified in individuals with non-obstructive azoospermia, with some cases also showing mild hematological abnormalities suggestive of previously unrecognized Fanconi anemia.[178–180] Understanding these overlapping genetic susceptibilities and pathway-specific risks helps tailor clinical surveillance, therapeutic strategies, and fertility preservation appropriately for patients with inherited mutations.

Management of individuals with germline predisposition to hematological malignancies requires coordinated multidisciplinary care involving hematologists, genetic counselors, transplant specialists, and psychosocial support providers. For individuals with an identified genetic predisposition, surveillance typically includes regular monitoring for early detection of disease progression or malignant transformation.[94,181] Recognizing these germline variants is crucial in selecting compatible donors and planning conditioning regimens, as unrecognized inherited conditions significantly increase risks for transplant-related complications, including severe graft-versus-host disease, organ toxicity, graft rejection, and secondary malignancies.[182,183] It is also important to identify affected family members when considering potential stem cell donors, to avoid transplant-related complications and improve outcomes. Identifying specific germline mutations enables tailored prophylactic measures and conditioning adjustments aimed at minimizing transplant-related complications and optimizing overall outcomes.[83,94,174,182]

Additionally, patients benefit from preventive measures including lifestyle modifications, infection prophylaxis, iron chelation therapy to mitigate transfusion-related iron overload, and fertility preservation strategies before initiating treatments that could impair reproductive capability.[94,174]

2.4.4 Diagnostic approaches and genetic testing

Diagnostic evaluation for germline predisposition to hematological malignancies involves a thorough clinical assessment, including detailed personal and family history, physical examination, and identification of patterns consistent with hereditary predisposition. Comprehensive next-generation sequencing panels targeting known predisposition genes and others are increasingly utilized to identify pathogenic or likely pathogenic variants, enabling accurate diagnosis and familial risk assessment.[83,94] Accurate tissue sampling is essential for differentiating germline from somatic variants; thus, testing typically involves cultured skin fibroblasts rather than peripheral blood or bone marrow samples, which may harbor somatic mutations.[94] Genetic counseling is critical throughout the diagnostic process, providing clarity about test results, implications for management, and importance of cascade testing among relatives.[85,174,181] Incorporating genetic testing into standard clinical practice facilitates timely diagnosis and personalized management strategies, significantly enhancing patient care.

2.4.5 Ethical considerations in genetic testing

Genetic counseling for germline predisposition to hematological malignancies involves addressing multiple ethical dimensions, including informed consent, confidentiality, and disclosure of genetic risk to family members. Genetic counselors face the challenge of balancing individual patient autonomy and privacy with ethical obligations toward informing at-risk relatives who could benefit from preventive surveillance or interventions.[184] Ethical issues also arise in managing uncertain or incidental genetic findings, such as uncovering unrelated hereditary cancer syndromes during broad panel testing, and navigating potential implications associated with hereditary diagnoses.[83,184] Counselors must ensure clear, comprehensive communication about medical, psychological, and social implications of genetic testing results, enabling informed decision-making.[174,184]

2.5 Hematopoietic stem cell transplantation (HSCT)

Hematopoietic stem cell transplantation has evolved dramatically over recent decades, becoming an essential and potentially curative treatment for a variety of malignant and non-malignant hematological diseases.[185,186] Originally developed over 60 years ago, HSCT utilizes hematopoietic stem cells derived from bone marrow, peripheral blood, or umbilical cord blood to reconstitute a patient's hematopoietic system following high-dose chemotherapy with or without radiation.[186]

The annual global activity in HSCT continues to increase, driven by advancements in donor selection, improved conditioning regimens, and enhanced supportive care strategies, leading to better patient outcomes and expanded eligibility.[186–189] Allogeneic HSCT has notably risen in prevalence due to improved techniques such as haploidentical transplants and the widespread adoption of peripheral blood stem cells as a primary stem cell source, reflecting greater donor availability and improved engraftment success.[185,186,188]

Today, indications for HSCT span a broad spectrum, including hematological malignancies as well as inherited conditions like bone marrow failure syndromes and hemoglobinopathies.[185,188,189] Despite advances, HSCT remains associated with significant risks and complications, such as graft-versus-host disease (GVHD), infections, graft failure, and late effects, highlighting the importance of multidisciplinary care and comprehensive patient management strategies to optimize outcomes and quality of life post-transplant.[190,191]

2.5.1 Indications and patient selection

Hematopoietic stem cell transplantation has become an essential therapeutic approach for a diverse array of hematological conditions.[192] Its indications broadly include malignant disorders such as acute leukemia, myelodysplastic syndromes, myeloproliferative neoplasms, and lymphomas, as well as nonmalignant disorders like bone marrow failure syndromes, hemoglobinopathies, inherited metabolic disorders, and immunodeficiencies.[188,189,192,193] The selection of appropriate candidates for transplantation involves careful assessment of both disease-related and patient-related factors.[192,194]

Patient selection criteria include disease risk stratification, patient's overall health status, age, and comorbidity profile. Tools such as the European Society for Blood and Marrow Transplantation (EBMT) Acute Leukemia score and the Disease Risk Index have been developed to predict post-transplant outcomes, helping clinicians identify patients who are most likely to benefit from HSCT.[194,195] For instance, the Disease Risk Index categorizes patients based on the specific disease, cytogenetics, and remission status at transplantation, which correlates strongly with overall survival and progression-free survival, primarily due to variations in relapse risk.[194]

Patient comorbidities significantly influence transplant-related risks.[196] The Hematopoietic Cell Transplantation Comorbidity Index quantifies these risks by categorizing patients into low, intermediate, and high-risk groups, predicting non-relapse mortality (NRM) and OS post-transplant.[197] This index is widely utilized to assess patient suitability and tailor transplant conditioning regimens accordingly.

Particularly in nonmalignant diseases, HSCT aims at correcting underlying hematologic deficiencies without the added benefit of graft-versus-leukemia effects; thus, reduced-intensity conditioning regimens and donor selection strategies are carefully employed to minimize complications.[198] Age alone is no longer an exclusion criterion; rather, biological age, assessed via comorbidities and performance status, is considered critical in decision-making.[197]

Overall, patient selection for HSCT is guided by an intricate balance between potential risks and the curative benefits, necessitating a comprehensive pre-transplant evaluation to optimize patient outcomes and resource utilization.[192,193]

2.5.2 Treatment protocol and conditioning regimens

Allogeneic hematopoietic stem cell transplantation involves a complex, multi-step clinical protocol, beginning with patient evaluation, donor selection, and stem cell procurement, followed by conditioning therapy, transplantation, and comprehensive post-transplant care.

Initially, patients undergo thorough clinical assessments to determine their suitability for transplantation, including evaluation of disease status, overall

physical health, comorbidities, and performance status. Optimal donor selection involves careful matching of human leukocyte antigen (HLA) types to reduce the risk of graft-versus-host disease and graft rejection.[199] Sources of hematopoietic stem cells include peripheral blood, bone marrow, or umbilical cord blood, each with distinct clinical considerations and implications for transplantation outcomes.[200,201]

Conditioning therapy is a crucial preparatory step designed to eradicate residual malignant cells, suppress the recipient's immune response to prevent rejection, and create a conducive bone marrow environment for engraftment. Conditioning regimens are broadly classified into myeloablative conditioning (MAC), reduced-intensity conditioning (RIC), and non-myeloablative conditioning.[202] MAC regimens involve high-dose chemotherapy, often combined with total body irradiation (TBI), and are typically reserved for younger, medically fit patients due to their potential for significant organ toxicity.[203–205] RIC and non-myeloablative conditioning regimens use lower chemotherapy doses, minimizing toxicity while maintaining sufficient immunosuppressive effects to facilitate engraftment.[206] These less-intensive regimens have expanded the eligibility of HSCT to include older patients and those with substantial comorbidities.[207]

Following conditioning, donor stem cells are administered intravenously, homing to the bone marrow and repopulating hematopoietic cells, a process known as engraftment. Successful engraftment is closely monitored through regular blood counts and assessments of donor chimerism. Post-transplant care involves rigorous management of potential complications such as GVHD, infections, and graft failure, alongside long-term follow-up to monitor disease recurrence and manage late effects of treatment.[190,191,208–210]

Comprehensive supportive care strategies, including prophylactic antibiotics, antiviral and antifungal therapies, nutritional support, and psychosocial care, are integral components of the HSCT process, essential for optimizing patient outcomes and quality of life post-transplant.[211,212]

2.5.3 Donor selection

Donor selection is a critical factor determining the success of allogeneic hematopoietic stem cell transplantation. The process involves careful evaluation of several key criteria to minimize transplant-related complications, optimize graft-versus-leukemia effects, and improve overall patient survival.

HLA matching remains the cornerstone of donor selection. Optimal outcomes are generally achieved with fully HLA-matched donors. Mismatches at the critical loci significantly increase the risk of GVHD, graft rejection, and overall transplant-related mortality.[199,213,214] When multiple equally matched donors are available, additional donor-specific characteristics such as age, gender, blood group

compatibility, and cytomegalovirus serostatus become important secondary considerations.[199,213,214]

Donor age is particularly influential, with younger donors (typically aged 18–30 years) associated with better transplant outcomes and improved overall survival.[215,216] Additionally, the sex of the donor can influence outcomes, as male donors have been associated with better event-free survival rates compared to female donors, irrespective of recipient sex.[199,215]

For patients without a fully matched sibling or unrelated donor, alternative donor sources such as haploidentical family donors and, to a lesser extent, umbilical cord blood provide viable options.[199,214] Haploidentical transplants have become increasingly popular due to the availability of effective post-transplant immunosuppressive regimens, significantly expanding the donor pool and offering comparable outcomes to matched unrelated donors.[199,214]

In summary, selecting the most suitable donor involves meticulous balancing of HLA compatibility, donor age, gender, viral serostatus, and emerging predictive modeling techniques, all aimed at enhancing transplantation outcomes and patient survival.

2.5.4 Complications and follow-up

Hematopoietic stem cell transplantation is associated with numerous complications that significantly impact patient outcomes and quality of life, requiring meticulous follow-up care and monitoring.[211,217,218] Complications following HSCT can be broadly categorized into acute and chronic, with varying presentations depending on patient-specific factors, donor type, conditioning regimen, and underlying disease.[211,218]

Acute complications typically occur within the first 100 days post-transplant and include infections, GVHD, organ toxicity, and graft failure.[218] GVHD, resulting from immune reactions between donor T-cells and recipient tissues, is one of the most severe complications, contributing substantially to transplant-related morbidity and mortality.[208,218] Prophylactic strategies using immunosuppressive agents, post-transplant cyclophosphamide, and graft manipulation have significantly reduced the incidence of severe GVHD but remain critical to manage optimally.[208]

Chronic complications represent a major concern for long-term survivors, often occurring months to years after transplantation. Among these, chronic GVHD remains a persistent risk factor for late morbidity.[219] Other chronic issues include secondary malignancies, organ dysfunction such as cardiac, pulmonary, hepatic, renal, endocrine disturbances, and musculoskeletal complications.[210,217]

Late mortality and secondary neoplasms constitute severe long-term risks for survivors.[217,220] NRM has decreased significantly over recent decades due to improved transplantation techniques, better supportive care, and refined GVHD

prophylaxis, yet remains considerable.[218,221] Post-transplant lymphoproliferative disorders, hematological malignancies, and solid tumors pose persistent threats to HSCT survivors, emphasizing the necessity for lifelong cancer surveillance.[191,210,217,220]

Hematopoietic stem cell transplantation, especially when preceded by conditioning regimens involving high-dose chemotherapy and TBI, represents one of the most gonadotoxic treatments, significantly increasing the risk of infertility and potentially causing irreversible ovarian and testicular dysfunction.[222–224] The cumulative dosage and the patient's age at the time of treatment are key determinants of infertility risk.[222,225] Young adult patients, who commonly undergo intense chemotherapy and HSCT, face especially high risks for infertility.[223,226,227] Studies have shown that the prevalence of infertility after HSCT exceeds 60% in female patients and ranges from 30% to 40% in male patients, depending on the underlying condition and intensity of the treatment.[223,228,229] The use of reduced-intensity conditioning has been explored to mitigate fertility risks, yet a significant proportion of patients still experience gonadal dysfunction even with milder regimens.[230,231]

In conclusion, successful long-term outcomes following hematopoietic stem cell transplantation depend on pre-treatment planning, proactive complication management, and structured, multidisciplinary follow-up care. Fertility preservation strategies should be integrated into the care of young patients prior to treatment.[230,232] International guidelines have established detailed recommendations for monitoring survivors, advocating a multidisciplinary approach to manage physical and psychological health comprehensively.[191,211] Given the risk of disease recurrence and diverse spectrum of acute and chronic complications lifelong surveillance is essential.[191,211]

2.5.5 Fertility preservation before HSCT

As survival rates improve for patients with hematological malignancies, fertility preservation has emerged as a crucial component of comprehensive patient care.[230] Fertility impairment following treatment for hematological malignancies necessitates proactive and thorough counseling about fertility preservation. Fertility preservation strategies aiming at safeguarding or restoring reproductive potential should be discussed and implemented promptly prior to initiating gonadotoxic treatments to maximize reproductive potential and enhance post-treatment quality of life.[226,227,233,234]

Established fertility preservation methods for post-pubertal patients include embryo and oocyte cryopreservation in females and sperm cryopreservation in males, all of which should be offered promptly prior to initiating gonadotoxic therapies.[223,230] In females, oocyte or embryo cryopreservation following controlled

ovarian stimulation is generally the preferred first-line strategy due to its high success rate and established clinical safety profile.[226,235] Ovarian tissue cryopreservation has shown increasing promise and is particularly valuable when urgent initiation of chemotherapy precludes conventional methods of fertility preservation.[235,236] Sperm cryopreservation remains the golden standard for male patients.[227]

Testicular tissue cryopreservation is an emerging fertility preservation strategy, particularly significant for prepubertal boys undergoing gonadotoxic cancer treatments who are unable to produce mature sperm for conventional sperm cryopreservation.[237,238] This experimental approach aims at preserving spermatogonial stem cells, the precursors for sperm production, which could potentially restore fertility through future transplantation or in vitro maturation techniques.[237,238]

Preclinical studies have demonstrated the potential feasibility and effectiveness of testicular tissue cryopreservation as a fertility restoration strategy in primates.[239] Human clinical experiences, although limited and still largely experimental, have also shown promising results in terms of graft survival and integrity of testicular tissue architecture after transplantation, despite not yet achieving mature sperm production in clinical settings.[240]

A critical aspect of evaluating the viability of testicular tissue cryopreservation as a fertility preservation strategy involves assessing the presence and quantity of spermatogonia in the preserved tissue.[241] Spermatogonial quantity and quality are essential metrics for evaluating fertility restoration potential.[242,243] Chemotherapy has been demonstrated to significantly impact spermatogonial counts, and preliminary studies have reported reduced numbers of spermatogonia in patients with hematological disorders even prior to initiating potentially sterilizing treatments, but as research evidence remains limited, definitive conclusions for specific patient groups cannot yet be drawn.[242,243] This reduction poses significant concerns about the effectiveness of testicular tissue cryopreservation as a fertility preservation option in these patients.[244]

Given the invasive nature and potential risks associated with testicular tissue cryopreservation, its application should be carefully considered and limited to patients most likely to benefit.[237,238] Ongoing research and advancements are required to refine this method and enhance its reliability, safety, and effectiveness in clinical practice.

3 Aims of the study

The aim of this thesis was to deepen the understanding of germline predisposition in malignant and severe hematological diseases, its clinical relevance in allogeneic hematopoietic stem cell transplantation, and its implications for fertility.

The specific aims were:

1. To investigate the prevalence of germline variants predisposing individuals to malignant and severe hematological disorders
2. To analyze the impact of germline mutations on the outcomes of allogeneic hematopoietic stem cell transplantation
3. To explore the fertility potential in male patients with severe inherited and acquired hematological diseases

4 Material and methods

4.1 Patients and tissue samples

4.1.1 Acute lymphoblastic leukemia patients (I)

Whole exome sequencing (WES) data from freshly collected or biobanked samples of 148 acute lymphoblastic leukemia patients, including 61 adults (16–70 years) and 87 pediatric patients (0–16 years), were analyzed to assess the prevalence of cancer-predisposing germline variants. The adult cohort was diagnosed at Helsinki University Hospital (HUH) between 2008 and 2019, and WES was performed on skin samples, except for one remission blood sample. The pediatric cohort consisted of patients treated with allogeneic hematopoietic stem cell transplantation according to HUH protocols between 1998 and 2019, with exome sequencing conducted on blood samples collected at the time of HLA typing for HSCT. Clinical data from all patients were available for analysis. Patient characteristics are summarized in Table 1.

Table 1. Characteristics of adult and pediatric patients with acute lymphoblastic leukemia.

Characteristic	Adults (n = 61)	Children (n = 87)
Number of patients	61	87
Male	40 (66%)	59 (68%)
Female	21 (34%)	28 (32%)
Age at diagnosis, years	16–70 (median 36)	0–16 (median 6)
ALL subtype		
B-ALL	36 (59%)	66 (76%)
T-ALL	17 (28%)	11 (13%)
Ph-ALL	8 (13%)	10 (11%)
Source of DNA	Skin*	Blood

ALL, acute lymphoblastic leukemia; Ph-ALL, Ph-positive B-ALL

*One adult patient's DNA was extracted from blood in remission

4.1.2 HSCT recipients and donors (II and IV)

We analyzed exome data from patients who had undergone hematopoietic stem cell transplantation to assess the prevalence of clinically relevant germline variants and their impact on transplantation outcomes. To validate findings on the most common variants, we included an additional cohort of patients with available single nucleotide polymorphism (SNP) array data. Clinical data from all patients were available for analysis, and patient characteristics are summarized in Table 2. Furthermore, we utilized a set of respective transplant donors, including their clinical data and available WES or SNP array data.

The first adult patient cohort (adult cohort 1) consisted of 141 patients who underwent hematopoietic stem cell transplantation and had provided samples to the Finnish Hematological Registry and Biobank (FHRB) or for academic research purposes. HSCT was performed between 1999 and 2019 at Helsinki University Hospital (n = 134) or Turku University Hospital (TUH, n = 7). At the time of transplantation, patients were between 17 and 70 years old (mean age 45.8 years, median 48.2 years). DNA for exome sequencing was extracted from skin biopsies (n = 138), bone marrow samples (n = 2), or peripheral blood samples (n = 1).

The second adult patient cohort (adult cohort 2) consisted of 138 patients from the Finnish Bone Marrow Transplantation Registry. HSCT was performed between 2002 and 2016 at Helsinki University Hospital (HUH, n = 47) or Turku University Hospital (TUH, n = 91). Patients who overlapped with the first adult cohort, as well as adult patients who underwent HSCT for solid cancer and pediatric patients, were excluded. At the time of transplantation, patients were between 16 and 69 years old (mean age 49.1 years, median 52.0 years). DNA for exome sequencing was extracted from blood samples collected for HLA typing prior to transplantation. The two adult patient cohorts did not differ significantly in terms of age, gender, or distribution of diagnoses.

We also analyzed a set of pediatric patients who underwent hematopoietic stem cell transplantation to compare the prevalence of germline variants and to further analyze the significance of these variants. The pediatric patient cohort consisted of 153 patients treated in Finland between 2001 and 2020 and for whom exome sequencing data was available covering 43.5% of all pediatric transplant recipients during that time. At the time of transplantation, patients were between 0 and 18 years old (mean age 8.7 years, median 8.2 years). DNA for exome sequencing was extracted from blood samples collected for HLA typing prior to transplantation. Clinical characteristics, including age, gender, and diagnosis distribution, did not differ significantly between patients with and without exome sequencing data.

To strengthen the analyses of the impact of two *CHEK2* variants, c.1229del and c.470T>C, we used an independent cohort (adult cohort 3) from the Finnish Bone Marrow Transplantation Registry, which consisted of 486 adult patients with SNP array data. HSCT was performed between 1993 and 2018 at HUH and TUH. At the

time of transplantation, patients were between 18 and 70 years old (mean age 47.9 years, median 50.1 years).

We included clinical and, when available, genetic data of the transplant donors in our analysis. The WES data was available for 130 donors of adult patients and 32 donors of pediatric patients. 507 transplant donors had SNP arrays performed. We used donors' clinical information gathered during the HSCT protocol for the analysis.

Table 2. Patient characteristics. Study II included all patients from pediatric cohort and adult cohort 1 and 2. Study IV included patients with malignant hematological disease from all cohorts (AA/BMF, immunodeficiencies and other diseases were excluded)

Characteristic	**Adult cohort 1 (n = 141)**	**Adult cohort 2 (n = 138)**	**Adult cohort 3 (n=486)**	**Pediatric cohort (n=153)**
Number of patients	141	138	486	153
Male	69 (49%)	68 (50%)	259 (53%)	102 (67%)
Female	72 (51%)	68 (50%)	227 (47%)	51 (33%)
NA	-	2	-	-
Age at HSCT, years (mean)	17 – 70 (45.8)	16 – 69 (49.1)	18 – 70 (47.9)	0 – 18 (8.7)
Diagnoses, n (%)				
Acute leukemia	97 (68.8%)	79 (57.2%)	249 (51.2%)	110 (71.9%)
Lymphoproliferative disease	12 (8.5%)	30 (21.7%)	112 (23.0%)	0 (0%)
Myeloproliferative neoplasms	16 11.3%)	11 (8.0%)	72 (14.8%)	6 (3.9%)
Myelodysplastic neoplasms	13 (9.2%)	10 (7.2%)	53 (10.9%)	7 (4.6%)
Aplastic anemia / Bone marrow failure	3 (2.1%)	8 (5.8%)	0 (0%)	11 (7.2%)
Primary immunodeficiency	0 (0%)	0 (0%)	0 (0%)	8 (5.2%)
Other	0 (0%)	0 (0%)	0 (0%)	11 (7.2%)
Source of transplant, n (MUD/MFD)	111/30	0/138	168/318	86/67
Type of transplant, n (PB/BM/CB/NA)	121/19/1/0	101/33/0/4	283/203/0/0	0/130/23/0
Conditioning, n (MAC/RIC/NA)	118/23/0	93/43/2	386/100/0	143/10/0

BM, bone marrow; CB, cord blood; MAC, myeloablative conditioning; MFD, matched family donor; MUD, matched unrelated donor; NA, not applicable, PB, peripheral blood; RIC, reduced-intensity conditioning

4.1.3 Pediatric patients undergoing fertility preservation (III)

We analyzed testicular tissue samples from 43 prepubertal patients (mean age 6.9 years, range 0.4–15.9 years) with severe hematological diseases who underwent testicular tissue cryopreservation prior to hematopoietic stem cell transplantation. Except for one patient with secondary MDS who had been treated for acute lymphoblastic leukemia, none had received chemotherapy before biopsy.

Clinical data provided by treating physicians — including diagnosis, genetic findings, and testicular volumes — were evaluated alongside histological analysis of testicular tissues. Samples were collected for research purposes with a focus on fertility preservation. Patients with a high risk of infection or bleeding were excluded.

A total of 18 patients were included in the Nordic fertility preservation program 'NORDFERTIL', while 25 participated in the German program 'Androprotect', based at the Centre of Reproductive Medicine and Andrology (Münster, Germany).

Patients were categorized into three groups based on disease phenotype: aplastic anemia/bone marrow failure syndrome (AA/BMFS), immunodeficiencies (IMMUNO), and myelodysplasia/myeloproliferative neoplasms (MDS/MPN). Patient characteristics are summarized in Table 3.

Table 3. Patient information prepubertal boys included in the study III.

Characteristic	AA/BMFS	IMMUNO	MDS/MPN
Number of patients	9	21	13
Age, years (median)	4.1–10.8 (7.7)	0.4–13.3 (4.8)	0.6–15.9 (7.2)
Diagnoses, n	Aplastic anemia (3) Bone marrow failure (1) Congenital amegakaryocytic thrombocytopenia (1) Diamond-Blackfan anemia (1) Fanconi anemia (3)	Chronic granulomatous disease (6) Chronic neutropenia (2) Hyper-IgE syndrome (1) Hyper IgM syndrome (3) Hyperinflammatory syndrome (5) IPEX syndrome (1) Lymphoproliferative syndrome (2) Severe combined immunodeficiency (1)	Juvenile myelomonocytic leukemia (3) Myelodysplastic syndrome (10)

4.1.4 Ethics declaration

Ethical approvals for the studies I, II, and IV were obtained from the Ethics Committees of Helsinki University Hospital and Turku University Hospital, and the Finnish National Supervisory Authority for Welfare and Health (Valvira) (adult patients from HUH, TUH and FHRB #206/13/03/03/2016, #303/13/03/01/2011; pediatric patients from HUH and TUH HUS/114/2018, HUS/284/2019, and V/3235/2019; Finnish Red Cross, Blood Service patients V/74832/2017, HUS/2152/2020, ETMK 78/2012). Samples and data were collected after written informed consent (living individuals), or authorization by the ethics committee (deceased patients).

Ethical approvals for use of testicular tissue samples (Study III) were obtained from the Ethics Board of Karolinska Institutet and the Regional Ethics Board in Stockholm (Dnr 2013-2129-31/3 and Dnr 2014-267-31/4), the Ethics Board of the University of Helsinki (426/13/03/03/2015, 192/13/03/03/2013) and Turku University Hospital (Dnr 1905-32/300/05), and the Ethics Committee of the Medical Faculty of Münster (no 2011-520-f-S).

4.2 Genetic analyses

4.2.1 Sample preparation and DNA sequencing (pediatric patients)

DNA for exome sequencing was extracted from blood samples collected for HLA typing before transplantation. Exome sequencing and sample preparation of pediatric patient samples were carried out at Blueprint Genetics (Helsinki, Finland). Genomic DNA was extracted from the samples using a spin column method. The extracted DNA underwent random fragmentation through an isothermal, non-contact sonochemistry approach, followed by purification with solid-phase reversible immobilization (SPRI) beads. The resulting DNA fragments were subjected to end-repair and sequencing adapters were ligated to both ends.

To obtain an optimal fragment size, adapter-ligated DNA libraries were size-selected using SPRI beads before undergoing amplification via ligation-mediated PCR. Post-amplification, the libraries were purified and subjected to target enrichment through a hybridization-based capture method. This step enabled the selective enrichment of the whole exome as well as predefined non-coding regions using the xGen Exome Research Panel and custom-designed capture probes (Integrated DNA Technologies, Coralville, Iowa, USA).

Library quality control involved verifying fragment size and concentration to ensure appropriate sequencing performance while eliminating any remaining primer-dimers. Illumina sequencing system with paired-end sequencing (2x150

base pairs) was used to sequence captured libraries. The raw sequencing reads underwent quality assessment before being aligned to the GRCh37 human reference genome using the Burrows-Wheeler Aligner algorithm.[245,246] GATK was used for duplicate read marking, local realignment of indels, base quality score recalibration, and variant calling.[247]

4.2.2 Sample preparation and DNA sequencing (adult patients)

DNA for exome sequencing was obtained from skin, blood, or bone marrow samples of adult patients. In adult cohort 1, DNA was extracted from cultured skin fibroblasts collected as germline controls or retrieved from the Finnish Hematological Registry and Biobank, and sequencing was performed at the Institute for Molecular Medicine Finland (FIMM) and the Biomedicum Functional Genomics Unit (FuGU). For three patients without available skin samples, DNA from blood or bone marrow sample was used. In adult cohort 2, DNA was extracted from blood samples collected for HLA typing prior to transplantation through the Finnish Bone Marrow Transplantation Registry and sequenced at the McGill Genome Centre.

Exomes produced at FIMM (Helsinki, Finland) were captured using Nimblegen SeqCap EZ MedExome kit (Roche NimbleGen, Madison, WI, USA), Agilent SureSelect v5 Exome, or Agilent SureSelect XT Clinical Research Exome (Agilent, Santa Clara, CA, USA) capture kits, and sequenced using Illumina HiSeq Sequencing System (Illumina, San Diego, CA, USA). Illumina reads were merged with SeqPrep (v0.4.5) and paired reads were trimmed using Q2. Reads shorter than 36 base pairs were discarded. Both paired and single-end reads were then aligned to the human reference genome GRCh37 with Burrows-Wheeler Aligner.[245,246] Reads that aligned to multiple genomic locations were removed. The Genome Analysis Toolkit (GATK) Indel Realignment (v2.2-16) was used to refine mapped reads, and duplicate reads arising from polymerase chain reaction (PCR) amplification were eliminated using Picard MarkDuplicates (v1.90). SAMtools mpileup (v0.1.19) was used for variant calling and alternative alleles were determined based on quality ratio.[248]

Exomes produced at FuGU (Helsinki, Finland) were captured using a Nimblegen SeqCap EZ MedExome kit (Roche NimbleGen, Madison, WI, USA and sequenced using Illumina HiSeq Sequencing System (Illumina, San Diego, CA, USA). Trimmomatic software was used to perform quality trimming on the exome sequencing data and then it was aligned to the GRCh37 human reference genome using the Burrows-Wheeler Aligner algorithm.[245,246] Picard MarkDuplicates (v2.8.1) was used to remove PCR duplicates. GATK Best Practices workflow, which

includes base quality score recalibration, was followed to refine mapped reads.[247,249] GATK HaplotypeCaller was used to call single-nucleotide and short-indel variants.

Exomes produced at the McGill Genome Centre (McGill University, Montreal, Canada) using a custom panel targeting the whole exome and sequenced using Illumina HiSeq 2000 system. The resulting reads were aligned to the GRCh37/hg19 reference genome. Variant calling was performed with GATK v3.2-2 HaplotypeCaller, generating aligned BAM, gVCF, and VCF files. Base quality score recalibration and SNP/INDEL discovery were carried out using GATK v3.6-0 VariantRecalibrator and ApplyRecalibration tools, following GATK Best Practices.[247,249,250] The data were further filtered using a genotype quality threshold of >18, selected to maximize genotype similarity between duplicated samples. Variant annotation was performed with snpEff v4.2 using default settings.

4.2.3 Single nucleotide polymorphism (SNP) array data

The SNP array data were produced at the Finnish Institute of Molecular Medicine in Helsinki, Finland, and the McGill Genome Centre, Montreal, Canada. At FIMM, the samples were genotyped using Illumina Immunochip v1, Immunoarray v2, Illumina Global Screening Array v2 or v3. At McGill Genome Centre, the samples were genotyped using exome sequencing pipeline. Data was harmonized with a lift-over to the GRCh38 human reference genome by genome-wide SNP imputation. THL Biobank's SISu v3 reference panel was used in lift-over and imputation.

4.2.4 Gene panels

We constructed a comprehensive list of 93 genes known to predispose individuals to acute lymphoblastic leukemia or other hematological malignancies based on published literature to determine the prevalence of cancer-predisposing gene variants in acute lymphoblastic leukemia. To further expand our analysis, we compiled an additional list of 70 genes associated with germline predisposition to cancer. This gene set was derived from two genomic resources: the Catalogue of Somatic Mutations in Cancer (COSMIC) Cancer Gene Census (https://cancer.sanger.ac.uk/census, version 94, Tier 1) and the National Human Genome Research Institute (NHGRI) Clinical Genomics Database (https://research.nhgri.nih.gov/CGD/, accessed 05/03/2021).[251] The COSMIC is a database that categorizes genes with strong experimental and clinical evidence of involvement in cancer development. It distinguishes genes that drive tumorigenesis through either germline or somatic mutations. The NHGRI Clinical Genomics Database is an evidence-based resource that links specific genes to clinical disorders, particularly those with a hereditary component. Both of these provide

curated information on genes implicated in inherited diseases by integrating findings from scientific literature and clinical genetic studies.

To identify germline variants with potential clinical significance that could impact the success of hematopoietic stem cell transplantation, we used three distinct gene panels. Variants in these genes may increase the risk of relapse or donor cell–derived leukemia in the setting of a related donor, predispose patients to secondary cancers particularly after intensive chemotherapy or radiation, and give rise to other conditions that require specific considerations in comprehensive patient management. First, we compiled a Hematology Panel consisting of 189 genes recognized to predispose to or cause hematological malignancies, cytopenia syndromes, and inborn errors of immunity. Second, we gathered an Oncology Panel of 114 genes linked to solid tumor predisposition. Third, we included a list of 73 genes based on the American College of Medical Genetics and Genomics (ACMG) Secondary Findings 3.0 (SF 3.0) Panel.[252] The ACMG SF Panel consists of genes for which pathogenic variants are known to cause serious but preventable conditions. These genes have been curated by ACMG to guide clinical decision-making, as they are associated with disorders that have established medical management recommendations.

4.2.5 Variant analyses

Variants were analyzed using BasePlayer, an interactive visualization and analysis tool designed for exploring next-generation sequencing data.[253] Findings were visually validated with BasePlayer, which enables efficient review of genomic variants, examination of read alignments, and validation of results through a graphical interface. When available, somatic exome sequencing data were used for further validation.

Genome Aggregation Database (GnomAD) non-cancer databases (whole database and Finnish population) were used as controls (versions 2.1 and 4.1.0).[254–256] Variants with a minor allele frequency higher than 0.05 were excluded. To filter out poor-quality variants, a 1000 Genomes mappability pilot mask track was applied, along with quality measures requiring genotype quality ≥ 20, QUAL ≥ 20, site coverage > 6 reads, and allelic fraction ≥ 30%.[257]

Variants were classified according to ACMG guidelines into benign, likely benign, variant of uncertain significance (VUS), likely pathogenic (LP), or pathogenic (P) using two different classification tools: InterVar (version 2.0.2) and VarSome (versions 10.1.1–10.2.3).[258,259] ACMG guidelines classify variants using a weighted system, where different pieces of evidence are combined to determine the likelihood of pathogenicity or benignity based on population frequency, computational predictions, functional studies, familial segregation analysis, type of genetic alteration, and literature evidence.[260]

For some patients, DNA was extracted from blood samples collected for HLA typing at different stages of the disease, requiring the filtering of somatic mutations. We filtered somatic mutations by considering the variant allele frequency, characteristics of the variant, and stage of the patient's disease. In addition, we used gnomAD (germline variants) and COSMIC (somatic variants) databases to evaluate the origin of the variant.

4.3 Testicular biopsy and analysis of spermatogonial numbers

4.3.1 Sample collection

The NORDFERTIL cohort comprises testicular samples collected from prepubertal boys in Sweden, Finland, and Iceland who were at a very high risk of infertility due to impending hematopoietic stem cell transplantation or testicular radiotherapy. These boys were offered an experimental procedure involving the cryopreservation of testicular tissue. Both the parents and, when appropriate, the patients themselves received verbal and written information about the research project and provided written informed consent. Clinical treatment details were documented and pseudo-anonymized before being entered into the research database. Testicular tissue was obtained through a unilateral open biopsy, with less than 20% of the volume of one testicle being sampled. Of the collected tissue, two-thirds were cryopreserved for potential future fertility preservation, and the remaining third was anonymized and transported to the NORDFERTIL research laboratory at Karolinska Institutet. The inclusion criteria for the study required participants to have a very high risk of therapy-related infertility and a testicular volume below 10 ml, as measured by an orchidometer and patients with a high risk of bleeding or infection were excluded from participation.[243]

Androprotect is an interdisciplinary network coordinated by the Centrum for Reproductive Medicine and Andrology at the University Hospital Münster, working in collaboration with pediatric oncology centers across Germany. The sample cohort comprises of testicular biopsies collected from prepubertal boys prior to the initiation of gonadotoxic therapy. During this procedure, a small volume of testicular tissue is collected and is immediately processed for cryopreservation. The cryopreservation of immature testicular tissue enables scientific research which aims to safeguard these spermatogonial stem cells to preserve fertility through refertilization techniques. The patients and their parents received verbal and written information about the research project and gave their written informed consent.[261]

4.3.2 Sample preparation and histological evaluation

Testicular biopsy samples obtained from the NORDFERTIL program were preserved using either formalin or Bouin's solution, and biopsies from the Androprotect program were fixed in Bouin's solution. Following fixation, the samples were embedded in paraffin and cut into 3–5 µm sections. Sections were stained using periodic acid-Schiff staining or immunostained using germ cell-specific marker MAGEA4, both of which have been previously validated as equally effective to detect spermatogonia.[243,262]

At Karolinska Institutet, image analysis was performed using a fluorescence microscope (Eclipse E800, Nikon, Japan) and in Münster, images were acquired with the PreciPoint M8 microscope/scanner and subsequently analyzed using the ViewPoint light software (1.0.0.9628, PreciPoint, Freising, Germany). Spermatogonia were identified based on their morphology, location, and MAGEA4 expression. To ensure an unbiased evaluation, a blinded approach was applied. All round tubular cross-sections within the tissue sections were examined and categorized into two groups: tubules containing spermatogonia and tubules composed solely of somatic Sertoli cells. To enable comparisons between samples, the mean number of spermatogonia per round tubular cross-section (S/T) was calculated. Additionally, the fertility index (FI) was determined as the percentage of tubular cross-sections that contained spermatogonia.

4.3.3 Reference values

To establish reference values for spermatogonial numbers across different age groups, we conducted a systematic review of published quantitative histological studies and combined reference values for different age groups from birth to adulthood.[262] The reference studies provided data on the number of spermatogonia per round tubular cross-section and the fertility index at various developmental stages.[263–266]

Reference values were determined separately for each year of age from birth to 10 years. For older individuals, we defined broader age groups: prepubertal (10–14 years), adolescent (14–18 years), and adult (25–40 years). The reference values for ages 0–10 and 10–14 years were derived from multiple datasets, whereas values for the older age groups (14–18 and 25–40 years) were based on a single study.[262]

In cases where studies reported median values and ranges instead of means and standard deviations (SD), we applied statistical conversion formulas to estimate these values.[267] When multiple datasets existed for the same age group, we calculated combined means and SDs using established statistical formulas for pooled data. Since raw S/T values were not normally distributed, we transformed the S/T values on a log-transformed scale using base-e log transformation.[268]

The reference dataset was based on published histological data of healthy prepubertal and adolescent males. To establish thresholds for low and severely depleted spermatogonial numbers, additional analyses included testicular tissue samples from patients with Klinefelter syndrome and from individuals exposed to alkylating chemotherapy, both known to significantly impact spermatogonial survival.[269] Patients with Klinefelter syndrome consistently exhibited S/T Z-scores below -7 SD, supporting this as a threshold for severe germ cell depletion. Similarly, patients who had received high cumulative doses of alkylating agents (>4,000 mg/m² cyclophosphamide equivalent dose) also had S/T Z-scores below -7 SD, further confirming the validity of this cutoff. This threshold represents a nearly complete absence of spermatogonia. In contrast, patients who had undergone non-alkylating chemotherapy typically had spermatogonial counts within -3 SD to the normal range, supporting the use of -3 SD as a clinically meaningful threshold for detecting moderate germ cell depletion.[262] This cutoff corresponds to a spermatogonial count below the 0.15th percentile of the reference population, indicating a significant reduction in the germ cell pool.

4.3.4 Z-scores for patient samples

After calculating the spermatogonial numbers in samples, we calculated Z-score for each sample by comparing individual S/T values to the corresponding reference mean and SD for their age group. The Z-score for each sample was calculated with following equation: $Z = (x - M)/SD$, where x is the individual sample value, M is the reference mean, and SD is the reference standard deviation.

By applying these Z-scores to testicular tissue samples from patients, we were able to compare patient samples across different developmental stages. This allowed us to quantify the impact of genetic conditions and chemotherapy exposure on spermatogonial numbers.

4.4 Statistical methods

Variant frequencies identified in adult and pediatric ALL patients were compared with those from population-matched Finnish controls (gnomAD Finns) using a two-sided Fisher's exact test. The Benjamini-Hochberg procedure was employed to correct for multiple testing.

A two-sided Fisher's exact test was also used to compare the frequencies of germline variants between adult and pediatric stem cell recipients and between stem cell recipients and the Finnish control population (gnomAD Finns). Additionally, a two-sided Fisher's exact test was employed to compare the prevalence of GVHD across patient groups. Clinical outcomes, including OS, relapse rate (RR), and NRM, were evaluated using Kaplan-Meier estimates and log-

rank tests. Odds ratios (OR) were calculated by dividing the odds in the first group by the odds in the second group, with 95 % confidence intervals (CI) computed using the Woolf method (logarithmic approximation). Multivariate analyses were performed using the Cox regression model. The proportional hazards assumption was evaluated using Schoenfeld residuals, and no major violations were observed.

Differences in spermatogonial quantities among patient phenotype groups were analyzed using the Pearson chi-squared test. Pearson correlation was also used to explore the relationship between S/T Z-scores and ferritin levels.

5 Results

5.1 Cancer-predisposing variants in acute lymphoblastic leukemia patients

5.1.1 Frequency and description of the germline variants

Pathogenic or likely pathogenic germline variants in genes associated with hematological malignancies or solid cancers were identified in 5 out of 61 (8%) adult patients and 10 out of 87 (11%) pediatric patients.[270] The majority of these variants had been previously linked to an increased cancer risk, occurring in 4/61 (8%) adults and 8/87 (9%) pediatric patients. The variants are listed in Table 4. Additionally, 6/61 adults and 11/87 pediatric patients carried heterozygous P/LP variants in genes typically associated with autosomal recessive inheritance.

A pathogenic variant in a high-risk leukemia predisposition gene, *RUNX1* p.Arg204Gln, was detected in one adult patient. In addition, a truncating variant in *SAMD9* (p.Ser844ValfsTer10) was identified in a pediatric patient. This variant was classified as a VUS due to the lack of functional data and segregation analysis. While most pathogenic *SAMD9* variants are missense, a few rare loss-of-function variants have also been reported in patients with myelodysplastic syndrome.[271,272] Germline variants in *RUNX1* and *SAMD9* are primarily associated with myeloid malignancies, though a predisposition to lymphoid malignancies has also been described.[273,274]

Among genes linked to syndromic predisposition to acute lymphoblastic leukemia, we identified two P/LP variants: one in *TP53* (p.Gly245Ser), associated with Li-Fraumeni syndrome, and one in *LZTR1* (c.2407-1G>A), linked to Noonan syndrome.

Additionally, a pediatric patient was compound heterozygous for two *MUTYH* hotspot variants (p.Gly396Asp and p.Tyr179Cys). In *PMS2*, we detected pathogenic heterozygous variants (p.Tyr255Ter and p.Glu109GlyfsTer30) in one adult and one pediatric patient, respectively, despite no known family history of colorectal cancer.

Furthermore, heterozygous P/LP variants were identified in genes associated with solid tumor predisposition, including *BRCA1* (n=1), *BRIP1* (n=1), and *CHEK2* (n=6), all of which are linked to breast cancer susceptibility. Additionally, *RET* (n=1) was identified, predisposing to multiple endocrine neoplasia, as well as *SDHB*

(n=1) and *SDHC* (n=1), both known to confer a risk for familial paraganglioma-pheochromocytoma.

Table 4. Pathogenic and likely pathogenic variants in autosomal dominant genes or compound heterozygous in patients with acute lymphoblastic leukemia

Patient	Gene	Inheritance	Germline disease association for gene	Conclusion of pathogenicity	Variant
A1	*BRCA1*	AR/AD	Fanconi anemia/ Breast cancer	Pathogenic	c.4097-2A>G (splice site variant)
P1	*BRIP1*	AR/AD	Fanconi anemia/ Breast cancer	Likely pathogenic	c.3440dup (p.Asn1147LysfsTer2)
P2*	*CHEK2*	AD	Tumor predisposition syndrome	Pathogenic	c.1100del (p.Thr367MetfsTer15)
P3	*CHEK2*	AD	Tumor predisposition syndrome	Pathogenic	c.1100del (p.Thr367MetfsTer15)
P4	*CHEK2*	AD	Tumor predisposition syndrome	Pathogenic	c.1100del (p.Thr367MetfsTer15)
P5	*CHEK2*	AD	Tumor predisposition syndrome	Pathogenic	c.1100del (p.Thr367MetfsTer15)
P6	*CHEK2*	AD	Tumor predisposition syndrome	Pathogenic	c.1100del (p.Thr367MetfsTer15)
A2	*CHEK2*	AD	Tumor predisposition syndrome	Pathogenic	c.1100del (p.Thr367MetfsTer15)
P2*	*LZTR1*	AR, AD/ AD	Noonan syndrome/ Schwannomatosis	Likely pathogenic	c.2407-1G>A (splice site variant)
P7*	*MUTYH*	AR	Familial adenomatous polyposis	Pathogenic	c.1187G>A (p.Gly396Asp)
				Pathogenic	c.536A>G (p.Tyr179Cys)
P8	*PMS2*	AD/AR	Lynch Syndrome/ Mismatch repair cancer syndrome	Pathogenic	c.765C>A (p.Tyr255Ter)
A3	*PMS2*	AD/AR	Lynch Syndrome/ Mismatch repair cancer syndrome	Pathogenic	c.325dup (p.Glu109GlyfsTer30)
A4*	*RET*	AD	Multiple endocrine neoplasia	Pathogenic	c.2410G>A (p.Val804Met)
A5	*RUNX1*	AD	Familial platelet disorder with predisposition to AML	Pathogenic	c.611G>A (p.Arg204Gln)
A4*	*SDHB*	AD	Pheochromocytoma/ Paraganglioma syndrome	Likely pathogenic	c.177G>C (p.Gln59His)
P9	*SDHC*	AD	Pheochromocytoma/ Paraganglioma syndrome	Likely pathogenic	c.380A>G (p.His127Arg)
P10	*TP53*	AD	Li-Fraumeni syndrome	Pathogenic	c.733G>A (p.Gly245Ser)

AD, autosomal dominant; AR autosomal recessive; BM, bone marrow; CB, cord blood; MAC, myeloablative conditioning; MFD, matched family donor; MUD, matched unrelated donor; NA, not applicable, PB, peripheral blood; RIC, reduced-intensity conditioning

5.1.2 Enrichment of the cancer-predisposing variants

We analyzed the prevalence of pathogenic or likely pathogenic germline variants in ALL patients and compared it to the non-cancer Finnish control data from GnomAD to assess the frequency of harmful germline variants. We observed a 2.6-fold enrichment (95% CI 1.5–4.2, p = 0.00071) of these variants in the combined adult and pediatric patient cohort. The enrichment was particularly pronounced in solid cancer predisposition genes among adults (4.2-fold, 95% CI 1.3–10.5, p = 0.0094) and in genes predisposing to hematological malignancies among children (2.5-fold, 95% CI 1.0–5.4, p = 0.031). In the entire acute lymphoblastic leukemia patient cohort, both hematological malignancy risk genes (OR 2.1, 95% CI 1.0–4.0, p = 0.035) and solid cancer risk genes (OR 3.0, 95% CI 1.3–6.0, p = 0.0046) were significantly enriched compared to the Finnish control population.

The most significant differences in the frequency of pathogenic and likely pathogenic variants between patients and population-matched controls were observed in *PMS2*, *DNAJC21*, and *MUTYH*, with ORs of 48.9 (95% CI 4.1–434.6, p = 0.0018), 4.1 (95% CI 1.3–10.0, p = 0.0098), and 5.1 (95% CI 1.8–11.7, p = 0.0016), respectively.

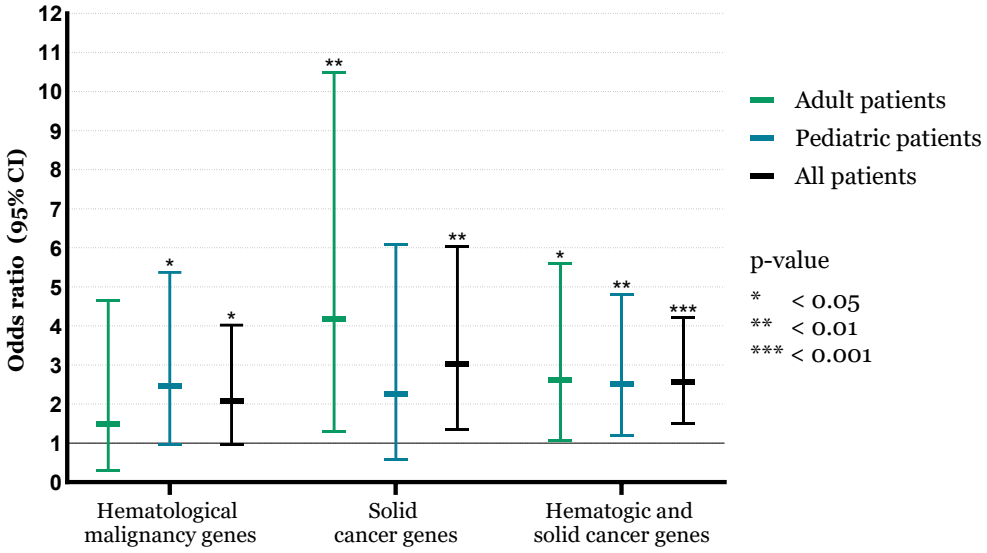


Figure 3. Enrichment of pathogenic variants in patients with acute lymphoblastic leukemia compared to healthy controls (gnomAD Finns) in genes with known predisposition to hematological malignancies and cancer-predisposition genes. CI, confidence interval.

5.2 Germline variants in hematopoietic stem cell recipients

5.2.1 Frequency and distribution of pathogenic variants in patients

In our study, the prevalence and clinical significance of pathogenic and likely pathogenic germline variants were investigated in 432 patients who had undergone allogeneic hematopoietic stem cell transplantation.[275] The patient cohort included both adults and children, and the analysis focused on genes associated with hematological malignancies, solid tumors, and significant secondary findings.

Overall, 17.8% of patients were found to carry at least one clinically relevant germline variant, with a higher prevalence in pediatric patients (22.9%) compared to adults (15.1%). These variants were identified in genes involved in hematological disease predisposition, solid tumor predisposition, and in genes included in the ACMG secondary findings list. The most frequently affected genes in the entire cohort were *CHEK2* (n = 13), *FANCM* (n = 11), *GATA2* (n = 5), and *HOXB13* (n = 5), and *ANKRD26* (n = 3). The most frequently mutated genes, *CHEK2* and *FANCM*, are both involved in DNA damage repair.

A significant finding of the study was that only 65% (11/17) of the pediatric patients and 50% (5/10) of the adult patients with a harmful germline variant in gene predisposing to the hematological disease had received a genetic diagnosis prior to the hematopoietic stem transplantation. This suggests that a considerable number of patients underwent transplantation without prior knowledge of their underlying genetic predisposition, which could have affected donor selection, conditioning regimens, and post-transplant outcomes and follow-up.

Among the genes predisposing to hematological malignancies, cytopenia, and inborn errors of immunity bone marrow failure syndromes, variants were identified in 5.4% of adult patients and 11.1% of pediatric patients. The prevalence was significantly higher in pediatric patients and was largely attributable to the higher frequency of variants in pediatric patients with inherited bone marrow failure syndromes and primary immunodeficiencies. In contrast, variants in genes predisposing to solid tumors were identified in 10.4% of adult patients and 12.4% of pediatric patients, with no significant difference between the two age groups.

In the ACMG secondary findings panel, which includes genes with clinically actionable mutations, pathogenic or likely pathogenic variants were identified in 2.2% of adult patients and 7.2% of pediatric patients, with a statistically significant difference between the groups. Ten of the seventeen identified variants were in genes predisposing to cancer but seven involved other non-cancer genes. These included three patients with truncating variants in the TTN gene, associated with dilated cardiomyopathy. Two pediatric patients were found to be homozygous for

the HFE p.C282Y variant, which is associated with hereditary hemochromatosis. Additionally, two patients were found to carry pathogenic variants in OTC, an X-linked gene associated with a urea cycle disorder.

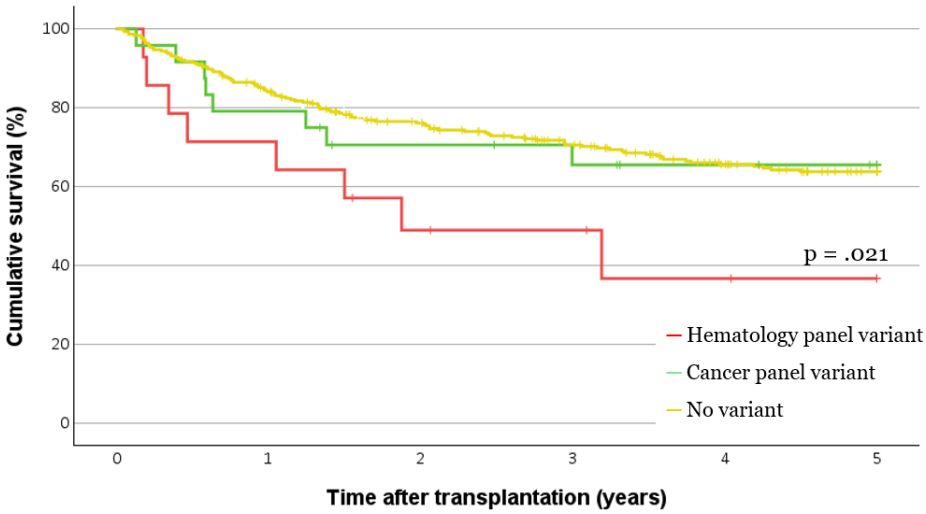
5.2.2 CHEK2 prevalence and association to hematological malignancies

Given the high prevalence of *CHEK2* variants in the Finnish population, their role in hematological malignancy risk and post-transplant outcomes was further examined in 877 HSCT recipients with malignant hematological disease.[276] In addition to the pathogenic c.1229del (p.Cys410SerfsTer4) variant, the analysis included c.470T>C (p.Ile157Thr), a variant with conflicting evidence regarding its pathogenicity. Both variants were significantly enriched in the patient population compared to Finnish controls. The c.1229del variant was found in 4.1% of patients and was associated with a 2.5-fold increased risk of hematological malignancies, while the c.470T>C variant was present in 9.4% of patients and was associated with a 1.9-fold increased risk.

5.2.3 Influence of germline mutations on HSCT outcomes

In our analysis of hematopoietic stem cell transplantation outcomes, only patients with malignant diseases were included. Among them, germline variants in genes associated with hematological malignancy predisposition (Hematology panel) were identified in 3.6% of cases (n = 14/386), while 17.6% (n = 68/386) carried a variant in the Oncology panel. The most prevalent variants within the Oncology panel were in *CHEK2* (11.4%, n = 44/386), and therefore, these patients were analyzed separately in further analyses.

Patients with Hematology panel variants had a significantly lower overall survival in Kaplan-Meier analysis (p = .021) compared to those without an identified germline variant (Figure 4). The reduced survival was driven by a slight but not statistically significant increase in early NRM and RR. Within this group, three patients died from transplant-related complications within six months post-HSCT. Five patients died after relapse during follow-up. These included two patients with acute lymphoblastic leukemia who carried *TP53* and *RUNX1* variants, and three patients with acute myeloid leukemia who carried *ERCC6L2*, *GATA2*, and *DDX41* variants. In contrast, the presence of a germline variant in the Oncology panel did not significantly impact OS, RR, or NRM in the five-year follow-up period.



No. at risk						
Hematology panel variant	14	10	6	5	3	2
Cancer panel variant	24	19	15	13	11	9
No variant	304	250	212	181	149	126

Figure 4. 5-year survival of patients in adult cohort 1, adult cohort 2 and pediatric cohort after allogeneic hematopoietic stem cell transplantation. Patients were grouped by identified germline variant into hematology panel and cancer panel variant groups and compared to patients with no identified variant.

No significant differences were observed in the incidence of acute (severe or all stages) or chronic (extensive or all stages) graft-versus-host disease between the groups analyzed (Figure 5).

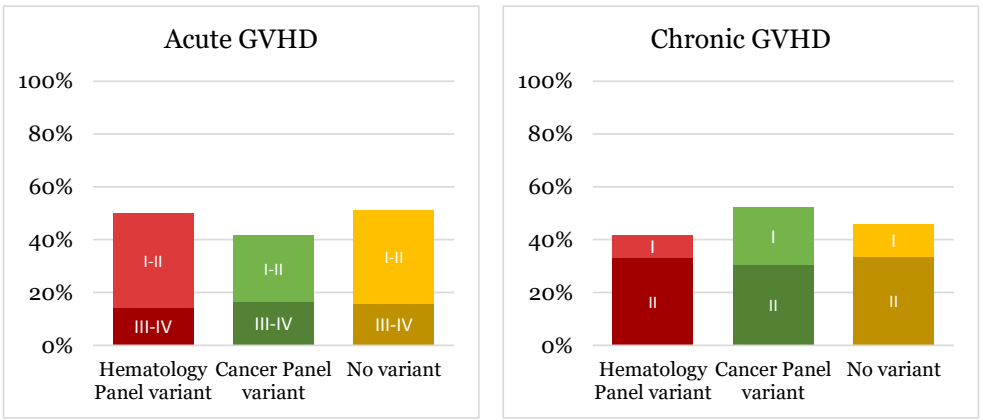


Figure 5. Rates of acute and chronic graft-versus-host disease (GVHD) by germline variant status in recipients

5.2.4 CHEK2 variants and HSCT outcomes

Despite the clear association between *CHEK2* variants and hematological malignancy susceptibility, the presence of these variants did not significantly impact OS, RR, or NRM following transplantation. Additionally, no association was detected between *CHEK2* variants and either acute or chronic graft-versus-host disease (Figure 6). Analyses were conducted on all patients carrying a *CHEK2* variant, as well as separately on those carrying either the c.1229del (n = 33) or c.470T>C (n = 79) variant, with three patients carrying both variants excluded from the analyses.

An exception to this finding was observed in homozygous carriers of the c.1229del variant, who exhibited an increased risk of early mortality. Both affected patients died within the first year post-HSCT due to non-relapse-related causes, suggesting that biallelic loss of *CHEK2* function may lead to heightened vulnerability to transplant-related complications. In contrast, among the five patients homozygous for the c.470T>C variant, two experienced relapse, leading to the death of one patient. The remaining four, including the one with relapse, survived until the end of the follow-up period. The median follow-up time for these patients were 5.2 years. These findings suggest that, unlike the c.1229del variant, homozygosity for c.470T>C did not appear to negatively impact overall prognosis following HSCT.

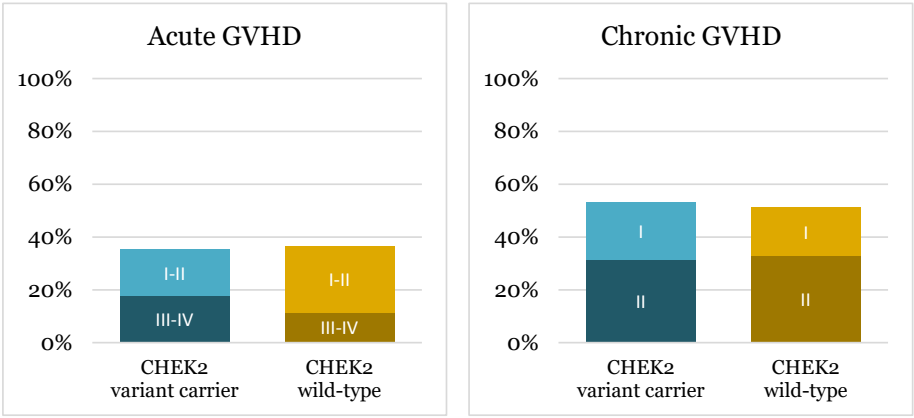


Figure 6. Rates of acute and chronic graft-versus-host disease (GVHD) by germline *CHEK2* variant status in recipients.

5.2.5 CHEK2 variants in transplant donors

To further investigate the relevance of *CHEK2* variants, the study also analyzed their prevalence in HSCT donors. Among 669 donors, 2.5% carried the c.1229del variant, while 7.0% carried the c.470T>C variant. The prevalence of these variants

in donors was higher than in Finnish population controls but lower than in HSCT recipients, reinforcing the notion that *CHEK2* carriers are at increased risk for hematological malignancies but are not necessarily unsuitable as donors. Recipients of grafts from *CHEK2* variant carriers did not show significant differences in OS, RR, or NRM compared to recipients of wild-type donor grafts, indicating that the presence of these variants in donors does not negatively impact transplant success.

An intriguing finding was that all three patients who received a transplant from homozygous CHEK2 variant carriers (one with c.1229del and two with c.470T>C) were alive at the end of the follow-up period (median follow-up time 6.5 years). Although one patient experienced relapse, the overall survival of these recipients suggests that, despite the small sample size, having a homozygous *CHEK2* carrier as a donor does not necessarily lead to a poor outcome.

5.3 Spermatogonial quantity in patients with severe hematological diseases

Our study examined testicular tissue samples from 43 prepubertal patients diagnosed with severe hematological diseases and were scheduled for hematopoietic stem cell transplantation. Since HSCT involves high-dose chemotherapy, sometimes accompanied by TBI, which can severely impact fertility, patients were offered cryopreservation of immature testicular tissue prior to gonadotoxic treatment. The patient cohort consisted of three major diagnostic groups: aplastic anemia/bone marrow failure syndromes (AA/BMFS), immunodeficiencies (IMMUNO), and myelodysplastic syndromes/myeloproliferative neoplasms (MDS/MPN).

5.3.1 Spermatogonial numbers

A key finding was that 49% (21/43) of prepubertal patients had spermatogonial numbers below the normal range (−3 SD), and 19% (8/43) had a severely depleted spermatogonial pool (−7 SD or lower). Notably, significant variation was observed between disease groups. The results are demonstrated in Figure 7.

Patients with Fanconi anemia showed markedly reduced spermatogonial numbers, with all samples from the three cases containing only individual cells or none at all, indicating near-total depletion of the spermatogonial pool. Patients with other inherited bone marrow failure syndromes, such as congenital amegakaryocytic thrombocytopenia and *ERCC6L2*-related BMFS, also showed reduced spermatogonial counts, with Z-scores of −3.2 and −6.8, respectively. In contrast, patients with acquired aplastic anemia had more variable spermatogonial numbers, ranging from normal to slightly reduced levels.

Among patients with immunodeficiencies, the median spermatogonial Z-score was −1.9, with considerable variation within the group. Patients with chronic granulomatous disease had moderately decreased spermatogonial numbers, with values ranging from −7.0 to −1.2. In contrast, patients with hyper-IgM and hyper-IgE syndromes had spermatogonial counts within the normal range, suggesting that certain immune disorders may have a minimal direct impact on spermatogenesis. Notably, two patients with X-linked lymphoproliferative syndrome (germline *SH2D1A* mutations) had significantly reduced spermatogonial numbers (Z-scores −3.4 and −7.2), raising the possibility that underlying immune dysregulation may affect germ cell survival.

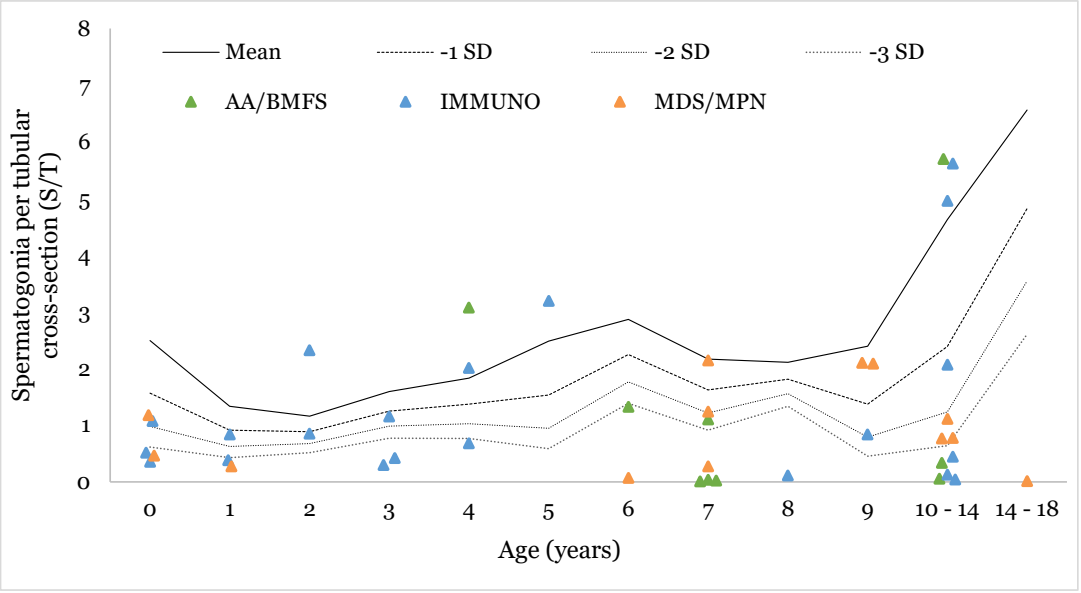


Figure 7. Number of spermatogonia per round tubular cross section in patients with severe hematological disease. Data are plotted on lines corresponding to the *Z*-scores for the reference mean values. AA/BMFS, aplastic anemia/bone marrow failure syndrome; IMMUNO, immunodeficiencies; MDS/MPN, myelodysplasia/myeloproliferative neoplasms (MDS/MPN).

In the MDS/MPN group, spermatogonial numbers were highly variable. While the median spermatogonial Z-score was −2.7, some patients had values as low as −20.5. Among the ten MDS patients, seven had normal spermatogonial counts, including one patient with *GATA2* mutation and another with secondary MDS following previous acute lymphoblastic leukemia treatment. Notably, no germline mutations were identified in JMML patients, and their spermatogonial counts were either normal or only slightly reduced.

5.3.2 Fertility index and the impact of blood transfusions and iron load

In addition to spermatogonial counts, the fertility index, which represents the proportion of tubules containing spermatogonia, was assessed (graph below). The FI values showed significant variability between disease groups. The AA/BMFS group had a median FI Z-score of −6.1, while the IMMUNO group had a median of −2.9, and the MDS/MPN group had a median of −4.2. Notably, patients with Fanconi anemia had the lowest FI values, further reinforcing the notion that germ cell depletion in FA patients begins early in life.

Interestingly, no clear correlation was observed between blood transfusion history and spermatogonial depletion, as 44% of regularly transfused patients had normal spermatogonial numbers, compared to 31% of those with infrequent or no transfusions.

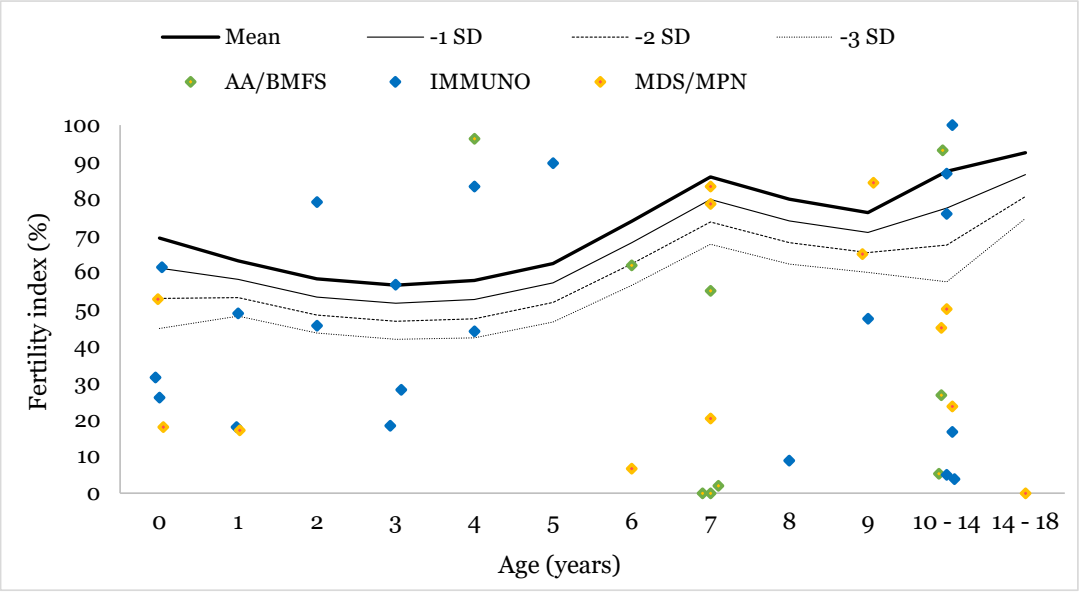


Figure 8. Fertility index in patients with severe hematological disease. Data are plotted on lines corresponding to the *Z*-scores for the reference mean values. AA/BMFS, aplastic anemia/bone marrow failure syndrome; IMMUNO, immunodeficiencies; MDS/MPN, myelodysplasia/myeloproliferative neoplasms (MDS/MPN).

5.3.3 Impact of underlying disease on spermatogonial numbers

These findings indicate that severe hematological diseases can significantly affect spermatogonial numbers even before exposure to gonadotoxic HSCT regimens. The degree of spermatogonial depletion appears to be largely dependent on the

underlying disease and its genetic basis, Fanconi anemia and inherited bone marrow failure syndromes showing the most severe germ cell loss. In contrast, most immunodeficiencies and acquired bone marrow failure conditions appear to have a lesser impact on spermatogonial numbers, preserving the potential for future fertility restoration in these patients.

6 Discussion

In recent years, inherited pathogenic variants in cancer predisposition genes have been increasingly recognized as important contributors to hematological malignancies in both children and adults. These variants may not only predispose individuals to the development of malignancies, but also carry significant clinical implications that extend throughout the entire diagnostic and treatment pathway.[83,94,174]

Despite growing recognition of germline predisposition syndromes, their role in routine clinical care has remained limited. The discovery of a germline variant can influence key decisions, such as the choice of stem cell donor, particularly when related donors are considered, the conditioning regimen, and long-term surveillance strategies for secondary malignancies or treatment-related complications.[174] In addition, inherited bone marrow failure syndromes and other predisposing conditions are associated with increased vulnerability to treatment-related toxicity, making early recognition essential.[51,183]

Equally important, many hematological diseases affect children and young adults, for whom long-term quality of life, including fertility, remains a major concern.[222,230] There is growing awareness that the underlying disease itself, even before initiation of cytotoxic therapy, may already impair reproductive capacity, particularly in prepubertal patients with inherited marrow failure or malignant disease.[222] However, data on spermatogonial counts and fertility preservation potential in these patients remain scarce.

The motivation for this thesis was to address these gaps. Using exome sequencing and clinical data, the studies aim to identify the frequency and spectrum of germline variants associated with hematological malignancies, evaluate their clinical relevance, and assess the impact of severe hematological diseases on spermatogonial development.

6.1 Broadening the spectrum of germline predisposition

Inherited variants, particularly in genes involved in DNA repair, transcription regulation, and hematopoietic development, have been shown to play a role in both pediatric and adult leukemogenesis.[81,82,84,87] While traditionally associated with rare familial syndromes, such variants are now being increasingly identified across

unselected patient populations, including those without suggestive family history.[81,84]

Multiple studies have shown that a notable proportion of patients with hematological malignancies carry pathogenic or likely pathogenic germline variants in known cancer predisposition genes.[82,83,85,87,183] In our data set of allogeneic hematopoietic stem cell transplant recipients, we found the prevalence of high-penetrance germline variants predisposing to hematological malignancies comparable to previous reports: 5.4% in adult patients and 11.1% in pediatric patients. In addition, we found that 10.4% and 12.4% of adult and pediatric HSCT recipients, respectively, have a pathogenic or likely pathogenic variant in genes predisposing to solid cancer.

In our cohort of over 870 patients undergoing allogeneic HSCT, including more than 110 individuals carrying a CHEK2 variant, representing the largest published series of hematological malignancy patients with CHEK2 variants to date, two variants (c.1229del and c.470T>C) were significantly enriched compared to population-matched controls, with odds ratios of 2.5 and 1.9, respectively. Although CHEK2 has traditionally been considered primarily a solid tumor risk gene, our study adds to the growing body of evidence supporting its relevance in hematological malignancy susceptibility. [90,91,182,277] Our findings support a moderate increase in hematological malignancy risk associated with CHEK2 variants, reinforcing its emerging role in hematology. CHEK2 is a key component of the DNA damage response pathway and plays a central role in maintaining genomic stability by regulating cell cycle arrest, DNA repair, and apoptosis in response to genotoxic stress.[278] Germline pathogenic variants in CHEK2 are well established as moderate-risk alleles for several solid tumors — most notably breast cancer, but also prostate, colorectal, and thyroid cancers — highlighting the complexity of managing individuals with such variants.[277,278] CHEK2 may represent one of the more common inherited risk factors for hematological malignancies, underscoring the need for gene-specific considerations in germline testing and long-term follow-up of affected individuals.

In acute lymphoblastic leukemia, germline predisposition has long been recognized in pediatric cohorts, but recent findings confirm that adult patients may also harbor germline variants that influence disease risk.[279] In our study, germline exome data from both children and adults with ALL revealed a 2.6-fold enrichment of pathogenic or likely pathogenic variants compared to the Finnish general population. This enrichment was most notable in solid tumor predisposition genes among adults, with a 4.2-fold increase compared to the general population, and in genes associated with hematological malignancies among children, where a 2.5-fold increase was observed. These findings support the hypothesis that inherited predisposition contributes meaningfully to leukemogenesis across age groups.

The increasing detection of germline variants raises important considerations for clinical practice. While germline variants may not always be the primary driver of malignancy, their presence may modulate disease biology, treatment sensitivity, and long-term outcomes.[182] For example, patients with inherited bone marrow failure syndromes are more likely to develop treatment-related complications and may require tailored conditioning regimens for HSCT.[51,183] Moreover, the discovery of a germline predisposition may have implications for at-risk family members, necessitating genetic counseling and cascade testing.[83,94]

Despite their clinical relevance, germline variants remain under-recognized in hematology. Many patients proceed to transplantation or high-intensity treatment without any prior germline evaluation. While current guidelines focus on selected high-risk groups, such as those with early-onset disease, strong family history, or suggestive clinical features, our findings challenge this narrow approach. We identified germline variants even in patients without these traditional risk indicators, highlighting that relying solely on predefined clinical criteria may miss a substantial proportion of carriers. These results support a broader, perhaps even universal, consideration of germline testing in patients with hematological malignancies, as identifying a predisposition can have significant implications for treatment planning, donor selection, family counseling, and long-term surveillance.

In conclusion, germline predisposition represents an underutilized yet highly informative layer of precision medicine in hematology. Broader awareness, clearer clinical guidelines, and integration of genetic testing into diagnostic workflows are essential steps toward improving patient care.

6.2 Secondary findings in allogeneic HSCT recipients

In our cohort of allogeneic HSCT recipients, clinically actionable germline variants in ACMG secondary findings genes were more frequent in pediatric patients than in adults. Most of the identified variants predisposed to cancer, but several involved non-cancer genes with potential relevance for transplant care. These variants may be more common in pediatric patients due to the high penetrance and early onset of genetic disorders that increase cancer risk, while other associated disorders may not yet have manifested at the time of diagnosis. For instance, truncating variants in *TTN*, observed in three patients, may increase susceptibility to dilated cardiomyopathy and warrant particular attention when cardiotoxic conditioning regimens are used. Two pediatric patients carried homozygous *HFE* variants, conferring risk of iron overload syndromes, which could contribute to hepatic complications after transplantation if not recognized and managed. Additionally, pathogenic variants in the X-linked *OTC* gene were identified in two patients; *OTC* deficiency can manifest as hyperammonemia under metabolic stress, suggesting that anticipatory metabolic management might be required in the transplant

setting. Together, these findings highlight the importance of considering actionable germline variants in HSCT recipients, especially among pediatric patients, where such variants may be more common and clinically more significant with longer expected post-transplant survival that increases the likelihood of clinical manifestations over time.

6.3 The role of genetic testing in stem cell transplantation

The role of germline genetic testing in optimizing HSCT outcomes is only beginning to be appreciated.[174] Our studies underscore the growing relevance of incorporating germline genetic testing, both for recipients and potential donors, into the pre-transplantation evaluation.

Germline variants identified in our cohort included those associated with hematological malignancy risk, cancer predisposition, and systemic conditions with potential transplant implications. These variants were present in approximately 18% of all patients, including 15% of adults and 23% of pediatric recipients. Although most transplant protocols today do not include routine germline testing outside of known inherited bone marrow failure syndromes, our findings suggest that this may leave a significant proportion of clinically relevant variants undetected at the time of transplant.

Some of these variants may directly influence transplant outcomes. In our data, patients carrying pathogenic or likely pathogenic variants in genes associated with hematological malignancies experienced significantly lower overall survival compared to those without such variants, driven by both increased relapse risk and NRM. This observation aligns with the idea that patients with inherited predisposition may require adapted conditioning regimens, such as reduced-intensity conditioning in the context of DNA repair disorders or bone marrow failure syndromes, to avoid excessive toxicity. Moreover, recognizing germline variants before HSCT allows clinicians to consider long-term surveillance strategies tailored to inherited cancer risks.[94,183]

A significant proportion of the patients in our cohort had not undergone genetic testing prior to HSCT, including many who had related donors. This is an important clinical gap: what is not tested for cannot be accounted for in transplant planning. In our data, more than half of the patients with a family donor had no prior germline evaluation, despite the possibility of shared inherited risk. As genetic testing becomes more widespread, germline predisposition is increasingly identified in patients without typical features of inherited disease, highlighting the importance of systematic genetic evaluation.[83,174,183]

While germline variants in cancer predisposition genes did not significantly affect five-year post-transplant outcomes in our cohorts, their potential impact on

long-term health should not be underestimated. These genes, particularly those involved in DNA repair pathways, may increase the risk of secondary malignancies following HSCT, especially in patients exposed to intensive chemotherapy and TBI.[277,278] *CHEK2* was among the most commonly observed variants in our studies, and while heterozygous carriers showed no difference in OS or RR, biallelic carriers had early post-transplant mortality. Given the cumulative genotoxic stress from both underlying disease and treatment, patients harboring such variants may benefit from tailored long-term surveillance and cancer prevention strategies as part of post-HSCT follow-up.[174]

Additionally, the detection of secondary finding variants offers opportunities for proactive management and genetic counseling — not only for the patient but potentially also for family members who may share the same germline risk. Incorporating ACMG SF variant analysis into pre-HSCT genetic testing can thus provide added value beyond disease-specific diagnostics. It supports a broader shift toward precision medicine and long-term survivorship care in hematology.

Although rare, our results also highlight the possibility of clinically relevant germline variants in stem cell donors. In our studies, we observed shared pathogenic variants in familial donors, confirming that inherited predisposition can complicate donor selection. Even unrelated donors may occasionally carry variants of concern, including *CHEK2*, which was relatively frequent in both patients and donors in our Finnish cohort. Although *CHEK2* carriership did not influence 5-year transplant outcomes, its long-term effects remain unknown and may require closer follow-up.

6.4 Fertility considerations in hematological disorders

Fertility preservation is an increasingly important aspect of care for pediatric and young adult patients undergoing treatment for hematological disorders.[226,227,230] While the gonadotoxic effects of chemotherapy and TBI used in conditioning regimens for HSCT are well known, our findings demonstrate that spermatogonial depletion may already be present before any cytotoxic therapy is initiated.[226,227] This suggests that underlying disease biology, genetic predisposition, and disease-associated factors, such as chronic inflammation or transfusion dependency, can impair spermatogenesis independently of treatment.[280]

In our multicenter study of prepubertal boys with severe hematologic conditions, testicular biopsies revealed decreased spermatogonial numbers in nearly half of the patients, with severely depleted germ cell pools observed in 19 %. Notably, the most pronounced depletion was seen in patients with Fanconi anemia and other inherited bone marrow failure syndromes. In contrast, boys with immunodeficiencies or aplastic anemia more often presented with germ cell counts within the normal age-adjusted range. These findings underscore the variability in

fertility preservation potential based on disease subtype and underlying pathophysiology.

This pre-existing impairment has critical implications for testicular tissue cryopreservation, which remains the only fertility preservation option available for prepubertal boys.[237] While the technique is still considered experimental, it is increasingly offered as part of fertility preservation programs worldwide.[237] The success of future fertility restoration efforts, whether through autologous grafting, in vitro spermatogenesis, or spermatogonial stem cell transplantation, will likely depend on the quantity and quality of spermatogonia in the cryopreserved tissue.

Given these considerations, fertility risk assessment should begin at the time of diagnosis, not only prior to initiating HSCT. Early referral to fertility preservation programs is essential, especially for patients with syndromic or genetically defined marrow failure conditions, where irreversible gonadal damage may already have occurred by the time of transplantation. For prepubertal boys with severe hematological diseases, appropriate counseling regarding the potential reduction in spermatogonial numbers is essential prior to testicular tissue cryopreservation. In conclusion, fertility preservation should be considered a core component of care in pediatric and young adult patients with hematological disorders.

6.5 Strengths and limitations

A major strength of this thesis is the use of large, population-based and clinically well-annotated cohorts, including over 870 patients who underwent HSCT and both pediatric and adult patients with acute lymphoblastic leukemia. The exome sequencing data was of high quality, and the analytical pipeline applied thorough classification criteria using several standardized methods enhancing the robustness and reproducibility of the findings. Additionally, the availability of donor genotyping in one study allowed preliminary evaluation of germline variants in stem cell donors which is an aspect rarely addressed in transplantation research.

Another important strength lies in the comprehensive scope of genetic analysis, which extended beyond traditional genes associated with risk of hematological disorders to include germline variants potentially compromising the outcome HSCT. Also, combining histological, genetic, and phenotypic data from patients with severe hematological disorders provides a unique perspective on a field that has received limited clinical attention.

However, several limitations must also be acknowledged. First, despite the relatively large sample size, the absolute number of patients with individual rare variants remains small, which limits the statistical power to draw conclusions about specific genes or variants, especially regarding outcome measures such as relapse and survival. Second, in the ALL cohort, pediatric samples were often collected from peripheral blood at the time of HLA typing, which introduces a small but inherent

risk of misclassifying somatic variants as germline despite careful filtering and manual review.

Finally, in the fertility preservation study, testicular samples were obtained in a clinical setting where some disease- or treatment-related factors may not have been fully controlled for. Furthermore, while the findings regarding spermatogonial depletion are compelling, the long-term fertility outcomes of these patients remain unknown, and the clinical applicability of testicular tissue cryopreservation remains experimental.

Nonetheless, these findings provide valuable insights for improving genetic testing, guiding donor selection, and enhancing comprehensive, long-term patient care.

6.6 Future directions and clinical recommendations

The growing recognition of germline predisposition in hematological malignancies opens new avenues for more personalized and risk-adapted clinical approaches. While current clinical guidelines recommend genetic testing primarily for individuals with inherited bone marrow failure syndromes or strong familial backgrounds, our findings demonstrate that clinically relevant germline variants can also be identified in patients without classical phenotypes or family history.[51,83,184,281] Given the therapeutic and donor-related implications, particularly in the setting of allogeneic hematopoietic stem cell transplantation, there is increasing justification for expanding germline testing beyond high-risk groups. Considering the emerging evidence and recent best-practice recommendations from the UK, current practices should be reconsidered, with broader germline testing extended especially to those undergoing allogeneic hematopoietic stem cell transplantation, and potentially to all patients with hematological malignancies.[174]

While *CHEK2* emerged as a particularly relevant gene in our data, its full clinical significance remains uncertain. Although no clear effect on short-term HSCT outcomes was observed, *CHEK2* and other cancer predisposition variants may still have important implications for long-term risk and more research is needed before definitive clinical conclusions can be drawn.[277,278] Future studies on *CHEK2* should focus on understanding the underlying mechanisms to explain the risk for hematological malignancies and long-term follow-up of affected individuals, to determine whether these variants have late effects. Such research will be essential for developing personalized surveillance strategies and improving long-term outcomes in this growing patient population.

While allogeneic hematopoietic stem cell transplantation replaces the recipient's hematopoietic system, it does not replace the bone marrow microenvironment, which continues to influence stem cell behavior.[282–284] The bone marrow niche

plays a key role in regulating hematopoiesis and may influence disease course.[283,284] Emerging research suggests that alterations in the niche can contribute not only to malignant transformation but also to chemoresistance and relapse.[283,284] This raises the possibility that, in some cases, inherited predisposition may not be limited to hematopoietic cells themselves but could also involve the surrounding stromal microenvironment. Future studies are needed to explore the contribution of the germline niche to long-term transplant outcomes and malignant progression.[283]

In the clinical context, fertility preservation should ideally be integrated early after diagnosis and supported by multidisciplinary collaboration. Establishing evidence-based guidelines and long-term follow-up strategies will help ensure that fertility care becomes a routine and equitable part of comprehensive care.[237] Future research should focus on uncovering the underlying mechanisms of spermatogonial depletion in patients with hematological disorders, including the roles of genetic predisposition, as well as disease- and treatment-related factors.[237] Also, further research is needed to confirm whether testicular tissue freezing is inadvisable for patients with Fanconi anemia. Standardized methods for evaluating and preserving testicular tissue, along with continued research into fertility restoration techniques, will be essential as these approaches move closer to clinical application.

In summary, this work outlines several concrete directions for future research and clinical development. While these areas are already visible priorities, the comprehensive clinical and genetic datasets collected in this research may also serve as a foundation for future investigations beyond the current scope. As long-term follow-up data continues to evolve, these resources will be invaluable in shaping more personalized, predictive, and preventive approaches in hematology.

7 Conclusions

This thesis explored the prevalence and clinical implications of germline variants in patients with severe hematological disorders, focusing on their relevance in hematopoietic stem cell transplantation and fertility preservation. Through comprehensive analyses of germline genetic data of diverse patient cohorts, the studies demonstrated that pathogenic germline variants are relatively common not only in pediatric cases but also in unselected adult patients. Notably, the findings extend beyond classical inherited syndromes, revealing that significant germline predisposition can exist even in patients without suggestive family history or syndromic features.

The identification of *CHEK2* as a recurrent variant associated with hematological malignancies in the Finnish population provides new insights into moderate-risk cancer predisposition. Although *CHEK2* did not affect short-term HSCT outcomes, its potential role in long-term risks, such as secondary malignancies, underlines the need for future surveillance strategies.

In the context of fertility preservation, this work provides novel evidence that spermatogonial depletion may occur before any treatment is initiated, particularly in patients with inherited bone marrow failure syndromes. These findings stress the importance of offering fertility preservation at diagnosis and call for further investigation into the mechanisms behind impaired spermatogenesis in hematological diseases.

In conclusion, this thesis highlights the clinical relevance of germline predisposition in hematological disorders and supports its integration into routine evaluation. Ultimately, close interdisciplinary collaboration between hematologists, geneticists, fertility specialists, and genetic counselors, along with standardized practices, will be key to translating genetic insights into individualized care and improved patient outcomes.

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