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PHARMACOGENETICS AND CELL BIOLOGY OF PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA

PhD thesis

Judit Csányiné Sági, MD

Doctoral School of Molecular Medicine

Semmelweis University

Supervisor: Ágnes Félné Semsei, Ph.D

Official reviewers: Zsolt Rónai, Ph.D

Zsolt Szelid, MD, Ph.D

Head of the Final Exam Committee: Péter András Lakatos, MD, D.Sc

Members of the Final Exam Committee: András Bors, Ph.D

Anna Sebestyén, Ph.D

Csaba Bödör, Ph.D

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Abbreviations

ABCA2: ATP binding cassette subfamily A member 2

ABCA3: ATP binding cassette subfamily A member 3

ABCB1: ATP binding cassette subfamily B member 1

ABCC1: ATP binding cassette subfamily C member 1

ABL1 (RUNX1): ABL proto-oncogene 1

ACT: anthracycline-induced cardiotoxicity

AE: acute encephalopathy

ALL: acute lymphoblastic leukemia

ALL-BFM: ALL Berlin-Frankfurt-Münster chemotherapy protocol

AML: Acute myeloid leukemia

ATE: Acute toxic encephalopathy

B-ALL: B-cell acute lymphoblastic leukemia

BCL-2: B-cell lymphoma 2

BCR: breakpoint cluster region protein

BIM (BCL2L11): BCL2 Like 11

BM: Bone marrow

BMT: Bone marrow transplantation

CAR-T cell: Chimeric antigen receptor T cell

CD7/20/22/38: Cluster of differentiation 7/20/22/38

CDK4/6: Cyclin-dependent kinase 4/6

CDKN2A/B: Cyclin dependent kinase inhibitor 2A/B

CEP72: centrosomal protein 72

CGH: Comparative genomic hybridization

CLL: Chronic lymphocytic leukemia

CNOT3: CCR4-NOT transcription complex subunit 3

CNS: Central nervous system

CR: Complete remission

CTLA4: Cytotoxic T-lymphocyte associated protein 4

CYP3A4: Cytochrome P450 family 3 subfamily A member 4

CYP3A5: Cytochrome P450 family 3 subfamily A member 5

DNA: Deoxyribonucleic acid

EED: Embryonic ectoderm development

EFS: Event-free survival

ETV6 (TEL): ETS variant transcription factor 6

EZH2: Enhancer of Zeste 2 polycomb repressive complex 2 subunit

FBXW7: F-Box and WD repeat domain containing 7

FISH: Fluorescence in situ hybridization

FLT3: Fms related receptor tyrosine kinase 3

FS: Fractional shortening

GSI: γ-secretase inhibitors

GSTP1: Glutathione S-Transferase Pi 1

GSTT1: Glutathione S-Transferase Theta 1

GWAS: Genome-wide association study

HLA: Human leukocyte antigen

HR: High risk

HSCT: Hematopoietic stem-cell transplantation

IKZF1: IKAROS family zinc finger 1

IL7R: Interleukin-7 receptor

JAK 1: Janus kinase 1

KDM6A: Lysine demethylase 6A

MAF: Minimal allele frequency

miR: MicroRNA

MLL: KMT2A, lysine methyltransferase 2A

6-MP: 6-mercaptopurine

MRD: Minimal residual disease

mTOR: Mechanistic target of rapamycin

MTX: Methotrexate

NF1: Neurofibromin 1

NGS: next-generation sequencing

NOTCH1: Notch receptor 1

OS: Overall survival

OSC: Osteosarcoma

PAX5: Paired box 5

PCR: Polymerase chain reaction

Ph+ ALL: Philadelphia chromosome–positive acute lymphoblastic leukemia

PharmGKB: The Pharmacogenomics Knowledge Base

PHF6: PHD finger protein 6

PRES: Posterior reversible encephalopathy syndrome

PTEN: Phosphatase and Tensin homolog

RNA: Ribonucleic acid

RPL5: Ribosomal protein L5

RUNX1 (ABL1): See at ABL1

SKY: Spectral karyotyping

SLS: Stroke-like syndrome

SNP: Single nucleotide polymorphism

SR: Standard risk

STAT: Signal transducer and activator of transcription

SUZ12: SUZ12 polycomb repressive complex 2 subunit

TAL1: TAL BHLH transcription factor 1

T-ALL: T-cell acute lymphoblastic

TEL (ETV6): See at ETV6

TLX1/3: T-cell leukemia homeobox protein 1/3

VEGFR: Vascular endothelial growth factor receptor

WBC: White blood cell

WES: Whole exome sequencing

WHO: World Health Organization

WT1: WT1 Transcription Factor

1. Introduction

In Europe, an average of 12-15 new patients is diagnosed with malignancies per 100,000 children a year (Steliarova-Foucher *et al.*, 2018). Among childhood cancers, the most common is acute lymphoblastic leukemia (ALL). Incidence is 1/2000 kids in the 1-15 years age group in developed countries, diagnosis peak is at 2-5 years. Males are more prone than females (Roganovic, 2013). Leukemia is the malignant transformation and proliferation of a lymphoid progenitor with differentiation blockade. In Hungary, 50-70 pediatric patients are diagnosed with ALL every year. The two main subgroups of ALL are T-cell and B-cell types. The cure rate is higher than 80-90% in the overall pediatric ALL population. However, complications of the therapy are responsible for the remaining 10-20% unfavorable outcome (Garami *et al.*, 2014, Gatt & Izraeli, 2008). Challenges related to ALL therapy are the focus of this thesis. Side effects of the treatment or the return of primary leukemia caused by ineffective chemotherapy reduce survival (Stary *et al.*, 2014). Recurrent leukemia cases are called relapses. Relapse cases presented extramedullary in the central nervous system (CNS) or after primary T-cell ALL (T-ALL) are outstanding limitations on survival (Locatelli *et al.*, 2012). Adverse effects of the used drugs for example cardiotoxicity or neurotoxicity are responsible for dose-limitation during treatment or for long-lasting dysfunction of the affected organs (Cole *et al.*, 2009, Lipshultz *et al.*, 2005, Magge & DeAngelis, 2015, Raj *et al.*, 2014). The response to certain drugs or the success of the treatment shows inter-individual differences which can be influenced by inherited germline variants or de novo mutations of the transformed cells (Cunningham & Aplenc, 2007, Girardi *et al.*, 2017). Reliable biomarkers for predicting these complications and for targeted therapy are still needed (Christenson *et al.*, 2015, Frishman-Levy & Izraeli, 2016, Girardi *et al.*, 2017, Lauschke *et al.*, 2019). After proper validation, biomarkers based on the genetic background of patients could support endeavors of personalized precision medicine also in the care of ALL (Marchiano *et al.*, 2021).

In the present thesis, our aim was to investigate the association of inherited single nucleotide variants with cardio-, and neurotoxicity or first CNS relapse and de novo mutations in the clonal evolution of the first relapse in T-ALL.

1.1. Pediatric acute lymphoblastic leukemia

Many factors influence the risk of ALL. The vast majority of primary ALL is sporadic and the result of acquired new mutations, however, 5% are inherited diseases or part of a family cancer syndrome like ataxia-telangiectasia, Bloom, or Li-Fraumeni syndrome. Down-syndrome increases the risk of ALL by 20%, and ALL occurs more often with congenital B-cell immune deficiency (Gatt & Izraeli, 2008). Known leukemic driver mutations affect *IKZF1* (IKAROS family zinc finger 1), which encodes the lymphoid transcription factor Ikaros; hematopoietic or B-cell developmental genes, like *TEL* (*ETV6*, ETS variant transcription factor 6), *ABL1 (RUNX1)* (ABL proto-oncogene 1), and *PAX5* (Paired Box 5). The etiology of ALL was also described by the two-hit hypothesis. According to this, there is an early defect in the fetal lymphopoiesis, which is the first hit and results in the proliferation of a pre-leukemic clone. Twins can share such cells through metastasis and this is why leukemia is concordant in twins. (Roganovic, 2013). Most of the diagnosed patients (70%) carry a pre-leukemic clone detected by Guthrie cards, however, only 1% of the clone carriers develop leukemia subsequently. The second hit on the pre-leukemic clone, which happens after birth, initiates the leukemia disease formation (Greaves, 2018, Hein *et al.*, 2019). This highlights the role of environmental factors next to the genetic variants: both are responsible for the development of ALL. Parental or offspring exposure to irradiation, pesticides, paints, alcohol, smoking, viral infections (Epstein-Barr virus, influenza, chickenpox, measles, mumps) are proved risk factors (Roganovic, 2013). Furthermore, the second hit can be the disturbed maturation of the immune system caused by the cleaner lifestyle of modern societies. Meeting fewer pathogens or delayed infections can be a trigger for the pre-leukemic clone to survive, to acquire more genetic rearrangements and to start forming definitive disease. Protective factors can be early infections through social interactions with other children like siblings or daycare contacts. In addition, the microbiome can influence the risk of ALL. Even breastfeeding and vaginal delivery can introduce a special microbiome to the baby which prevents ALL (Greaves, 2018). Disturbed gut microbiome - at the overuse of antibiotics - without the presence of an infection, together with inherited leukemic susceptibility is also a predisposing factor for the development of leukemia (Gradel & Kærlev, 2015, Vicente-Duenãs *et al.*, 2020). A higher risk for ALL was associated with increased birth

weight or previous intrauterine death in former children (Roganovic, 2013). Adenovirus and Streptococcus infections were supposed to cause clusters of leukemia cases, although, ALL was not the direct effect of the infections in this studies (Francis *et al.*, 2012, Heath & Hasterlik, 1963).

B-lineage ALL represents 80% of childhood ALL, the remaining 20% includes most of all T-ALL cases but have the same clinical appearance. The clinical signs of ALL appear 3-4 weeks before diagnosis. Possible symptoms are tachycardia, dyspnoea, petechia, purpura, bleeding from mucosa, fever, infection, ulceration of buccal mucosa. These common marks are related to different organ involvements. Bone marrow (BM) failure, in which more than 25% of the blast cells are present in the marrow, causes anemia, thrombocytopenia, and neutropenia; however, serious hemorrhage is sporadic. Bone pain or weight loss can be present, as well. Lymphadenopathy, hepato-, and splenomegaly are not mandatory signs. Rare cases are present with respiratory distress or vena cava superior syndrome caused by larger mediastinal lymph nodes. CNS involvement is present in 5% of cases showing symptoms like headache, papilledema, seizure, cranial nerve palsy. Extramedullary manifestations of the disease can affect other organs like testicles, ovaries, eyes, skin, lungs, heart, kidneys, and gastrointestinal tract. Relapse or refractory disease are frequent in these organs. The nonspecific symptoms of ALL can be confused with congenital or acquired neutropenia, thrombocytopenia, anemia, viral infections, rheumatoid arthritis, pertussis, acute myelogenous leukemia, and tumors affecting BM. Differential diagnosis is supported by BM aspirate, which is always needed for the diagnosis of ALL. In the absence of leukopenia, blasts can be detected in peripheral blood smear, as well (Figure 1). If the BM has high density, the biopsy is the gold standard. Important laboratorial findings are initial white blood cell (WBC) count (> 10.000/mm^3) which supports the diagnosis in half of the cases. Normochromic and normocytic anemia with a low number of reticulocytes is characteristic (hemoglobin < 10g/dL). Elevated uric acid level caused by nucleic acid catabolism can damage the kidneys, which has to be controlled carefully (tumor lysis syndrome). Cerebrospinal fluid cytocentrifugation is always required at the diagnosis to test if CNS involvement of leukemia is present. Cerebrospinal fluid categorization determines 3 groups: CNS-1 status (<5 WBC/mm^3 and no blasts), CNS-2 status (<5 WBC/mm^3 with blasts), and CNS-3 status (≥5 WBC/ mm^3 and blasts or cranial nerve involvement or presence of cerebral mass). ALL classification

has many aspects, like morphology, immunophenotype, cytogenetic, or molecular genetic factors that determine the therapeutic plan and prognosis. WHO classification of acute leukemias revised in 2016 is in use until today (Arber *et al.*, 2016).

Risk stratification is an important task at the therapy initiation and in the treatment continuation. Based on the age and WBC count at diagnosis, sex, race, possible extramedullary manifestation, immunophenotype, and cytogenetics, patients are stratified into three groups: standard- (SR), intermediate- (MR), and high-risk (HR) group. Considering the early answer to the induction therapy represented by minimal residual disease (MRD), the risk group of the patient can be changed. Rapid early responders have the best survival, while residual leukemic cells on day 14 of induction (<5% blasts in the BM) predispose for relapsed or refractory disease. MRD status is controlled regularly during the therapy. Less than 10^{-4} blast count is the goal to be achieved (Roganovic, 2013). Complete remission (CR) is achieved when less than 5% of leukemic blasts are present in BM and there are no tumor cells in the peripheral blood and no signs or symptoms of leukemia (Sung & Jang, 2014). Genetic classification is also an important aspect of the treatment and prognosis. Polymerase chain reaction (PCR), fluorescence in situ hybridization (FISH), spectral karyotyping, (SKY) and comparative genomic hybridization (CGH) are the key methods for patients' stratification. Such variants are for example *TEL/AML1* translocation which means a better prognosis, while Philadelphia chromosome-positive ALL predicts an inferior outcome (Roganovic, 2013).

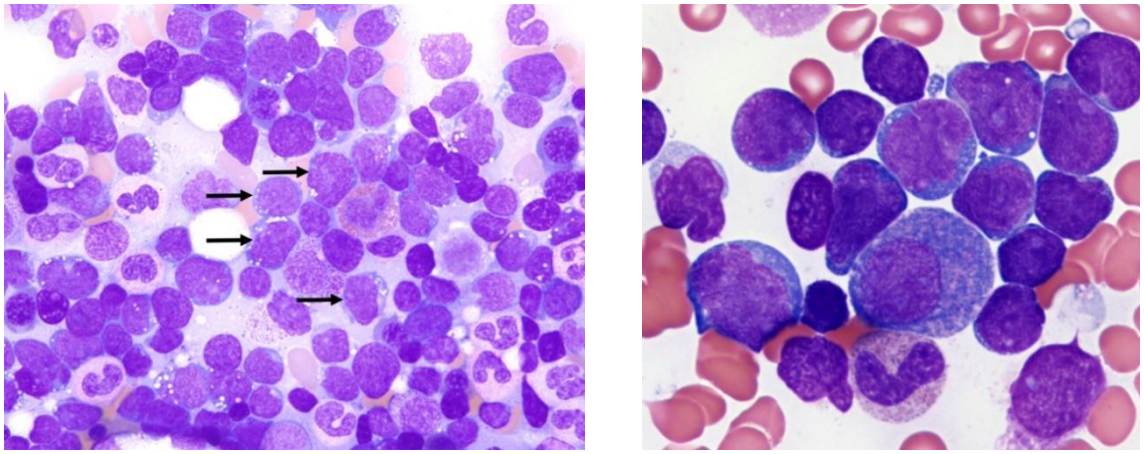

Figure 1. Bone marrow aspirate smears with increased blasts in pediatric acute lymphoid leukemia ('Atlasgeneticsoncology', n.d., 'Atlasoncology_Down', n.d.)

Based on the previous factors, the treatment strategy of ALL will be chosen. Instead of a single medication, a combined regimen of chemotherapy drugs is used in various phases of the treatment. After an intensive, intravenous period, patients get maintenance therapy for more than two years (Figure 2).

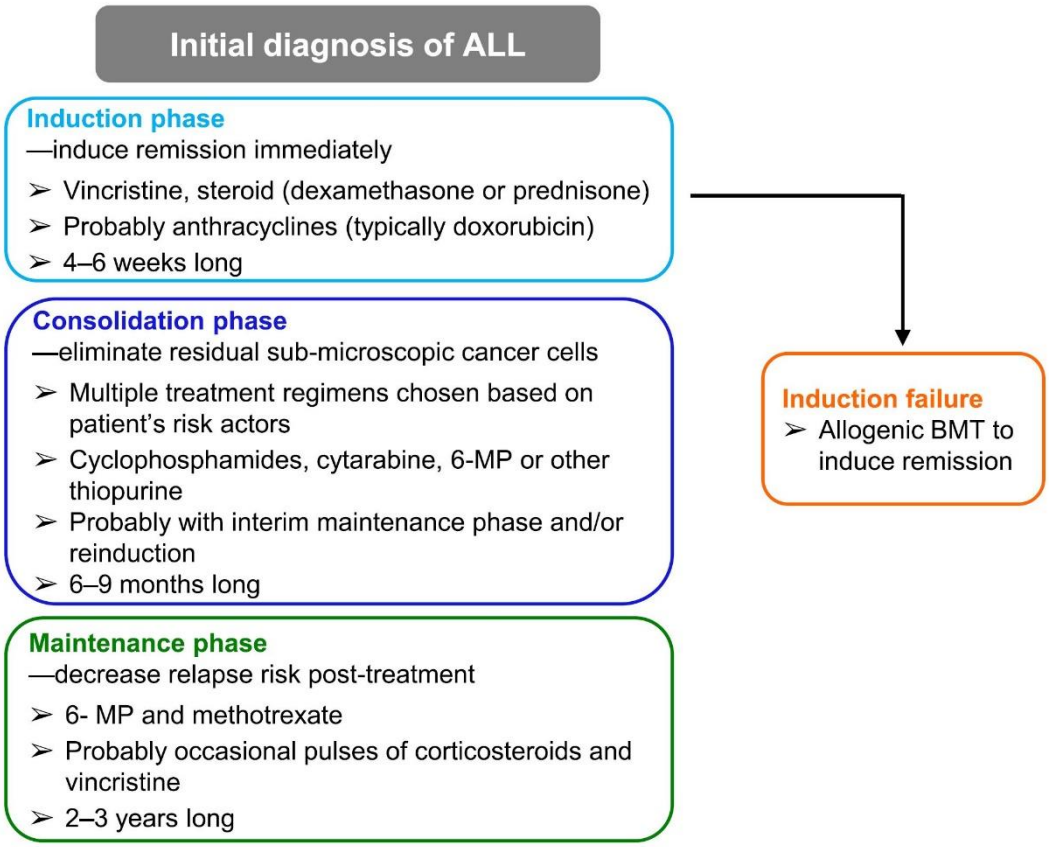

Figure 2. Treatment strategy of ALL (Rudin *et al.*, 2017). Aim of the phases of the treatment and the main drugs used are indicated for each phase.

Abbreviation: BMT- bone marrow transplantation, MP-mercaptopurine.

Many protocols are used worldwide for treating ALL. Usually, trials are also part of the protocols to investigate augmentation therapies or other opportunities to achieve better survival for patients with high risk or avoid treatment side effects. In Hungary, the ALL IC-BFM 2009 protocol is in use currently. Therefore, I focus on ALL IC-BFM 2009 to give a brief introduction of ALL therapies. The protocol includes five main steps: induction, early intensification, consolidation, reinduction, and maintenance. Anti-tumor drugs are applied intravenously, intrathecal, or per os (occasionally intramuscular). CNS

prophylaxis is an important part of the protocol. It includes high-dose systemic methotrexate (more doses per week) in combination with intrathecal methotrexate alone or together with cytarabine and hydrocortisone. In the induction, the main aims are the elimination of most of the blasts, and to achieve remission. Medications used in this phase are glucocorticoids (prednisone/ dexamethasone) as a single therapy in the first weeks for a slow tumor lysis. After this additional vincristine, L-asparaginase and anthracyclines are administered together. This phase is complete with further intrathecal chemotherapy. After induction in the early intensification, the applied drugs are 6-mercaptopurine, cyclophosphamide, and cytarabine with intrathecal chemotherapeutics. This is followed by consolidation or a so-called CNS-directed therapy against the tumor cells in sanctuaries, where the leukemia cells can hide from the systemic chemotherapy. The final intensive step is the reinduction phase, where the remaining blasts will be targeted using dexamethasone, daunorubicin, and thioguanine. Children with high-risk or inappropriate induction responses are candidates for allogeneic stem cell transplantation. After 6 or 12 months of intensive treatment, immunosuppressive maintenance therapy will keep patients in remission and prevent marrow from relapse using the combination of oral 6-mercaptopurine and oral, plus intrathecal methotrexate for further 24 months (Inaba & Mullighan, 2020, Kaplan, 2019).

Novel treatment strategies are targeted molecules and immunotherapy. Most of them are used in refractory or relapsed ALL. Targeted therapies for ALL are imatinib mesylate, a selective inhibitor of BCR-ABL1 (breakpoint cluster region protein, ABL proto-oncogene 1) protein kinase in Ph+ ALL or lestaurtinib, a selective small-molecule FLT3 (Fms-like tyrosine kinase 3) tyrosine kinase inhibitor is used in infant ALL with *MLL (KMT2A*, lysine methyltransferase 2A) rearrangements. BCL-2 (B-cell lymphoma 2) protein mimetic venetoclax, mTOR (Mechanistic target of rapamycin) inhibitor sirolimus, bortezomib a selective inhibitor of the 26S proteasome are promising agents, too. Monoclonal antibodies against CD20 or CD22 (rituximab and epratuzumab, respectively) or histone deacetylase inhibitors like vorinostat are also in clinical trials with encouraging results. The aim of these new therapies is to be more effective and less toxic (Cooper & Brown, 2015, Lato *et al.*, 2021, Roganovic, 2013). The use of nelarabine, etoposide, and cyclophosphamide improved the survival of relapsed T-ALL. The γ-secretase inhibitors (GSIs) are inhibitors of NOTCH receptors. Monoclonal antibody

therapy is also promising against human NOTCH1 (Notch receptor 1). CDK4/6 (cyclin-dependent kinase 4/6) inhibitors like palbociclib are also already in clinical trials which are causing cell-cycle arrest in malignant leukoblasts. Ruxolitinib and tofacitinib are also in the test phase for investigating their effect to decrease the activity of ILR7 (Interleukin 7 receptor alpha chain) and JAK (Janus kinase)–STAT (signal transducer and activator of transcription) pathway. Tyrosine kinase inhibitors imatinib, dasatinib, and nilotinib can have a clinical impact on this disease, as well. CAR-T cell therapy is effective in B-cell leukemia, however, is difficult to introduce in T-ALL. Reasons are self-targeting, contamination of modified cells with malignant ones, or the problematic differentiation between modified, normal, and abnormal cells. Daratumumab is a human monoclonal antibody that binds to CD38. It was applied in multiple myeloma primarily and showed good results in T-ALL patient-derived xenografts, too (Lato *et al.*, 2021). BiTE is a bispecific T-cell engager antibody construct. Blinatumomab is such a therapy option that targets CD19 on B-cell leukemia blasts (Von Stackelberg *et al.*, 2016). Immune checkpoint inhibitors (ICIs) are potent agents, too, to target CTLA4 or PD1 function like nivolumab which binds the PD-1 receptor (Batlevi,Connie Lee *et al.*, 2016). Monoclonal antibodies can be conjugated to cytotoxic drugs, toxins of bacteria, or radioisotopes to make them more sufficient (Pui & Jeha, 2007). Resistance or toxicity related to these alternative therapies still need to be addressed, however, they are potent agents to improve the survival of relapsed T-ALL in a good combination and involving supportive care (Lato *et al.*, 2021). Pre-B-cell ALL, girls, and intermediate age group has better survival than patients with mature T-cell ALL, boys, patients under 1 year or older than 10 years of age, or being obese at the start of diagnosis (Orgel *et al.*, 2021, Roganovic, 2013).

1.2. Challenges and pharmacogenomics in the therapy of pediatric acute lymphoblastic leukemia

Despite the excellent cure rate of ALL, the challenges of therapy are not negligible. These are treatment failures, complications related to the chemotherapy, refractory or relapsed disease, and adverse events, like toxicities. Therapy-related side effects can be life-threatening and can affect almost every organ (Zawitkowska *et al.*, 2020), can have an

impact on the quality of life even in the long run or influence survival by causing delays or discontinuation of the therapy. Serious complications are the relapse of primary leukemia after an unsuccessful cure or acute and late adverse effects related to the overall treatment. Most common side effects may appear as neurotoxicity; anthracycline-induced cardiotoxicity; BM toxicity (osteonecrosis and reduced bone mineral density); endocrine problems (growth delay, gonad dysfunction, obesity, and metabolic syndrome); second neoplasms like brain tumors or hematopoietic neoplasms or neuropsychological symptoms. Irradiation, if administered, is the biggest risk for such events. The toxic effects can be associated with a special group of drugs used in ALL, but in most cases more drugs are supposed to cause the same or similar adverse event.

Patients treated with the polychemotherapeutic approach show inter-individual differences in the presence or absence of adverse events or in the grade of the symptoms (Franca *et al.*, 2020, Kaplan, 2019). Drug dosing is usually based on body weight or body surface area in ALL protocols. However, pharmacokinetics and pharmacodynamics are influenced not only by age or weight but by germline variants, as well (Kearns *et al.*, 2003). Inter-individual differences in the treatment response can be based on the patients' genetic background. Genetic variations have been proven to influence the metabolism, effect, and side effects of chemotherapeutic drugs. This genetic diversity in the human genome can lead to the different manifestations of toxicities (Stanulla *et al.*, 2005). The most relevant pharmacogenetic risk factors are known for more than a decade and are prognostic biomarkers associated with clinical outcomes also (Cunningham & Aplenc, 2007). In cancer treatment, some drug labels are already included recommendations for dosing based on the patients' genetics (J. A. Kim *et al.*, 2021). For pediatric ALL, the highest evidence (level 1A) drug-gene annotations are known for *TPMT* (Thiopurine S-methyltransferase) or *NUDT15* (Nudix Hydrolase 15), but they are still a long way from the clinical implementation (Maamari *et al.*, 2020). Level 1A means the strongest evidence which is the Clinical Annotation Levels of Evidence according to PharmGKB, where the levels are 1A, 1B, 2A, 2B, 3, 4 (PharmPKB, n.d.). Predictive markers for determining the risk of side effects are important factors in personalized treatment plans. Germline variants are single nucleotide polymorphisms (SNPs) that can help predispose patients for adverse events or relapse tailoring their therapy based on risk alleles (Franca *et al.*, 2020, Kaplan, 2019). Several SNPs are described in predicting toxicity and relapse

in ALL without using them in the clinical practice (B. Leonardi *et al.*, 2017, R. Hough & Vora, 2017, Stanulla *et al.*, 2005). Candidate gene association studies, genome-wide association studies (GWAS), and whole exome sequencing studies (WES) already proposed new variants for clinical use in this area (Roganovic, 2013) (Figure 3).

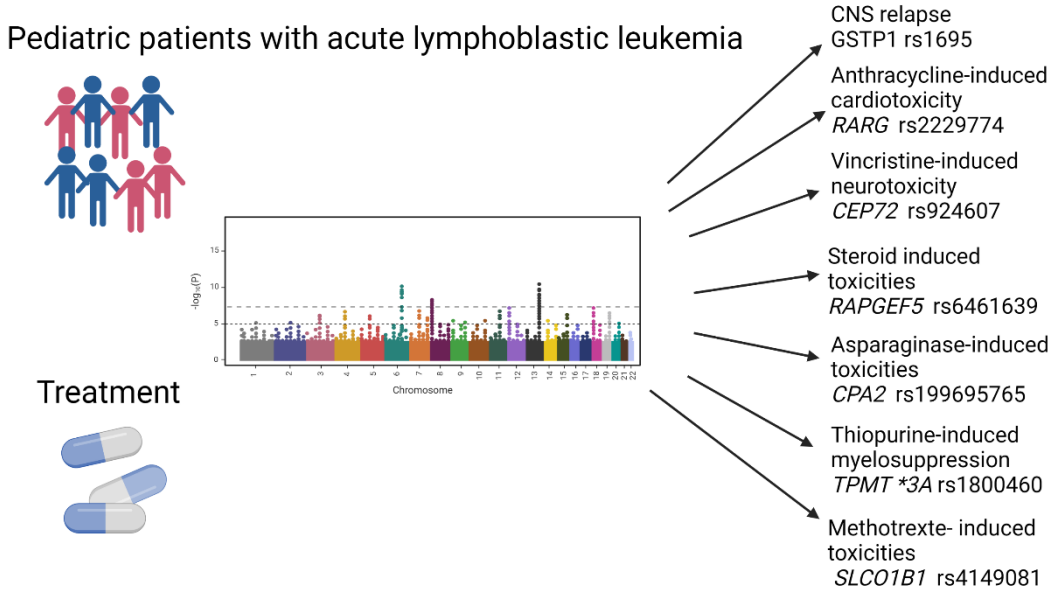

Figure 3. Main toxic side effects of ALL therapy and recommended SNPs for a personalized approach. (Created with BioRender.com.) Abbreviations: *CEP72*- Centrosomal Protein 72, *CPA2*- carboxypeptidase A2, *GSTP1*- Glutathione S-Transferase Pi 1, *RAPGEF5*- Rap Guanine Nucleotide Exchange Factor 5, *RARG*- Retinoic Acid Receptor Gamma, *SLCO1B1*- Solute Carrier Organic Anion Transporter Family Member 1B1, *TPMT*- Thiopurine S-Methyltransferase

1.2.1. Cardiotoxicity

In the backbone of cancer therapy, anthracyclines are key elements. They are very effective components extracted from Streptomyces bacterium. Anthracyclines intercalate to the DNA and inhibit topoisomerase II. Metabolite of anthracyclines accumulates in the cardiomyocytes and reduces heart contractility by inhibiting Ca2+ and Na+/K+ pumps (Blanco *et al.*, 2012). The main anthracycline compounds used in ALL treatment are doxorubicin and daunorubicin. Short and long-term cardiac side effects or secondary malignancies can appear after the administration of anthracyclines and influence the quality of life (Marinello *et al.*, 2018). Acute, early-and late-onset manifestations are

known, symptoms evolve within hours or days, or within 1 year or more than 1 year after treatment. Progressive left ventricular dysfunction can lead to congestive heart failure (Petrykey *et al.*, 2020). Early prevention of symptomatic cardiac failure is essential. For a prompt treatment, detection of cardiotoxicity in the asymptomatic stadium is part of the clinical care of patients with malignancies. Regular screening means physical examination (blood pressure, BMI (body mass index), B-type natriuretic peptide (BNP) measurement, examination of the ocular fundus, the jugular pulse), and the application of imaging tools. The most frequently used is echocardiography, supporting tools are CT, MRI, and ECG. Clinical manifestation of anthracycline-induced cardiotoxicity (ACT) can vary from mild symptoms to stage A-D heart failure (Carver *et al.*, 2013). ACT is diagnosed, if a greater than 10% reduction in left ventricular ejection fraction (LVEF) value is observed compared to the level measured at the start of treatment or left ventricular fractional shortening (FS) was ≤ 28%. Typical symptoms of ACT can be the reduced capacity for physical exercise, chest pain, and shortness of breath (Petrykey *et al.*, 2020). Patients with osteosarcoma (OSC) are treated with anthracyclines, too. Cardiac toxicity is also known in this group of patients regarding the higher administration doses of doxorubicin in this cohort are at 375–600 mg/m^2. Several studies analyzing OSC together with other malignancies for a sufficient number of cases (Claudia Maria Hattinger *et al.*, 2020). Pediatric cancer patients usually suffer from restrictive cardiomyopathy with diastolic dysfunction but with intact systolic function (Carver *et al.*, 2013). Cardiovascular mortality of patients diagnosed with cancer is eight times higher than that of the average population (Petrykey *et al.*, 2020). Anthracycline-induced cardiotoxicity is more often among patients at younger age, girls, after higher cumulative anthracycline dose (> 550 mg/m^2), and with additional radiotherapy of the cardiac region. However, a prominent increase in the prevalence of ACT has shown to present at 550 mg/m^2, histopathologic changes in the cardiac biopsies are found already at 250 mg/m^2 (Bristow *et al.*, 1978, von Hoff *et al.*, 1979). The inter-individual differences in the appearance and severity of cardiotoxicity are also influenced by genetic variants (Petrykey *et al.*, 2020). SNPs in transporters *ABCC1 (*ATP binding cassette subfamily C member 1) (rs3743527 and rs246221), in *ABCC5 (*ATP binding cassette subfamily C member 5) gene (rs7627754) and *SLC28A3* (Solute Carrier Family 28 Member 3) gene rs7853758 were in association with chemotherapy-related cardiotoxicity in pediatric

patients with ALL (M. Krajinovic *et al.*, 2016, Semsei *et al.*, 2012, Henk Visscher *et al.*, 2012). *CBR3* (Carbonyl reductase 3) metabolizing enzyme gene polymorphism, rs1056892 G allele was detected more often among patients with anthracycline-related congestive heart failure (Blanco *et al.*, 2008). *SLC22A17* and *SLC22A7* were associated with anthracycline-induced cardiotoxicity in children (Henk Visscher *et al.*, 2015). Protective SNPs against ACT were rs10836235 in the antioxidative enzyme *CAT* (Catalase), rs1799983 in nitric oxide synthase *NOS3* (Nitric oxide synthase 3), rs9327264 in *PLCE1* (Phospholipase C epsilon 1) and rs17249754 in *ATP2B1* (ATPase plasma membrane Ca2+ transporting 1) (Hildebrandt *et al.*, 2017, M. Krajinovic *et al.*, 2016, Rajić *et al.*, 2009). *HFE* (Homeostatic iron regulator) rs1800562 and *HAS3* (Hyaluronan synthase 3) rs2232228, *RARG* (Retinoic acid receptor gamma) rs2229774, *CELF4* (CUGBP Elav-like family member 4) rs1786814, and *GPR35* rs12468485 were a risk factor for ACT (Aminkeng *et al.*, 2015, Lipshultz *et al.*, 2013, Ruiz-Pinto *et al.*, 2017, Wang *et al.*, 2014, 2016). Rs17863783 in *UGT1A6* (UDP glucuronosyltransferase family 1 member A6) was also a predictor for cardiotoxicity (H. Visscher *et al.*, 2013). Recommendations of drug-gene annotations with moderate evidence for pediatric patients with malignancies treated with anthracyclines imply three variants: *RARG* rs2229774, *SLC28A3* rs7853758, and *UGT1A6*4* rs17863783 (Aminkeng *et al.*, 2016, Loucks *et al.*, 2021). Troponin, natriuretic peptides, microRNAs, peripheral blood mononuclear cell gene expression profile are further possible biomarker options for detecting cardiotoxicity (Tan & Lyon, 2018). If we could identify patients with a higher risk for heart failure, the use of pegylated liposomal anthracyclines would be one option to avoid cardiac toxicity in this group. Other preventive possibilities are the administration of cardioprotective dexrazoxane and statins (Upshaw, 2020). In doxorubicin-induced cardiotoxicity, exosomes are promising biomarkers and drug delivering-agents (Tian *et al.*, 2021). Exercise training can help patients with subclinical cardiomyopathy to improve cardiovascular reserve capacity (Smith *et al.*, 2014). Topoisomerase 2 β could be a potential target for preventing cardiotoxicity (Vejpongsa & Yeh, 2014). Dexrazoxane has the potential for preventing this adverse effect of doxorubicin (Geidel *et al.*, 1991, Kopp *et al.*, 2019).

1.2.2. Neurotoxicity in the central nervous system

Regarding the central nervous system (CNS), challenges are treatment-related toxicity and leukemia involvement (Kembhavi *et al.*, 2012). Neurotoxicity is the second among adverse effects which indicate dose-reduction during ALL treatment (Magge & DeAngelis, 2015). The prevalence of acute CNS toxicity related to ALL therapy is 3–13% (Parasole *et al.*, 2010). Systemic and CNS-directed treatment aims to avoid leukemia involvement in the CNS, although, it is considered to be neurotoxic in the short-, and long-term in children with ALL (Frishman-Levy & Izraeli, 2016, Fulbright *et al.*, 2011). Children treated only with chemotherapy (without cranial radiotherapy) have already risk to experience neurocognitive deficit (Cheung & Krull, 2015, Conklin *et al.*, 2015, Nassar *et al.*, 2017). Acute CNS symptoms with any reason are diverse like vomiting, headache, seizure, severe aphasia, impaired level of consciousness, and others depending on the affected brain areas. Clinical manifestations might appear as mild symptoms, as a severe, reversible focal deficit, or even as death. (Özütemiz *et al.*, 2019, Schmidt *et al.*, 2008, Vagace *et al.*, 2012). The discrimination between causes of neurologic complications in ALL might be difficult, but still important: many of them have a specific cure and early intervention is needed (Berg & Nand, 2017, J. Liu *et al.*, 2017). One adverse event of cancer treatment is acute toxic encephalopathy. Its differential diagnosis is based on clinical features and specific MRI findings (Özütemiz *et al.*, 2019) and needs the exclusion of peripheral neuropathy, CNS infection or malignancy, intracranial bleeding or stroke, sedative medication effect, or metabolic disturbance e.g. liver failure causing hyperammonemia, blood glucose abnormalities, or SIADH (syndrome of inappropriate antidiuretic hormone secretion) with low potassium level (Beitinjaneh *et al.*, 2011). Regarding a nomenclature published in 2016, CNS toxic events involve stroke-like syndrome (SLS), seizures, posterior reversible encephalopathy (PRES), depressed level of consciousness, thromboembolism (Schmiegelow *et al.*, 2016). PRES and SLS have distinct clinical and MRI features and therapeutic guidelines. (Parasole *et al.*, 2010, Schmiegelow *et al.*, 2016). Toxic encephalopathy might be followed by neurocognitive impairment, poor survival in some protocols, or long-term neurobehavioral problems (Banerjee *et al.*, 2018, Cheung *et al.*, 2016, Cole *et al.*, 2009). The detection of CNS toxicity has to be quick and accurate to avoid long-lasting lesions in severe cases

(Parasole *et al.*, 2010). The chemotherapy includes several drugs with possible neurotoxic effects. Among the administered drugs, vincristine, methotrexate, cytarabine, L-asparaginase, and glucocorticoids: prednisone and dexamethasone were found to be associated with central neurotoxicity (Fulbright *et al.*, 2011, Vagace *et al.*, 2012). Anticancer drug combinations, CRT, CNS leukemia, inherited genetic variants all can influence blood-brain barrier function and the development of neurotoxicity (Vagace *et al.*, 2012). Genetic association studies in finding prognostic factors for acute CNS toxicity in ALL have controversial results. (de Carvalho *et al.*, 2018, Vagace *et al.*, 2012). Pharmacogenetic risk factors for neurotoxicity in ALL are variations mainly in methotrexate- or vincristine pathways, such as *MTHFR* (methylenetetrahydrofolate reductase) (Mahadeo *et al.*, 2010), *CYP3A5* (cytochrome P450 family 3 subfamily A member 5) (Egbelakin *et al.*, 2011). CNS neurotoxicity associated with genes like *ABCB1* (ATP Binding Cassette Subfamily B Member 1), *ABCG2* (ATP Binding Cassette Subfamily G Member 2), *SLCO1A2* (Solute Carrier Organic Anion Transporter Family Member 1A2), *SLCO1B1* (Solute Carrier Organic Anion Transporter Family Member 1B1), *APOE* (Apolipoprotein E) (de Carvalho *et al.*, 2018, Froklage *et al.*, 2011). Variants in *VDR* (Vitamin D Receptor), *GSTP1* (glutathione S-transferase 1) genes were in association with central or peripheral neurotoxicity (Erdilyi *et al.*, 2008, Kishi *et al.*, 2007). Vincristine-pathway genes, *ACTG1* (Actin Gamma 1), *CAPG* (Capping Actin Protein, Gelsolin Like), and *ABCB1* were in association with peripheral neurotoxicity (Ceppi *et al.*, 2014). *ABCB1* rs1045642 with ATE or CNS relapse correlated oppositely (Erdilyi *et al.*, 2008, Stanulla *et al.*, 2005). If a transporter or enzyme eliminates less drug, prevents relapse, but predisposes for toxicity and vice versa. The balance between therapy, CNS relapse, and toxicity is shown on Figure 4.

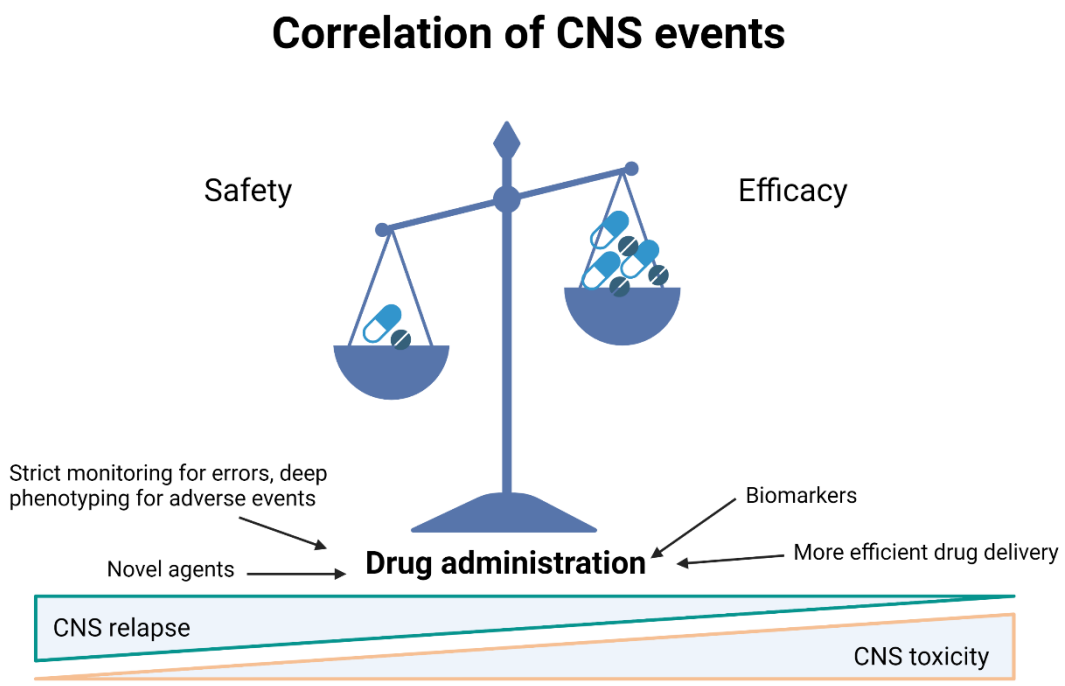

Figure 4. Drug administration in the aspect of CNS events. (Created with BioRender.com) The use of less drug can prevent toxicity and so result in a safer therapy, however, gives a chance for the return of the disease with an ineffective therapy.

1.2.3. Relapse in the central nervous system

Relapse of ALL is the recurrence of the disease and affects 20% of the patients initially diagnosed with ALL. The survival of patients with relapse is much lower than the survival of de novo cases: 5-year event-free survival (EFS) after the first relapse is around 50% (Lawson *et al.*, 2000, Roy *et al.*, 2005). The main sites of relapse presentation are the BM, which is called medullary relapse; or extramedullary sites like the central nervous system or testes. Rare manifestations appear in ovaries, eyes, ears, uterus, kidney, bone, muscle, tonsil, mediastinum, pleura, or paranasal sinus. Relapse can be present at one site or can be combined (more sites at once); early after the initial diagnosis or later. Early BM relapse returns within 36 months after initial diagnosis or within 6 months of completion of the therapy (Medscape, n.d.). Central nervous system involvement occurs in 5% of primary leukemia and 30 % of relapse cases. Blasts leukocyte count at diagnosis, CNS involvement at diagnosis, male gender, T-ALL associated with poor prognosis in relapse (Cancela *et al.*, 2012, N. Kim *et al.*, 2008). Extramedullary, combined, or late

manifestation relapses show better treatment response than the early or medullary ones, these latter ones remain great obstacles. The molecular behavior of the second or umpteenth disease is important for the treatment plan which has to be more intensive and precise than at initial leukemia. Systemic chemotherapy, CNS- directed therapy (irradiation and intrathecal drug administration), targeted therapy, immunotherapy, and hematopoietic stem cell transplantation are the treatment options for relapse (Locatelli *et al.*, 2012).

Because of the special anatomical features, CNS is a sanctuary for leukemic clones to survive the non-specific polychemotherapy and build up a relapse. Risk factors for CNS involvement are known for a long time like higher alkaline phosphatase, lactate dehydrogenase, bilirubin, creatinine or low albumin levels; older age, t(1;19) translocation, t(9;22) translocation, peripheral hyperleukocytosis, T cell immunophenotype, or mixed-lineage leukemia (Kantarjian *et al.*, 1992, Lenk *et al.*, 2020, Shihadeh *et al.*, 2012). Progression-free survival of CNS leukemia is around 40 % thanks to the intensified systematic chemotherapy and the more frequent CNS- directed intrathecal treatment (Krishnan *et al.*, 2010, Masurekar *et al.*, 2014). CNS leukemia is an intensively studied field, its diagnosis and therapy are still challenging. For a better diagnostic efficacy multicolor flow cytometry was tested, promising soluble biomarkers for CNS relapse are VEGFR (Vascular endothelial growth factor receptor), WT1 (Wilms' tumor suppressor gene1), IL7R (Alsadeq *et al.*, 2018, Popov *et al.*, 2019, Ramirez *et al.*, 2003, Tang *et al.*, 2013). Exosomes and miRs are convincing new factors in its pathomechanism (Heidari *et al.*, 2016, Kinjyo *et al.*, 2019). Intrathecal dose intensification by CNS status at diagnosis has improved the prevention of CNS involvement, although, results for using irradiation for the same aim are controversial (Fukano *et al.*, 2014, R. W. Gao *et al.*, 2018, Jastaniah *et al.*, 2019, Laver *et al.*, 2000, Piette *et al.*, 2020, Pui *et al.*, 1998). Targeted, immune-, CAR T-cell therapies are new approaches arising as possible, precise treatments for CNS involvement (Lenk *et al.*, 2020). Inherited genetic risk factors for CNS relapse or overall relapse were *GSTT1* (Glutathione S-Transferase Theta 1) presenting genotype against null genotype, variants in *GSTP1* (glutathione S-transferase 1), and *ABCB1* genes (Anderer *et al.*, 2000, Stanulla *et al.*, 2005, Talaat *et al.*, 2018).

1.2.4. T-cell acute lymphoblastic leukemia

T-ALL represents 20% of all acute lymphoblastic leukemia cases. In its primary disease, the EFS is around 85% nowadays, which below the 90% B-ALL survival. Unfortunately, 15–20% of children with T-ALL develop relapse. Relapse after T-ALL has a significantly dismal prognosis, EFS is below 25% (Barredo *et al.*, 2006, Krishnan *et al.*, 2010, Locatelli *et al.*, 2012). T-ALL relapse appears most often in the BM (50% of the cases), in CNS (21%), or in testes (5%) (Lato *et al.*, 2021). The pathogenesis of relapse disease is diverse. The relapse-promoting clone can bear similar biological features with the therapy-resistant primary leukemia or may be present at diagnosis as a sub-clone carrying different somatic mutation-spectrum. This sub-clone may originate from a pre-leukemic clone and thus form a second disease, or a malignant cell line presents at diagnosis, which acquires new mutations and is selected out during chemotherapy. This phenomenon is called clonal evolution (Ding *et al.*, 2012, Mullighan *et al.*, 2008, Vicente & Cools, 2015) (Figure 5).

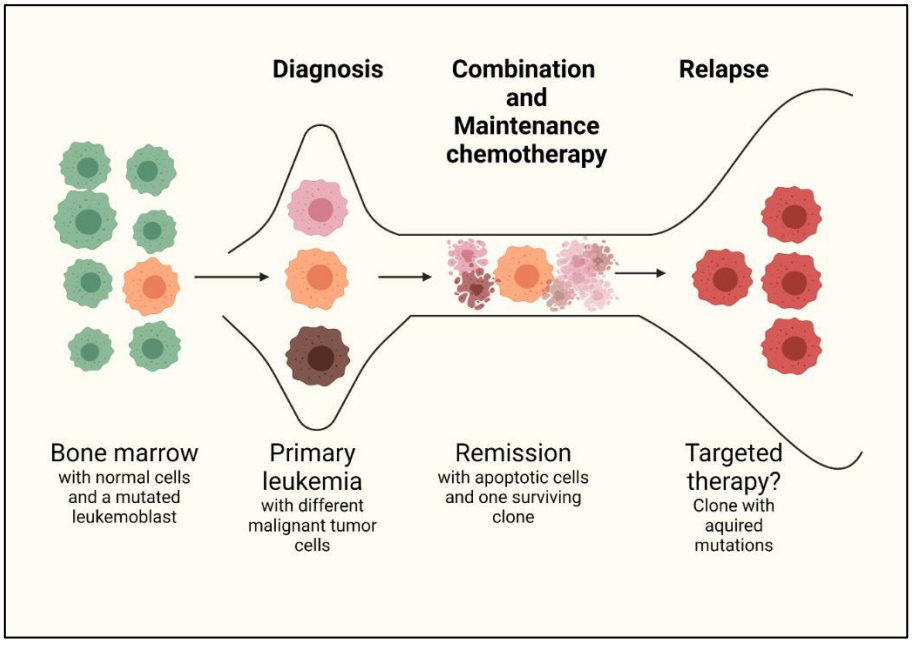

Figure 5. Clonal evolution of a leukemic clone building up primary leukemia and relapse (Ding *et al.*, 2012)

Gene expression studies, or next-generation sequencing technologies are key in the understanding of the molecular behavior of T-ALL (Veenstra *et al.*, 2019). Abnormal T-cell function with uncontrolled proliferation and survival results in leukemia. Key mechanisms of T-ALL initiation are described in the following. Inactivation of *CDKN2A* (p16) (Cyclin-dependent kinase inhibitor 2A) and *CDKN2B* (p15) (Cyclin-dependent kinase inhibitor 2B) and translocations in the T-cell receptor gene were the first administered alterations in this disease (Omura-Minamisawa *et al.*, 2000). Overexpression of transcription factors such as *TAL1* (TAL BHLH transcription factor 1), *TLX1* (T cell leukemia homeobox 1), activating mutations of *NOTCH1*, *IL7R*, and the JAK/STAT pathway are major oncogenic events in T-cell development and dysregulation of many target genes (Y. Liu *et al.*, 2017). FBXW7 (F-box and WD repeat domain containing 7) normal function initiates proteasomal degradation of NOTCH1. *KDM6A* (Lysine demethylase 6A) demethylation causing inactivating lesions enhance *NOTCH1*-driven leukemia (Girardi *et al.*, 2017). Epigenetic lesions are also common in T-ALL, like inactivation of *PHF6* (PHD finger protein 6), affecting transcriptional regulation, ribosome biogenesis, DNA damage response, and cell cycle regulation. Loss-of-function mutations of enzymes regulating methylation (*EZH2* (Enhancer of zeste 2 polycomb repressive complex 2 subunit), *SUZ12* (SUZ12 polycomb repressive complex 2 subunit), and *EED* (Embryonic ectoderm development)) also support tumor formation (D'Angelo *et al.*, 2015). Mutations in ribosomal protein gene *RPL5* (Ribosomal protein L5) or transcriptional regulator *CNOT3* (CCR4-NOT transcription complex subunit 3) are also involved (De Keersmaecker *et al.*, 2013). MiRNAs (miR-19b, miR-20a, miR-26a, miR-92, miR-223) inhibit the transcription of *IKZF1*, *PTEN* (Phosphatase and tensin homolog), *BIM* (*BCL2L11*, BCL2 Like 11), *PHF6*, *NF1* (Neurofibromin 1), and *FBXW7*. These events can parallel happen and work together in T-ALL formation (Girardi *et al.*, 2017). Treatment of T-ALL was improved with appropriate intensive care and targeted therapies, however, chemotherapy-resistant clones with new alterations end up in relapse of T-ALL. In vitro cell line investigations, mouse models, and studies on patient samples help to understand the resistance mechanisms of T-ALL. Downregulation of *ABCC1*, overexpression of *ABCA2 (*ATP binding cassette subfamily A member 2), *ABCA3*, (ATP binding cassette subfamily A member 3) *ABCB1* were determined in association with resistance against dexamethasone, vincristine, and cytarabine (Aberuyi *et al.*, 2020). The

traditional approach, such as combined chemotherapy cycles, even if followed by allogeneic HSCT (Hematopoietic stem-cell transplantation), is still not sufficient resolution for the cure of all patients with relapsed T-ALL. The goal is the administration of targeted therapies to achieve remission before HSCT which is still the most effective therapy of T-ALL. Relapse of T-ALL is an aggressive, therapy-resistant disease, so the investigations of relapse-specific somatic alterations made it possible to target specific molecules for better treatment (Pierro *et al.*, 2017). The targeted therapies already in use in the clinical practice of T-ALL are summarized in Table 1.

Table 1. Drugs in clinical practice for pediatric T-ALL based on OpenTargets database (Ochoa *et al.*, 2021)

Drug	Type	Mechanism Of Action	Action Type	Target
ALEMTUZUMAB	Antibody	CAMPATH-1 antigen inhibitor	Inhibitor	CD52
ALLOPURINOL	Small molecule	Xanthine dehydrogenase inhibitor	Inhibitor	XDH
BORTEZOMIB	Small molecule	26S proteasome inhibitor	Inhibitor	PSMB5
CYTARABINE	Small molecule	DNA polymerase (alpha/delta/epsilon) inhibitor	Inhibitor	POLE, POLE3, POLE2, POLA2, PRIM2, POLA1, POLD1, POLD2, PRIM1, POLD4, POLD3, POLA2.
DAUNORUBICIN	Small molecule	DNA topoisomerase II alpha inhibitor	Inhibitor	TOP2A
DEXAMETHASONE	Small molecule	Glucocorticoid receptor agonist	Agonist	NR3C1
DOXORUBICIN	Small molecule	DNA topoisomerase II alpha inhibitor	Inhibitor	TOP2A
ETOPOSIDE	Small molecule	DNA topoisomerase II alpha inhibitor	Inhibitor	TOP2A
FILGRASTIM	Protein	Granulocyte colony stimulating factor receptor agonist	Agonist	CSF3R
HYDROCORTISONE	Small molecule	Glucocorticoid receptor agonist	Agonist	NR3C1
IMATINIB	Small molecule	Platelet-derived growth factor receptor beta inhibitor, Tyrosine-protein kinase ABL inhibitor, Stem cell growth factor receptor inhibitor	Inhibitor	PDGFRB, ABL1, KIT
MERCAPTOPURINE	Small molecule	Amidophosphoribosyltransferase inhibitor	Inhibitor	PPAT
METHOTREXATE	Small molecule	Dihydrofolate reductase inhibitor	Inhibitor	DHFR
PREDNISONE	Small molecule	Glucocorticoid receptor agonist	Agonist	NR3C1
SIROLIMUS	Small molecule	FK506-binding protein 1A inhibitor	Inhibitor	FKBP1A
TACROLIMUS	Small molecule	FK506-binding protein 1A inhibitor	Inhibitor	FKBP1A
VINCRISTINE	Small molecule	Tubulin inhibitor	Inhibitor	TUBB and Class 1, 2, 3, 4, 6, 8.

2 Objectives

The aims of my research were the followings:

1. Evaluation of single nucleotide polymorphisms as possible pharmacogenetic markers in anthracycline-related cardiotoxicity. To test the association between genotypes of germline SNPs and left ventricular parameters indicating cardiotoxicity induced by anthracyclines appeared during or after the treatment of pediatric primary ALL (T-, and B- cell subtypes) and OSC.

2. Evaluation of single nucleotide polymorphisms as possible pharmacogenetic markers in neurotoxicity. To analyze if germline SNPs of metabolizing enzymes and transporters of the blood-brain barrier have a significant contribution to the appearance of neurotoxic events caused by chemotherapy in pediatric patients with primary ALL (T-, and B-cell subtypes).

3. Evaluation of single nucleotide polymorphisms as possible pharmacogenetic markers in association with the first relapse presented in the central nervous system. To investigate the association between germline SNPs of metabolizing enzymes and transporters of the blood-brain barrier and the presence of relapse in the central nervous system in pediatric patients with ALL (T-, and B-cell subtypes).

4. Searching for somatic mutations as possible prognostic markers in the relapse of T-cell acute lymphoblastic leukemia. To evaluate their role in the survival of relapse-specific SNVs, small insertions, and deletions in T- cell ALL tumor-specific samples collected at initial and relapse time points.

3 Methods

3.1. Patients

Hungarian pediatric patients diagnosed with acute lympoblastic leukemia or osteosarcoma were enrolled in the cardiotoxicity, neurotoxicity and CNS relapse studies (n=661, n=626 and n=533, respectively). All of them were treated in one of the 6 pediatric oncology centers of Hungary. Their DNA was stored in the Hungarian Pediatric Oncohematology Biobank at the Semmelweis University, Department of Genetics, Cell- and Immunobiology due to the successful collaboration with the Hungarian Pediatric Oncology Network. Retrospective clinical data collection was conducted from medical records of enrolled patients or from the National Pediatric Cancer Registry of Hungary (Garami *et al.*, 2014). Chemotherapeutic drugs and doses used in treatment protocols were summarized in previous articles of our research group (Erdilyi *et al.*, 2008, Hegyi *et al.*, 2017). Further patients data were collected and analyzed in international collaborations. The researches were conducted according to the principles expressed in the Declaration of Helsinki. Written informed consent was requested from all patients or the parents or guardians of the minors involved in the studies. The ethical committees of the participating countries approved the study.

Cardiotoxicity study group

Patients aged 0-18 years and diagnosed between 1989 and 2015 with ALL or OSC in Hungary were enrolled into the cardiotoxicity study group. Characteristics of the patients are detailed in Table 2. Exclusion criteria were preceding cardiac problems, congenital cardiac abnormalities, or inherited genetic diseases which could influence the cardiac and genetic markers of the individuals (n=19). Dosages of anthracyclines were administered in the intravenous part of the chemotherapy. Low and intermediate-risk patients received a cumulative dose between 180-240 mg/m^2 while patients with high risk or relapse got a cumulative dose between 240-380 mg /m^2. Cumulative doxorubicin doses of OSC patients were 360 mg/m^2 for standard-risk or 180 mg/m^2 for high-risk. Twenty-nine percent of the cohort was treated with 12 Gy cranial radiotherapy (regarding the corresponding BFM protocol). Patients were checked for left ventricular function after anthracycline therapy regularly. Echocardiography (ECHO) was used to control these parameters. Left ventricular end-diastolic-diameter (LVEDD) and left ventricular end-

systolic diameter (LVESD) data were collected retrospectively for the study. Left ventricular ejection fraction (EF) and left ventricular fractional shortening (FS) were calculated as EF = (LVEDD3-LVESD3)/LVEDD3 and FS = (LVEDD-LVESD)/ LVEDD and included in the analysis.

Table 2. Characteristics of the cardiotoxicity study populations (Ságі *et al.*, 2018)

	Patients with ALL	**Patients with OSC**	**Total**
Number of patients	622	39	661
Gender *n* (%)			
Male	372 (60)	27 (69)	399 (60)
Female	250 (40)	12 (31)	262 (40)
Age at diagnosis (%)			
<1 yr *n*	7 (1)	0	7 (1)
1-10 yr *n*	505 (81)	9 (23)	515 (78)
>10 yr *n*	109 (18)	30 (77)	138 (21)
Mean ±SD *yr*	6.39 (±4.3)	13.1 (±3.5)	6.6 (±4.3)
Median (range) *yr*	5.2 (0-18)	13.2 (5-18)	5.3 (0-18)
Risk group *n* (%)			
SR	165 (27)	3 (7)	168 (25)
IR	355 (57)	24 (62)	379 (58)
HR	100 (16)	12 (31)	112 (17)
Chemotherapy protocol *n* (%)			
Protocols before 2000[1]	325 (52)	–	325 (52)
Protocols after 2000[2]	297 (48)	–	297 (48)
OSC protocols[3]	–	39	39
Anthracycline dose[4] (range, mg/m^2)	60-840	180-360	60-840
Anthracycline dose *n* (%)			
≤ 240 mg/m^2	457 (74)	6 (15)	463 (70)
> 240 mg/m^2	163 (26)	33 (85)	196 (30)
Patients with pathological FS[5] *n*	18	2	20

Data are reported as numbers with percentages unless mentioned otherwise. Abbreviations: ALL, acute lymphoid leukemia; OSC, osteosarcoma; SD, standard deviation; SR, standard-risk; IR, intermediate-risk; HR, high-risk; FS, left ventricular fractional shortening. [1]Patients with ALL treated with ALL BFM 88, ALL BFM 90, ALL BFM 95, Interfant 98, NHL BFM 90 or NHL BFM 95 protocol. [2]Patients with ALL treated with ALL IC BFM 2002, ALL IC BFM 2009, or Interfant 2006 protocol. [3]COSS (German-Austrian-Swiss osteosarcoma study group) -86 and COSS-96 protocols. [4]Cumulative anthracycline dose in doxorubicin or daunorubicin equivalent doses during the treatment according to protocol. [5]FS below 28%.

Clinical checkpoints for echocardiography were before the start of the therapy, during and after the intravenous treatment on several occasions, repeatedly. In this study, patients were grouped by follow-up categories and their FS and EF values were analyzed within the group, respectively. These follow-up categories were: 1) at the diagnosis (used as a control) (n=387); 2) in acute phase: during the intensive chemotherapy phase (n=280); 3) during oral maintenance chemotherapy (n=49); 4) at the end of the treatment (n=315), which is after the oral maintenance chemotherapy period completed 2 or 3 years after the diagnosis; 5) from the end of the treatment until 5 years after the diagnosis (n=264); 6) 5– 10 years after the diagnosis (n=301); 7) 10–15 years after the diagnosis (n=152); 8) more than 15 years after the diagnosis (n=32). During the long follow-up, not all ECHO data were accessible. Always the latest ECHO data were included in the analysis of each follow-up category. For the case-control study, the worst recorded ECHO data were used by every patient. Echocardiograms with FS ≤ 28% were determined as pathological FS cases (n=20), controls had FS > 28% throughout the follow-up time (n=641). The change in the FS value was studied as a dichotomous variable: the difference of FS at diagnosis from the end of the treatment was registered. The change in FS value from the diagnosis to the last follow-up was also analyzed. Decreasing FS cases (n=105, n=170) were studied in comparison with increasing FS cases (n=94, n=152), respectively. In this analysis, power was ≥75% for all of the results (Ságiet *al.*, 2018). The study design of this analysis is shown in Table 3.

Table 3. Cardiotoxicity study design

	Total cohort (OSC, ALL)		**ALL subpopulation**	
Type of analysis	Case-control	Multi-adjusted logistic regression	Case-control	Multi-adjusted logistic regression
	Follow-up (8 categories)	Multi-adjusted general linear model	Follow-up (8 categories)	Multi-adjusted general linear model
	FS alteration (2 categories)	Multi-adjusted logistic regression	FS alteration (2 categories)	Multi-adjusted logistic regression

Neurotoxicity study group

Our research group (Medical Genomics Research Group, Semmelweis University, Department of Genetics, Cell- and Immunobiology) studied chemotherapy-related ATE

among patients diagnosed with ALL in the period of 1995-2005. In my research, I extended this cohort with additional ATE cases among patients diagnosed with ALL between 2005 and 2015. The newly collected population and the original one were analyzed together to test associations between SNPs of blood-brain barrier enzymes and transporters (n=580) (*Discovery cohort*). Our previously published results with *ABCB1* rs1045642 and with its combination with *ABCG2* rs2231142 and ATE and further candidate SNPs were investigated on the extended population. To validate the association of five chosen SNPs with ATE, a European international collaboration was organized. Matched case-control cohort was set up with Austrian, Czech, and Northern (Nordic Society of Pediatric Hematology and Oncology (NOPHO) Group) patients collected from national study groups. The same enrolment criteria were used for all of the study groups when selecting patients for the *Joined validation cohort*. The *Joined validation cohort* included 107 ATE cases and 211 controls. ATE and its subphenotypes were also studied on the total *Combined cohort* of ATE which contained a matched Hungarian population and the *Joined validation cohort*. Studying complications of the central nervous system in pediatric ALL, cohorts included children with 0-18 years of age at the time of diagnosis (1-18 years for neurotoxicity study; 0-18 years for relapse study) from Hungary, Austria, Czech Republic and countries of the NOPHO Group (Denmark, Norway, Sweden, Finland, Iceland, Lithuania, Estonia) ('NOPHO Participating institutions', n.d.). Clinical data collection source were data collection sheets of the PdL 'Retrospective Investigation of Children with ALL/LBL with Central Neurotoxicity Related to Therapy' study (with complements to Christina Halsey and the Ponte di Legno Toxicity Working Group). All four study groups were involved in this study. See Table 4. for patients' characteristics. Complications of the central nervous system in ALL, such as acute encephalopathy (AE) and CNS relapse were in the focus of this study. AE included adverse CNS symptoms at least grade 3 regarding the Common Terminology Criteria for Adverse Events (CTCAE) v.4.0 ('NIH. Common terminology criteria for adverse events v4.0', 2009). Acute symptoms, appearing from the start of the treatment until 3 weeks after the i.v. chemotherapy, were analyzed [49]. Exclusion criteria for neurotoxicity study were any previous chemotherapy, any major deviations from ALL therapy, previous CNS diseases, uncertain or mild neurologic symptoms.

Table 4. Basic characteristics of the studied populations with acute encephalopathy (AE) and acute toxic encephalopathy (ATE) (Ságі *et al.*, 2021)

Study cohort	**Hungarian**			**Austrian**	**Czech**	**NOPHO**	**Combined**
				Joined validation cohort			
	Non-matched		Matched	Matched			Matched
Phenotype	**AE**	**ATE**	**ATE**	**ATE**			**ATE**
Number of patients *n*	626	580	108	62	119	137	426
ATE Cases/controls *n* (%)	82/544 (13/87)	36/544 (6/94)	36/72 (33/67)	21/41 (34/66)	39/80 (49/51)	47/90 (34/66)	143/283 (34/66)
Seizure only *n*	21	20	20	8	10	6	44
SLS[1] *n*	6	6	6	1	6	7	20
Toxic PRES[2] *n*	3	3	3	12	18	33	66
Gender *n* (%) Male	339 (54)	317 (55)	52 (48)	26 (42)	53 (45)	74 (54)	205 (48)
Period of ALL diagnosis *y*	1990-2015	1990-2015	1992-2015	2010-2018	2003-2017	2008-2015	1992-2018
Age at diagnosis *n* (%) >10 *yr n*	104 (17)	88 (15)	35 (32)	30 (48)	42 (35)	29 (21)	136 (32)
Median (range) *yr*	5.0 (1-18)	5.0 (1-18)	7.7 (1-18)	9.9(1.8-17.7)	7.1(1.3-18)	7.0 (1-16)	7.6 (1-18)
Risk group (HR[3]) *n* (%)	75 (12)	69 (12)	17 (16)	29 (47)	15 (13)	41 (30)	102 (24)
Chemotherapy protocols	ALL BFM 90, ALL BFM 95, ALLIC BFM 2002, ALLIC BFM 2009, IDH-ALL-91[4]	ALL BFM[4] 90, ALL BFM 95, ALLIC BFM 2002, ALLIC BFM 2009, IDH[5]-ALL-91	ALL BFM 90, ALL BFM 95, ALLIC BFM 2002, ALLIC BFM 2009	AIEOP-BFM[6] ALL 2009	ALL-BFM 2000, ALL IC-BFM 2002, AIEOP-BFM ALL 2009	NOPHO[7] ALL2008	All

Abbreviations: AE: acute encephalopathy; ATE: acute toxic encephalopathy; [1]SLS: Stroke-like syndrome; [2]PRES: Posterior reversible encephalopathy syndrome; [3]HR: high risk, as per patient's treatment protocol; [4] ALL BFM: Acute lymphoid leukemia Berlin-Frankfurt-Münster; [5]IDH: Italian-Dutch–Hungarian; [6]AIEOP BFM: Associazione Italiana Ematologia Oncologia Pediatrica (Italian) Berlin-Frankfurt Münster; [7]NOPHO: Nordic Society for Pediatric Hematology and Oncology.

AE cases were CNS-linked cerebrovascular events, like infections or leukemia not in remission, systemic metabolic disturbances (e.g. hepatic encephalopathy, hypoglycemia, or diabetic ketoacidosis), or the result of insufficient CNS circulation (e.g. hypertensive encephalopathy, increased intracranial pressure, hypotension, or hypoxia). See more details in Supplementary Patient Criteria of Ságі et al, 2021 (Ságі *et al.*, 2021).

Subphenotype groups of AE were also investigated. Events with no known secondary etiology were called acute toxic encephalopathy (ATE) and were considered as direct adverse effects of chemotherapeutic drugs affecting the blood-brain barrier. CNS symptoms with known causes were excluded from this cohort. AE and ATE cases were categorized into overlapping Delphi consensus phenotypes as a stroke-like syndrome (SLS), seizures with no other symptoms, depressed level of consciousness, posterior

reversible encephalopathy syndrome (PRES) defined by the PdL Toxicity Working Group in 2016. These phenotypes could appear with or without known origin which was the basis of their classification in the analysis (Schmiegelow *et al.*, 2016) (Figure 6). Two controls were matched to one case in the total cohort. Controls were pediatric patients with ALL who experienced none of these events, had no comorbidities, medical history, or co-medication that may have influenced the occurrence of CNS complications or drug pharmacokinetics.

AE events occurred during the whole intravenous therapy which was divided into 4 treatment phases in the analyses: 'Induction-like' (including steroid, L-asparaginase, vincristine, +/- anthracycline); 'High-dose methotrexate' (including methotrexate 2-5 g/m2 iv with mercaptopurine); 'BFM-consolidation' (including cyclophosphamide, cytarabine, 6-mercaptopurine or thioguanine) and 'Other' (e.g. BFM high risk (HR) 1, HR2, HR3 cycles) (Ságі *et al.*, 2021).

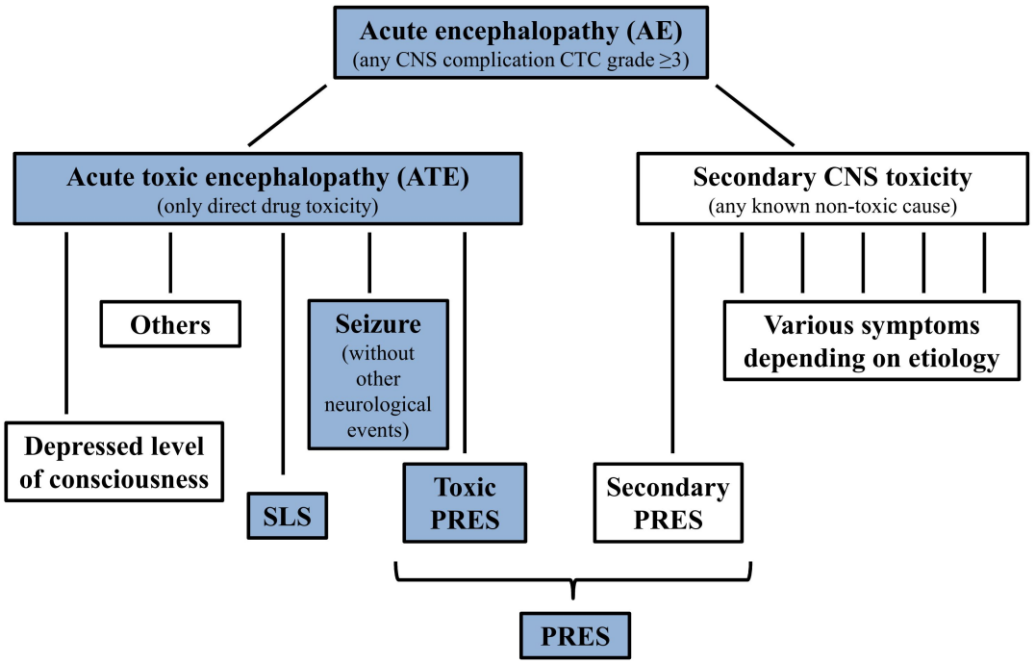

Figure 6. Acute CNS adverse events during ALL intravenous therapy (Ságі *et al.*, 2021). Analyzed phenotype categories are marked with blue. Symptoms of AE and ATE subgroups and the subgroups themselves may overlap (Schmiegelow *et al.*, 2016). Uncommon types of ATE e.g. steroid psychosis, are not present on this map. (The PRES analysis is not introduced in this dissertation.)

CNS relapse study group

CNS relapse was studied on a non-matched population of Hungarian patients as a discovery population. After this, we established a further international cohort for studying

CNS relapse on a second *Combined cohort* with patients of the four study groups. Into the CNS relapse study patients were enrolled with 1st relapse affecting only the CNS as isolated cases, and combined CNS relapse cases with additional medullary or extramedullary ALL. In the analysis two non-relapsed ALL cases and one isolated BM first relapse case were controls matched to one CNS relapse case. See for more details Supplementary Patient Criteria of Sági et al, 2021 (Sági *et al.*, 2021). The features of the studied cohort shown in Table 5. Into the *Combined cohort* of CNS relapse 86 CNS relapse cases (isolated or combined), 105 isolated bone-marrow (BM) relapse cases, and 129 relapse-free controls were enrolled and three controls per case were matched. The number of patients in the validation cohort was designed based on the results found on the discovery population with the statistical power of 0.8 (Sági *et al.*, 2021). For the steps and design of the CNS study in ALL see Figure 7.

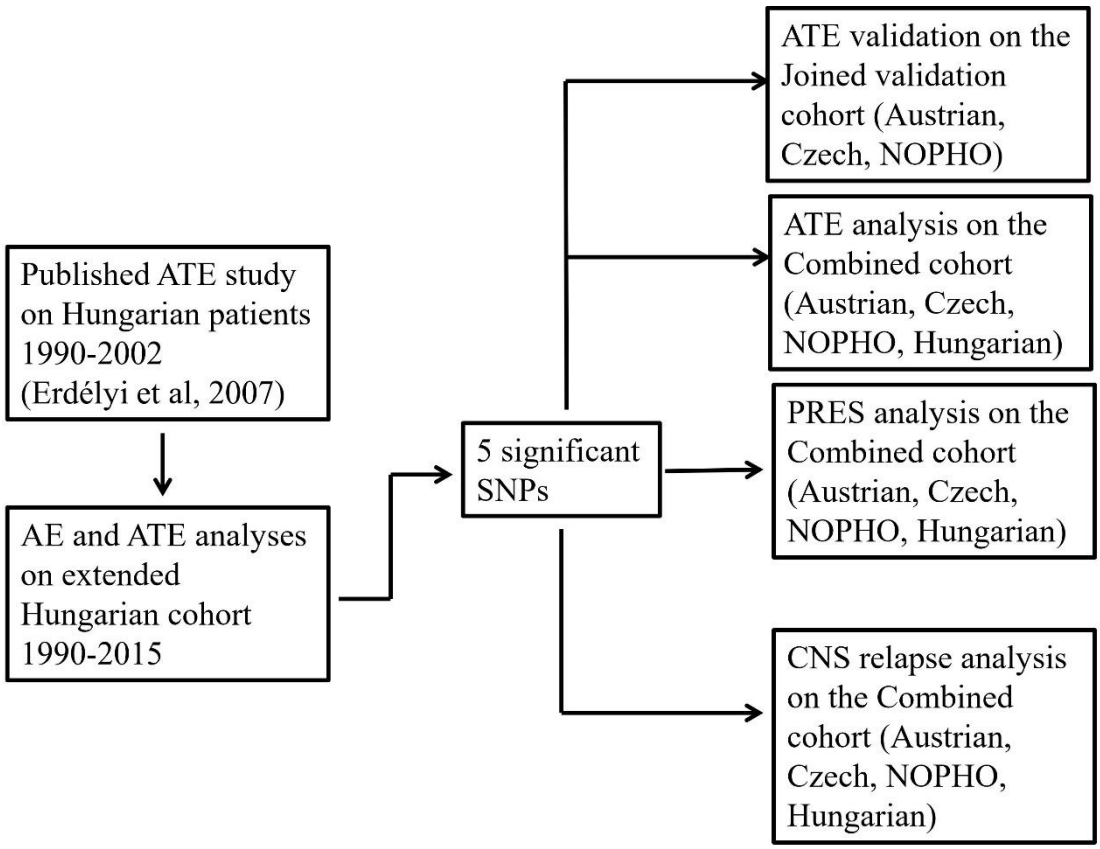

Figure 7. Steps of the CNS study in ALL (Sági *et al.*, 2021)

Table 5. Basic characteristics of the studied population of central nervous system first relapse (CNS relapse)

Study cohorts	**All Hungarian relapses (isolated and combined CNS, isolated BM), Non-relapsed controls**	**Austrian**	**Czech**	**Hungarian**	**NOPHO**	**Combined**
	Non-matched cohorts	Matched cohorts				
Number of patients *n*	533	8	152	60	100	320
Isolated CNS[1] relapse	4	1	10	4	19	35
Combined CNS relapse	12	2	26	12	12	51
Isolated BM[2] relapse	55	5	54	16	30	105
Relapse- free controls	462	0	62	28	39	129
Gender *n* (%)	301	4	102	42	62	210
Male	(56)	(50)	(67)	(70)	(62)	(66)
Period of ALL diagnosis *y*	1990-2014	2010-2014	1996-2017	1992-2013	2008-2015	1992-2017
Age at diagnosis *n* (%)						
>10 yr *n*	94 (18)	3 (40)	29 (19)	22 (37)	24 (24)	78 (24)
Median (range) *yr*	4.62 (0.16-17.95)	9.5 (5.8-15.9)	4.2 (0.1-17.8)	7.4 (1-17)	5.0 (1-16)	4.9 (0.1-17.8)
Risk group (HR[3]) *n*	71	5	38	17	27	87
(%)	(13)	(63)	(25)	(28)	(27)	(27)
Chemotherapy protocols	ALL BFM 88, ALL BFM 90, ALL BFM 95, ALLIC BFM 2002, ALLIC BFM 2009, Interfant 98, Interfant 2006	AIEOP-BFM[4] ALL 2009	ALL BFM[5] 95, INTERFANT, EsPHALL[6], ALL-BFM 2000, ALL IC-BFM[7] 2002, AIEOP-BFM ALL 2009	ALL BFM 90, ALL BFM 95, ALLIC BFM 2002, ALLIC BFM 2009	NOPHO[8] ALL2008	All

Abbreviations: [1]CNS: central nervous system; [2]BM: bone marrow; [3]HR: high risk; [4]AIEOP BFM: Associazione Italiana Ematologia Oncologia Pediatrica (Italian) Berlin-Frankfurt Münster; [5]ALL BFM: Acute lymphoid leukemia Berlin-Frankfurt Münster; [6]EsPHALL: Philadelphia-chromosome-positive acute lymphoblastic leukemia; [7] ALL IC-BFM: Randomized Trial of the I-BFM-SG; [8]NOPHO: Nordic Society for Pediatric Hematology and Oncology.

T-ALL study group

Samples at the time of initial diagnosis were collected from 147 patients with T-cell acute lymphoblastic leukemia. These samples were called initial samples. Further non-matched 66 samples of patients with T-ALL were collected at the diagnosis of relapse. Patients with primary leukemia or relapse were treated according to ALL-BFM 2000, AIEOP-BFM ALL 2009, or ALL-REZ BFM 2002 protocols. Features of the analyzed cohorts are shown in Table 6. The study was approved by the institutional review boards of the Charité Universitätsmedizin Berlin and the Medical Faculty Heidelberg. Informed consent was obtained in accordance with the Declaration of Helsinki (Richter-Pechańska *et al.*, 2017).

Table 6. Characteristics of the cohorts diagnosed with primary or relapsed T-ALL (Richter-Pechańska *et al.*, 2017)

	Primary disease		**Relapse disease**	
Characteristics	#	(%)	#	(%)
Total	147		66	
Gender				
male	113	77%	51	77%
female	34	23%	15	23%
Immunophenotype				
NA	3	2%	0	
pre-T-ALL	41	28%	14	21%
cortical T	85	58%	25	38%
mature T-ALL	18	12%	27	41%
Age at diagnosis				
1-9 years	74	50%		
≥10 years	73	50%		
Risk Group				
SR	16	11%		
MR	70	48%		
HR	61	41%		
Time to relapse				
very early			33	50%
early			17	26%
late			16	24%
Site of relapse				
BM isolated			47	71%
BM combined			19	29%
Response				
early			23	35%
normal/late			20	30%
nonresponse			18	27%
NA			25	38%
Outcome				
CCR			17	26%
death in CR			8	12%
secondary malignancy			1	2%
second relapse			18	27%
non-response			18	27%
induction death			4	6%

Risk Group - risk group assignment in AIEOP-BFM ALL 2000 defined as follows: MRD standard-risk (SR); MRD high-risk (HR); MRD intermediate-risk (IR).

3.2. Laboratory approaches

DNA isolation

DNA was isolated from mononuclear cells extracted from BM or peripheral blood samples collected at initial diagnosis or remission. DNA isolation was performed using Qiagen isolation kits according to the manufacturer's instructions (QIAmp DNA Blood Midi or Maxi Kit, Qiagen, Hilden, Germany) or the Gentra Puregene Cell Kit (Qiagen, Hilden, Germany). For one isolated extramedullary relapse case, DNA was extracted from a lymph node.

SNP selection

Seventy SNPs in 26 genes were selected for genotyping. Based on previous publications, we collected variants in transporters or metabolizing enzymes related to chemotherapeutic drugs and further variants of potential new candidate genes. The functionality of SNPs was prioritized during selection: non-synonymous SNPs, SNPs in the promoter and the 3'-UTR (3′-untranslated region) region, synonymous SNPs, and intronic SNPs. Minor allele frequency data were established with the HapMap database No. 27 and the CEU population (CEPH: Utah residents with ancestry from northern and western Europe) (NCBI, n.d.). For more details of selected SNPs see Table 7.

Genotyping of the Hungarian samples

DNA samples of Hungarian patients were genotyped using TaqMan® OpenArray™ Genotyping System (Thermo Fisher Scientific, Waltham, MA, USA) by Zsolt Rónai and his workgroup at the Semmelweis University, Department of Medical Chemistry, Molecular Biology and Pathobiochemistry. The results were visualized using the TaqMan Genotyper SoftwareTM (Applied Biosystems). A portion of the Hungarian samples was genotyped using KASPar (KBioscience Competitive Allele-Specific Polymerase chain reaction)-on-Demand prevalidated assays (LGC Genomics, Berlin, Germany) on 7900HT Fast Real-Time PCR System (Thermo Fisher Scientific Waltham, MA, USA) following the manufacturer's instructions by our group at the Semmelweis University, Department of Genetics, Cell- and Immunobiology (Banlaki *et al.*, 2015). Genotypes of the samples collected internationally were provided by collaborators.

Table 7. Information about the studied SNPs

					MAF		
Gene	**SNP**	**Function**	**Major allele**	**Minor allele**	**Cardio-toxicity cohort**	**ATE Combined cohort**	**CNS-relapse Combined cohort**
ABCB1	rs3842	3'UTR	A	G	0.128	0.13	0.13
ABCB1	rs1045642	Ile1145Ile	T	C	0.450	0.45	0.45
ABCB1	rs10280101	intron	A	C	0.090	0.09	0.09
ABCB1	rs2032582	Ala893Ser/Ala893Thr	G	T/A	0.454/0.028	0.46/0.02	0.46/0.02
ABCB1	rs11760837	intron	T	C	0.087	0.09	0.09
ABCB1	rs1128503	Gly412Gly	C	T	0.468	0.46	0.46
ABCB1	rs1202179	intron	A	G	0.272	0.28	0.28
ABCB1	rs9282564	Asn21Asp	A	G	0.111	0.1	0.09
ABCC1	rs35587	Asn354Asn	T	C	0.329	0.31	0.32
ABCC1	rs35605	Leu562Leu	C	T	0.187	0.17	0.17
ABCC1	rs8187858	Tyr568Tyr	C	T	0.087	0.08	0.08
ABCC1	rs4148350	intron	G	T	0.079	0.08	0.08
ABCC1	rs35626	intron	G	T	0.335	0.33	0.34
ABCC1	rs212087	intron	C	T	0.420	0.42	0.43
ABCC1	rs3743527	3'UTR	C	T	0.247	0.25	0.24
ABCC1	rs212090	3'UTR	T	A	0.431	0.43	0.44
ABCC1	rs212091	3'UTR	A	G	0.135	0.13	0.12
ABCC1	rs212093	downstream	A	G	0.417	0.42	0.41
ABCC2	rs717620	5'UTR	G	A	0.163	0.17	0.16
ABCC2	rs2273697	Val417Ile	G	A	0.216	0.21	0.21
ABCC2	rs3740066	Ile1324Ile	G	A	0.328	0.33	0.33
ABCG2	rs2231142	Gln141Lys	C	A	0.097	0.1	0.11
ABCG2	rs1564481	intron	C	T	0.388	0.38	0.38
AKR1A1	rs9147	5'UTR	T	C	0.454	na	na
AKR1A1	rs2934859	intron	G	A	0.451	na	na
AKR1C3	rs3763676	5'UTR	T	C	0.282	na	na
AKR1C3	rs7741	Pro30Pro	G	A	0.292	na	na
AKR1C3	rs12387	Lys104Lys	A	G	0.170	na	na
AKR1C3	rs10508293	intron	T	C	0.195	na	na
AKR1C3	rs3209896	3'UTR	A	G	0.448	na	na
BCL2	rs4987853	3'UTR	A	G	0.160	0.15	0.16
BCL2	rs1564483	3'UTR	G	A	0.283	0.27	0.28
BCL2	rs4987845	3'UTR	G	A	0.081	0.07	0.08
BCL2	rs4941195	intron	C	A	0.440	0.44	0.44
BCL2	rs1893806	intron	T	G	0.433	0.44	0.45
CEP72	rs4956954	intron	A	G	0.339	0.34	0.34
CEP72	rs924607	intron	C	T	0.434	0.42	0.43
CEP72	rs12522955	Pro412Thr	C	A	0.195	0.21	0.2
CEP72	rs868649	Thr509Ala	T	C	0.230	0.22	0.22

Continued on next page.

					MAF		
Gene	**SNP**	**Function**	**Major allele**	**Minor allele**	**Cardio-toxicity cohort**	**ATE Combined cohort**	**CNS-relapse Combined cohort**
CEP72; TPPP	rs2458815	3'UTR	A	G	0.216	0.21	0.21
CHTF8; HAS3	rs2232228	Ala93Ala	A	G	0.442	0.44	0.44
CYP3A4	rs3735451	intron	A	G	0.139	0.14	0.14
CYP3A4	rs2246709	intron	A	G	0.274	0.27	0.28
CYP3A5	rs4646450	intron	C	T	0.155	0.15	0.15
CYP3A5	rs776746	intron	G	A	0.087	0.08	0.08
CYP3A5	rs28365067	intron	C	T	0.076	0.09	0.08
GSTP1	rs6591256	5'UTR	A	G	0.360	0.35	0.35
GSTP1	rs4147581	intron	us			na	na
GSTP1	rs1695	Ile105Val	A	G	0.297	0.31	0.32
GSTP1	rs749174	intron	C	T	0.304	0.3	0.3
*GSTT1**		deletion			na	na	0.09
*GSTM1**		deletion			na	na	0.23
HTATSF1P2; NQO2	rs1143684	Phe47Leu	T	C	0.234	0.23	0.23
HTATSF1P2; NQO2	rs6955	3'UTR	G	C	0.162	0.17	0.16
KDSR; BCL2	rs1801018	Thr7Thr	us			na	na
LINC01011; NQO2	rs2071002	5'UTR	us			na	na
MTHFR	rs1801131	Glu470Ala	A	C	0.318	0.32	0.31
NFAT5; NQO1	rs1043470	3'UTR	C	T	0.115	0.11	0.11
NQO1	rs1800566	Pro187Ser	C	T	0.180	0.18	0.19
NQO1	rs1469908	5'UTR	A	G	0.393	0.4	0.4
NQO2	rs4149352	intron	C	T	0.226	0.23	0.23
RARG; ITGB7	rs2229774	Ser427Leu	C	T	0.084	0.08	0.08
SLC22A17	rs12882406	downstream	G	C	0.370	0.39	0.37
SLC22A17	rs4982753	downstream	C	T	0.258	0.27	0.26
SLC22A6	rs10897310	downstream	T	C	0.376	0.36	0.36
SLC22A6	rs6591722	intron	T	A	0.315	0.33	0.32
SLC22A7; CRIP3	rs2270860	Ser425Ser	G	A	0.334	0.32	0.33
SLC22A7; CRIP3	rs4149178	intron	A	G	0.165	0.15	0.16
SLC22A8	rs2276299	Thr241Thr	A	T	0.179	0.17	0.17
SLC28A3	rs7853758	Leu461Leu	G	A	0.139	0.14	0.14
SLC28A3	rs885004	intron	G	A	0.138	0.14	0.13
SLC28A3	rs7867504	Thr89Thr	T	C	0.297	0.3	0.31

Minor/major alleles according to HapMap-CEU, Abbreviations: MAF: global minor allele frequency; na: not analyzed; SNP: single nucleotide polymorphism; HWE: Hardy-Weinberg Equilibrium; us: unsuccessful, *: Only genotyped in the Hungarian non-matched relapse cohort.

Selection of genes for target sequencing in samples of patients with T-ALL

We wanted to evaluate repetitive T-ALL relapse-specific somatic alterations in association with prognosis and clinical features of the studied patient group. During target design, we collected candidate genes from the literature. We searched genes in association with T-cell leukemia, which have been found in initial and relapsed disease or only in relapse. We excluded the too large genes and the results of cell line studies. We included genes from our previous results (Bandapalli *et al.*, 2014, Bernasconi *et al.*, 2005, Cario *et al.*, 2010, Cools, 2010, Graubert *et al.*, 2012, Kalender Atak *et al.*, 2013, De Keersmaecker *et al.*, 2013, Kunz *et al.*, 2015, Malcovati *et al.*, 2011, Mullighan *et al.*, 2007, Neumann *et al.*, 2013, Shochat *et al.*, 2014, Tzoneva *et al.*, 2013, Vlierberghe *et al.*, 2011, Van Vlierberghe & Ferrando, 2012, J. Zhang *et al.*, 2012, Zuurbier *et al.*, 2012), as well. With these screening, we could collect 324 possible candidates for further analysis (Table 8). I have searched for genes in the literature, looked hotspots of the selected genes in COSMIC ('COSMIC v94, Catalogue Of Somatic Mutations In Cancer', n.d.), ranked them with Endeavour ('ENDEAVOUR: A web resource for gene prioritization in multiple species', n.d.), and finally looked for the function and role of these genes in GeneCards ('GeneCards v5.3.0: The Human Gene Database', n.d.).

Mutation identification in T-ALL samples

After the gene selection step, we designed a Haloplex Panel to target regions of interest. Haloplex Target Enrichment Kit (Agilent, Santa Clara, CA, USA) was used for library preparation and covered the selected genes, 3.04 Mbp target sequence. After DNA quantification (Qubit dsDNA BR Assay kit (Life Technologies, Darmstadt, Germany), 112.5 ng genomic DNA was used. Libraries were pooled in batches and were sequenced as 100 bp paired reads on one lane using an Illumina HiSeq 2000 instrument (Illumina, San Diego, CA, USA). VarScan26 was used to detect both SNVs and small insertions and deletions (Koboldt *et al.*, 2009). To control the NGS (next-generation sequencing) results, we also used conventional Sanger-sequencing of the *NOTCH1* and *PTEN* genes in 144 patients.

Table 8. Haploplex- Targeted genes															
ABCA5	ATXN1	CASC5	CNOT3	DNMT3A	EZH2	HOXA9	KDM6A	MAPK10	NAALAD 2	NT5C2	PIK3CD	PTPRO	SECISBP 2L	SUZ12	TYRO3
ABL1	BAG6	CASP8A P2	CNOT6	DOCK2	FAT1	HPS5	KDR	MAPK13	NAT10	OBSCN	PIK3R1	PTPRT	SEPSEC S	SYT16	U2AF1
ACP2	BANP	CBL	COL24A 1	DOK4	FAT3	HRAS	KIF21B	MECP2	NAV2	OCA2	PIM1	PUM1	SETD2	TADA2B	UNC5D
ADCY6	BAP1	CCDC14 6	COL4A2	DOT1L	FBXW7	HS3ST6	KIF22	MED12	NCAM1	ODZ2	PLEKHO 2	RASGRP 2	SF3B1	TAPBPL	USH2A
AFF1	BAT3	CCDC88 A	CPNE2	DUSP7	FDFT1	HTRA3	KIT	MEF2C	NCK2	OPN1LW	PMM2	RB1	SF3B2	TBCK	USP7
AKAP2	BCL11B	CCND2	CREBBP	ECT2L	FGFR2	IGF1R	KPNA1	MET	NCOA2	OPN5	PMS2	RBL2	SH2B3	TCEA1	USP9X
AKAP3	BCLAF1	CCND3	CRLF2	EED	FGFR3	IGFL1	KPNA3	MKRN1	NCOR2	OR2T12	PPP1R13 L	RELN	SHROO M3	TET1	VCPIP1
AKAP8	BCOR	CDH23	CRLF2	EFEMP1	FICD	IKZF1	KRAS	MLH1	NDUFB8	OR2T2	PRDM2	RET	SLC45A1	TET2	WHSC1
AKT1	BCORL1	CDK8	CTCF	EHMT1	FLNA	IKZF2	L3MBTL 1	MLL	NEK9	OXER1	PRKCG	ROCK1	SMARC A4	TET3	WT1
ALK	BICD2	CDKN1B	CTNNB1	EIF2AK3	FLT3	IL7R	LCK	MLL2	NF1	PAX5	PRKDC	RPH3A	SMARC C1	TIFAB	WWC1
ALOX15 B	BIN1	CDKN2A	CTNND1	EIF4EBP 3	FOXJ2	INSR	LEF1	MLLT3	NF2	PAX5	PSMB11	RPL10	SMG8	TLR9	XIRP2
ANK3	BLM	CDKN2B	DAPK3	ELOVL4	FOXN4	IPO9	LSM4	MPL	NFKB2	PCDHAC 2	PTEN	RPL11	SOX1	TMEM17 4	XPO1
ANKHD1	BNC1	CEBPA	DCST1	ENOSF1	GATA2	IRF1	MAGEC 3	MSH2	NINL	PDE1B	PTK2B	RPL22	SPRED2	TMEM99	YES1
ANKRD1 1	BPIFC	CELF2	DGCR2	EP300	GATA3	IRS2	MAGI2	MSH3	NKTR	PDGFRA	PTPN11	RPL5	SPRY3	TP53	YY1AP1
ANXA11	BRAF	CHD8	DHX8	EPHA10	GPSM1	IRX4	MAML3	MSH6	NOTCH1	PDGFRA	PTPN13	RSPO1	SPRY4	TP73	ZBP1
APC	BRF1	CIR1	DIP2C	EPHB2	H3F3A	ITGAM	MAP2K3	MST1R	NOTCH2	PDGFRB	PTPN14	RUNX1	SRSF2	TRIP13	ZFP36L1
ARMCX 4	BTG1	CLASRP	DMD	ERBB2	HADHA	JAK1	MAP2K4	MTHFS	NOTCH3	PEG3	PTPN2	RYK	STAT3	TRIP6	ZMYM2
ASPM	C10ORF 71	CLCF1	DNAH11	ESRRG	HCLS1	JAK2	MAP3K1 1	MTOR	NRAS	PHF6	PTPN9	SAFB2	STAT5A	TRNT1	ZNHIT6
ATP6V1 B1	C17ORF 80	CLTC	DNM2	ETV6	HERC1	JAK3	MAP3K2	MYB	NRG1	PHIP	PTPRC	SART3	STAT5B	TSHR	
ATRN	C19ORF 70	CNOT1	DNMT3A	EYS	HFM1	JPH2	MAP3K7	MYC	NT5C1B	PIK3CA	PTPRE	SCN9A	STAT5B	TYK2	

3.3. Statistical analysis

In the Hungarian population, significant violation from Hardy-Weinberg equilibrium was tested with online software https://ihg.gsf.de/cgi-bin/hw/hwa1.pl. A multi-adjusted general linear model was used to study associations of SNPs and left ventricular parameters as continuous variables (such as FS or EF). Alterations of FS were studied as categorical variables using univariate or multivariate logistic regression. Potential confounders were age at the time of diagnosis (years), gender (male-female), chemotherapy protocols (before 2000, after 2000 and OSC protocols; also reflects radiotherapy), risk groups (standard, intermediate, high-risk), and cumulative dose of anthracycline ($\leq$ or $>$ 240 mg/m^2). Conditional logistic regression models were applied to study the association of genotype frequencies and the presence of neurotoxic events (case-control study design). Cox proportional hazard regression models for nested case-control data were used to analyze overall survival (OS) and event-free survival (EFS) in every CNS event cohort. Confidence intervals (CI) of odds ratio (OR) or hazard ratio (HR) were calculated at the 95% level. Confounders of the CNS study are shown in Table 9. We studied the genotypes separately (11 vs. 12; 11 vs. 22), using dominant (11 vs. 12/22) or recessive (11/12 vs. 22) models. Common homozygotes were described with 11 and the rare homozygotes with 22, which was supposed to be the dominant allele. For the correction of multiple comparisons the Benjamini-Hochberg false discovery rate (FDR) method with a type I error rate of 10 % or 13% was used, respectively. Alpha levels of $p \leq 8.90E\text{-}03$ or $p \leq 1.13E\text{-}02$ were considered significant after FDR correction (Benjamini & Hochberg, 1995, Storey J.D., 2002). EF and FS are given always with the standard error (SE) of the estimate of the mean. Analyses and figures were prepared in IBM SPSS Statistics 23.0 or 25.0 (IBM Corporation, Armonk, NY, USA) and RStudio Version 1.0.136 or version Version 3.6.3 (RStudio, Boston, MA, USA) programs. The clogit function of the survival package of R was used for the conditional logistic regression analyses (Therneau, 2020). MultipleNCC package was applied for the Cox proportional hazards regression analyses for nested case-control data (Stoer & Samuelsen, 2020). Power analysis was performed with PS: Power and Sample Size Calculation 3.1.2 or was estimated at a significance level of 0.05 using SPSS Statistics 23.0 or 25.0 programs. In

the T-ALL population, statistical analyses were performed using GraphPad Prism 6.0, or R (Package: Survival).

Table 9. Confounders and stratification listed for the analyses in the CNS study

			Analysis	
Population	**Phenotype**		**Case-control**	**Survival**
Hungarian cohort	**AE/ATE**	**Confounders**	Gender Age at diagnosis ALL phenotype Risk group Iv. MTX doses	Gender Age at diagnosis ALL phenotype Risk group
Validation joined cohort	**ATE**	**Confounders**	Not included any	Not analyzed
Combined cohort	**ATE**	**Confounders**	Not included any*	Age at diagnosis ALL phenotype Risk group Study group
		Stratification	Cycles of treatment	Cycles of treatment
Combined cohort	**CNS relapse**	**Confounders**	Not included any*	Gender Age at diagnosis ALL phenotype Risk group Study group

*In the analyses of the validation joined cohort and of all patients, covariates were included in the model only if they were significant. More details of the confounders are: age at diagnosis (years); ALL phenotype (B or T-cell leukemia); CNS status at diagnosis (CNS 1, 2, or 3); gender (male-female); iv. MTX doses (2 or 5 g/m2); Cycles of treatment (Induction-like (steroid, L-asparaginase, vincristine, +/- anthracycline), High-dose methotrexate (high dose methotrexate (2-5 g/m2)), BFM-consolidation (cyclophosphamide, cytarabine, 6-mercaptopurine) or Other); risk group (high risk or non-high risk); study group (Hungary, Austria, Czech Republic or NOPHO group). Abbreviations: AE: acute encephalopathy; ATE: acute toxic encephalopathy; CNS: central nervous system.

4. Results

4.1. Genotype data for studying cardiotoxicity and CNS complications

In the Hungarian population, 70 SNPs were genotyped. MAF of the SNPs are presented in Table 7. Genotyping was not successful for 3 SNPs and genotype distribution was not in Hardy-Weinberg equilibrium in case of one SNP (*AKR1A1* (Aldo-Keto Reductase Family 1 Member A1) rs2934859. These 4 SNPs were excluded from further analyses.

4.1.1 Single nucleotide polymorphisms associated with cardiotoxicity

Sixty-six SNPs were studied in association with left ventricular function parameters (ejection fraction (EF), fractional shortening (FS)) of pediatric patients with ALL or OSC. Case-control and follow-up types of studies were used to explore the genetic background of EF and FS. In my dissertation, I focus on the follow-up analysis of the total, of the ALL subpopulation and the alteration of FS. *ABCC2* rs3740066 was associated with worse FS in the acute phase of the therapy in the total cohort and at 5–10 years after the diagnosis, and also in this 5-10 years phase in the ALL subgroup (p=0.00738, p=0.000711, p=0.0045 respectively). *NQO1* (NAD(P)H Quinone Dehydrogenase 1) rs1043470 associated significantly with worse FS on the total population in these phases and in the acute phase in the ALL subpopulation (p=0.00428, p=0.00582, p=0.0026, respectively). *SLC22A6* (Solute Carrier Family 22 Member 6) gene rs6591722 was associated with lower mean FS at 5–10 years after the diagnosis on the total population and patients with ALL (p=0.00171, p=0.0059, respectively) (Figure 8 and Table 10). Ejection fraction results showed the same tendency as FS. Alterations of FS were studied in association with SNPs observing two intervals: between the time of diagnosis and the end of the treatment or the last follow-up (Table 11). In the ALL subpopulation, SNPs were found in significant association with cardiac parameters, however, these associations were not significant on the total cohort. *CYP3A4* (Cytochrome P450 Family 3 Subfamily A Member 4) rs3735451, *CYP3A5* rs776746 were in association with a shift in FS values analyzing the period between the time of diagnosis and the end of the treatment (p=0.0057, p=0.0038, respectively) (Table 11). Studying the time of diagnosis and the last registered FS value, *NQO1* rs1043470 was in association with FS alteration

(p=0.0089). The change in FS was below or over 3% in 67–71% in all groups (Ságі *et al.*, 2018).

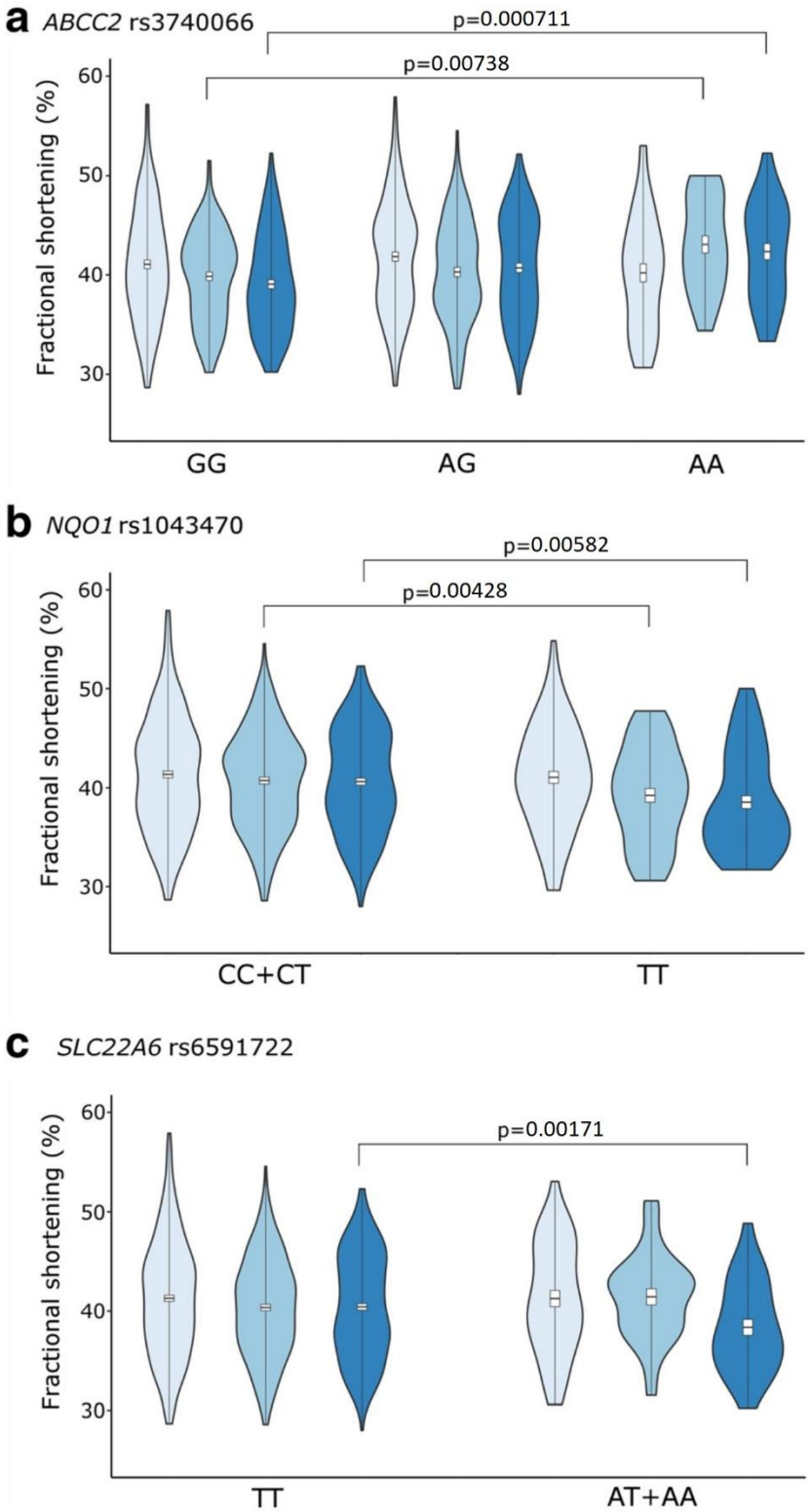

Figure 8. Violin plot of fractional shortening in the total population (Ságі *et al.*, 2018). FS (%) by genotypes is shown in different follow-up categories. Light blue is the time of diagnosis, medium blue is the time of the anthracycline administration (acute phase), dark blue is the follow-up 5–10 years after therapy. FS is indicated in box plots, box is mean ± S.D., whiskers are means ±3 S.D. Violin plot describes the distribution of FS data, records out of mean± 3SD are not shown. A: *ABCC2* rs3740066; B: *NQO1* rs1043470; C: *SLC22A6* rs6591722

Table 10. Significant results of the follow-up analysis in the acute lymphoid leukemia population (Ságiet *al.*, 2018)

Gene	SNP	Genotype group 1 / group 2	Mean FS % ± SE genotype group 1 (N)	Mean FS % ± SE genotype group 2 (N)	P value	Follow-up category
ABCB1	rs9282564	AA / AG+GG	41.5 ± 0.7 (100)	37.9 ± 1.1 (29)	0.0025	10-15 years after Dx
ABCC1	rs35626	GG / GT+TT	41.0 ± 0.6 (92)	39.0 ± 0.6 (127)	0.0079	2- 5 years after Dx
ABCC2	rs3740066	GG / GA / AA	39.5 ± 0.5 (112)	40.8 ± 0.5 (112) / 42.9 ± 0.9 (33)	0.0045	5-10 years after Dx
NQO1	rs1043470	CC / CT + TT	40.9 ± 0.5 (198)	38.1 ± 0.9 (53)	0.0026	acute phase
SLC22A6	rs6591722	TT+TA / AA	40.7 ± 0.4 (227)	37.8 ± 1.0 (28)	0.0059	5-10 years after Dx
SLC28A3	rs7853758	GG / GA+AA	41.3 ± 0.7 (96)	38.4 ± 1.1 (36)	0.0048	10-15 years after Dx
SLC28A3	rs885004	GG / GA+AA	41.3 ± 0.7 (95)	38.0 ± 1.1 (33)	0.0025	10-15 years after Dx

Results are from a multivariate general linear model performed on the ALL cohort adjusted for potential confounders. Abbreviations: Dx, diagnosis; FS, fractional shortening; N, number; SE, standard error

Table 11. Significant results of the analysis of FS alteration in the acute lymphoid leukemia population (Sági *et al.*, 2018)

Gene	SNP	Genotype group 1 / group 2	Patients with decreased FS in genotype groups N (%)	Patients with increased FS in genotype groups N (%)	P value	OR (CI 95%)
Alteration of FS: diagnosis vs. end of therapy						
CYP3A4	rs3735451	AA / AG + GG	74 (82) / 16 (18)	52 (63) / 31 (37)	0.0057	0.36 (0.18-0.74)
CYP3A5	rs776746	GG / GA + AA	81 (91) / 8 (9)	60 (73) / 22 (27)	0.0038	0.26 (0.11-0.65)
Alteration of FS: diagnosis vs. last echocardiography						
Gene	**SNP**	**Genotype group 1 / group 2**	**Patients with decreased FS in genotype groups N (%)**	**Patients with increased FS in genotype groups N (%)**	**P value**	**OR (CI 95%)**
NQO1	rs1043470	CC / CT + TT	111 (85) / 41 (15)	112 (73) / 20 (27)	0.0089	0.44 (0.24-0.81)

Results are from logistic regression performed on the ALL cohort adjusted for potential confounders. Abbreviations: CI, confidence interval; FS, fractional shortening; N, number; OR, odds ratio

4.1.2 Single nucleotide polymorphisms associated with central nervous system toxicity

We evaluated the association between 60 SNPs in 20 genes in the transport and metabolism of chemotherapeutic drugs and CNS toxicity. The presence of AE and ATE in Hungarian pediatric patients with ALL (discovery population) were studied in the case-control analysis. We validated the results (5 SNPs) related to CNS toxicity on an international validation cohort. CNS relapse and the same SNP-set were investigated on the cohort of all nations (Austria, Czech Republic, Hungary, and NOPHO Group). The study design is shown in Figure 7.

In the *Hungarian discovery cohort*, AE and ATE significantly associated with *ABCB1* rs1045642 (p=0.011, p=0.047). The association both with AE and ATE remained significant when analyzing *ABCB1* rs1045642 in combination with *ABCG2* rs2231142 (p=0.010, p=0.003). ATE associated with further *ABCB1* SNPs: rs1128503 and rs2032582 (p=0.043, p=0.026, respectively). *GSTP1* rs1695 was also in association with both AE and ATE (p=0.0005, p=0.004).

We investigated the above results with the presence of ATE in an international validation study. In the *Joined cohort* (Austria, Czech Republic, NOPHO Group) we found only *GSTP1* rs1695 in an opposite association with ATE compared to results of the *Hungarian cohort* (p=0.029).

Studying the *Combined cohort* of every patient, ATE was not in association with any of the studied SNPs (Table 12). However, the extended number of patients allowed the analysis of subpopulations of ATE in this cohort. Seizure was associated with *ABCB1* rs1045642, rs1128503 and rs2032582 polymorphisms, respectively (p=0.011, p=0.034, p=0.019) in a subpopulation of 44 patients with seizure and 89 matched controls. Analyzing only seizure cases in the therapy phase, in Induction-like chemotherapy cycles (n=28/57) associations remained unambiguous for the same SNPs (p=0.010, p=0.027, p=0.007) (Ságiet *al.*, 2021). We investigated also the survival and effect of the 5 SNPs on the Hungarian and *Combined* AE and ATE cohorts. Worse OS was associated with AE in Hungarian cohort (n=76/626) (p=0.005, HR=2.51, CI 95% (1.32-4.76)). Two neurotoxicity-induced deaths were registered in our database of 82 AE cases (9.5% of all exits).

Table 12. Results of the case-control analysis of AE and ATE (Ságiet *al.*, 2021)

			Hungarian discovery cohort		Joined validation cohort*	Combined cohort *
			AE	**ATE**	**ATE**	**ATE**
Cases/Controls			**n=86/544**	**n=36/590**	**n=107/211**	**n=143/283**
Gene	**SNP**	**Comparisons**	**OR (CI 95%)**			
ABCB1	**rs1045642**	**CC+CT vs. TT**	**0.46 (0.25-0.84)**	***0.45 (0.21-0.99)***	1.13 (0.68-1.89)	0.88 (0.57-1.35)
		CT vs. TT	**0.39 (0.19-0.77)**	0.43 (0.18-1.0)	1.28 (0.75-2.18)	0.93 (0.59-1.47)
	rs1128503	**TT vs. CC+CT**	1.87 (1.0-3.50)	***2.25 (1.03-4.92)***	1.21 (0.69-2.15)	1.51 (0.93-2.47)
	rs2032582	**TT vs. GG**	1.63 (0.76-3.49)	***3.55 (1.17-10.78)***	1.11 (0.56-2.19)	1.60 (0.89-2.88)
ABCB1 rs1045642 TT and ABCG2 rs2231142 CA+AA vs. others			**3.87 (1.38-10.84)**	**5.75 (1.81-18.25)**	1 (0.33-3.0)	1.07 (0.44-2.61)
GSTP1	**rs1695**	**GG+AG vs. AA**	**0.33 (0.17-0.64)**	***0.35 (0.15-0.82)***	1.60 (0.97-2.64)	1.15 (0.75-1.76)
		AG vs. AA	**0.22 (0.09-0.51)**	**0.16 (0.05-0.57)**	***1.76 (1.06-2.94)***	1.19 (0.76-1.85)

*The number of controls in matched populations was twice as much as cases; Hungarian discovery cohort: the unmatched set of Hungarian cases and controls; Joined cohort: Austrian-Czech-NOPHO matched case-control ATE validation population; Combined cohort of ATE: the full patient set of the matched Hungarian ATE population (including cases only with CNS toxicity and matched controls) and the Joined cohort of ATE validation cohort. Results with $p \leq 0.05$ are shown with bold italics characters, significant results with $p \leq 1.13E-02$ are shown with bold characters.

Survival of AE or ATE Hungarian populations were influenced by *CYP3A5* rs4646450 and *CYP3A4* rs3735451. *CYP3A5* rs4646450 associated with worse OS and EFS in AE and ATE cohorts (p=0.0001, HR=2.80, CI 95% (1.70-4.60); p=0.0003, HR=2.27, CI 95% (1.46-3.53); p=0.001, HR=2.43, CI 95% (1.43-4.13); p=0.004, HR=2.00, CI 95% (1.25-3.18), respectively). *CYP3A4* rs3735451 associated also with worse OS and EFS in AE and ATE cohorts (p=0.007, HR=5.15, CI 95% (1.56-17.00), p=0.001, HR=5.54, CI 95% (1.97-15.56), p=0.004, HR=5.91, CI 95% (1.77-19.72), p=0.001, HR=6.20, CI 95% (2.19-17.52), respectively). For more details see Table 13.

OS in *Combined matched cohort* (n=29/81) of ATE patients showed significant association with *GSTP1* rs1695 also when evaluating only ATE cases in Induction- like phase (n=18/49) (p=0.005, HR=0.23, CI 95% (0.08-0.64); p=0.005; HR=0.22, CI 95%

(0.08-0.63)). OS associated with rs1695 in the seizure subgroup (n=14/37), as well (p=0.007; HR=0.15, CI 95% (0.04-0.59). These associations remained significant after FDR correction (Sági *et al.*, 2021).

Table 13. Survival analysis on the Hungarian CNS adverse event case-control cohort (Sági *et al.*, 2021)

			Hungarian discovery cohort			
			AE		**ATE**	
			OS	**EFS**	**OS**	**EFS**
Event/Total population (n)			**78/630**	**103/630**	**76/626**	**101/626**
Gene	**SNP**	**Comparisons**	**HR (CI 95%)**			
CYP3A5	**rs4646450**	**TT+CT vs. CC**	**2.80 (1.70-4.60)**	**2.27 (1.46-3.53)**	**2.43 (1.43-4.13)**	**2.00 (1.25-3.18)**
		TT vs. CC+CT	**4.16 (1.65-10.53)**	2.97 (1.19-7.39)	3.74 (1.33-10.54)	2.63 (0.95-7.28)
		CT vs. CC	**2.55 (1.51-4.30)**	**2.13 (1.34-3.38)**	**2.22 (1.27-3.88)**	1.89 (1.16-3.07)
		TT vs. CC	**5.76 (2.21-15.00)**	**3.75 (1.48-9.52)**	**4.84 (1.68-13.97)**	3.16 (1.13-8.88)
CYP3A4	**rs3735451**	**GG+AG vs. AA**	1.69 (1.02-2.81)	1.70 (1.09-2.66)	1.89 (1.10-3.24)	**1.87 (1.17-2.98)**
		GG vs. AA+AG	4.61 (1.41-15.05)	**4.95 (1.78-13.76)**	**5.13 (1.56-16.87)**	**5.39 (1.93-15.0)**
		AG vs. AA	1.53 (0.90-2.61)	1.53 (0.96-2.45)	1.69 (0.96-2.99)	1.67 (1.03-2.73)
		GG vs. AA	**5.15 (1.56-17.00)**	**5.54 (1.97-15.56)**	**5.91 (1.77-19.72)**	**6.20 (2.19-17.5)**

Hungarian discovery cohort: the unmatched set of Hungarian cases and controls; Results with $p \leq 0.01$ are shown with bold italics characters, significant results with $p \leq 1.13E-02$ are shown with bold characters.

4.1.3 Single nucleotide polymorphisms associated with risk of CNS relapse

CNS relapse was analyzed on the non-matched population of Hungarian patients as a discovery population. We analyzed the impact of SNPs on CNS relapse comparing patients with isolated or combined CNS relapse to non-relapsed controls. The results of this analysis are shown in Table 14. Next to this OS and EFS were calculated in this population (Table 15).

Table 14. Results of Hungarian CNS relapse cohort case- control analysis

Cases / Controls	SNP	OR CI 95%	p-value
Patients with CNS-relapse / Non-relapsed controls	***CEP72*** rs12522955	0.07 (0.01-0.52)	0.009
	CYP3A4 rs3735451	4.01 (1.40-11.52)	0.01
	SLC22A7 rs4149178	13.12 (2.21-78.06)	0.005
All relapses (isolated and combined CNS, BM) / Non-relapsed controls	***ABCC1*** rs3743527	0.41 (0.21-0.79)	0.008
	BCL2 rs4987853	4.28 (1.48-12.41)	0.007
	CYP3A5 rs4646450	2.34 (1.32-4.17)	0.004
	SLC22A7 rs4149178	8.07 (2.23-29.19)	0.002

Abbreviations: CNS: central nervous system, BM: bone marrow.

Table 15. Results of Hungarian CNS relapse cohort survival analysis

	SNP	HR CI 95%	p-value
Overall survival	***CYP3A5*** rs776746	6.76 (2.40-19.04)	0.0003
Event-free survival	***CYP3A5*** rs776746	6.05 (2.17-16.86)	0.0006
	SLC22A7 rs4149178	3.84 (1.75-8.44)	0.0008

We analyzed the impact of SNPs on CNS relapse comparing patients with isolated or combined CNS relapse to non-relapsed controls on the *Combined cohort* (n=86/129) also. *ABCB1* rs2032582 and rs1128503 showed significant association with CNS relapse when relapse cases were compared to patients having no relapse (p=0.019, p=0.038). After FDR correction these associations were not significant. OS and EFS of the *Combined cohort* with CNS-relapse did not show association with any of the SNPs.

4.2. Inverse association between CNS toxicity and relapse

Inverse association of two SNPs of *ABCB1* (rs1128503 and rs2032582) with chemotherapy-related adverse neurological events and CNS relapse was found on the *Combined cohorts* of ATE and CNS relapse. Patients having risk for toxicity-related seizures had a lower chance for CNS relapse and vice-versa (Table 16 and Figure 9) (Ságiet *al.*, 2021).

Table 16. Summary of the results of toxic seizure and CNS relapse analyses in *Combined cohort* (Sági *et al.,* 2021)

Study cohorts (Cases/Controls)			**Seizure (n=44/89)**	**CNS relapse cases vs. patients without relapse (n=86/129)**
Gene	**SNP**	**Comparisons**	**OR (CI95%)**	
ABCB1	**rs1128503**	**TT+CT vs. CC**	2.10 (0.82-5.39)	***0.48 (0.24-0.96)***
		TT vs. CC+CT	2.49 (0.99-6.26)	0.74 (0.33-1.64)
		CT vs. CC	1.67 (0.61-4.52)	0.48 (0.23-1.01)
		TT vs. CC	***3.50 (1.10-11.12)***	0.46 (0.18-1.16)
	rs2032582 (triallelic)	**AG vs. GG**	nv	0.54 (0.10-2.97)
		TA vs. GG	2.16 (0.16-28.70)	nv
		TT vs. GG	***3.71 (1.23-11.17)***	0.59 (0.25-1.40)
		GT vs. GG	1.37 (0.50-3.75)	***0.41 (0.20-0.87)***

Abbreviations: nv: not valid; CNS: central nervous system; REL: relapse. Results with $p \leq 0.05$ are shown with bold italics characters, significant results with $p \leq 1.13E-02$ are shown with bold characters.

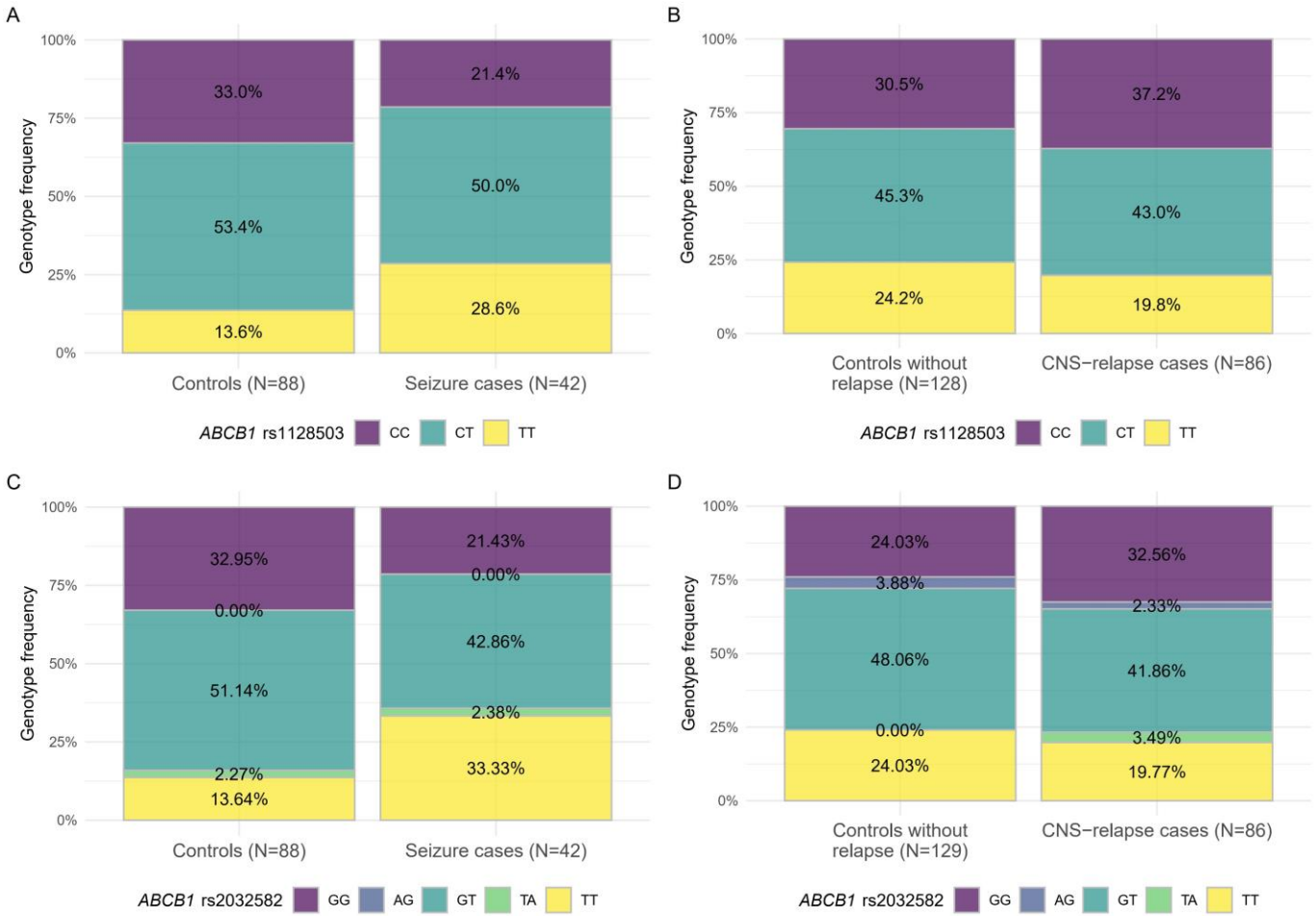

Figure 9. Inverse associations of blood-brain-barrier SNPs with toxic seizure or CNS relapse in case-control analyses (Sági *et al.*, 2021).
The studied populations were the Combined case-control cohorts of ATE and CNS relapse, respectively. A: Genotype frequencies between cases and controls regarding the association of *ABCB1* rs1128503 and seizure, B: Genotype frequencies between cases and controls regarding the association of *ABCB1* rs1128503 and CNS relapse, C: Genotype frequencies between cases and controls regarding the association of *ABCB1* rs2032582 (triallelic) and seizure, D: Genotype frequencies between cases and controls regarding the association of *ABCB1* rs2032582 (triallelic) and CNS relapse. Colors refer to genotypes.

4.3. Genetic events associated with risk of T-ALL relapse and survival

To identify T-ALL relapse-specific genes, the Haloplex target capture technique was used. The designed Haloplex panel contained exons of 324 genes; from these 313 genes were sufficiently covered on the T-ALL samples. The average coverage of the exons was 424 (median = 417), and 97% of them were covered more than 30-fold. We have found SNVs and small insertions and deletions (InDels) in the targeted sequences. Filtering out the false positives were a crucial point of the study. We excluded variants with allele frequency >1% and found in 1000 Genome Project release 2014 or in dbsnp138. Integrative Genomic Viewer (IGV) was used for the quality control of every suspected variant and the found sequencing artifacts were excluded. In the T-ALL population results of NGS of *NOTCH1* and *PTEN* genes was compared to Sanger-sequencing; the sensitivity of the NGS was above 90% for both genes.

The mean number of single nucleotide variations (SNVs) or small insertions and deletions (InDels) per sample was seven. Frequencies were similar in primary and relapsed samples regarding SNVs found in leukemia-associated genes. InDels were present more often in relapse samples which assumed that InDels may be acquired by chemotherapy. Cytosine deamination (exchanges of C>T) was the most frequent mutagenic event among nonsynonymous alterations. The majority of mutations were supposed to be heterozygote. Previously identified leukemia driver genes showed mutation densities similar to the known. SNVs were found most often in *NOTCH1*, *PHF6*, *FBXW7*, and *PTEN* (55-12%); alterations in *DNM2*, *XIRP2*, and *CDH23* were found in more than 10% of the samples (Figure 10).

Our aim was to find new genes in the development of T-ALL relapse and possible prognostic markers. To distinguish between driver and passenger genes, we have determined the mutation density (mutations per Mbp): the number of nonsynonymous SNVs and InDels were divided by the length of the targeted exons per gene. The mean density was 1.8/Mbp. The majority of the genes were below this value. We could confirm driver genes in this setting such as *NOTCH1*, *PTEN*, or *NT5C2*. In *NT5C2* 20 SNVs were detected in 24% of all relapse samples and one SNV in one initial diagnosis sample (p= 0.0001, Fisher's exact test). *NT5C2* had no impact on survival in this analysis, although, mutations of *NT5C2* were more often carried by patients with early relapses (p=0.01, Fisher's exact test) (Richter-Pechańska *et al.*, 2017).

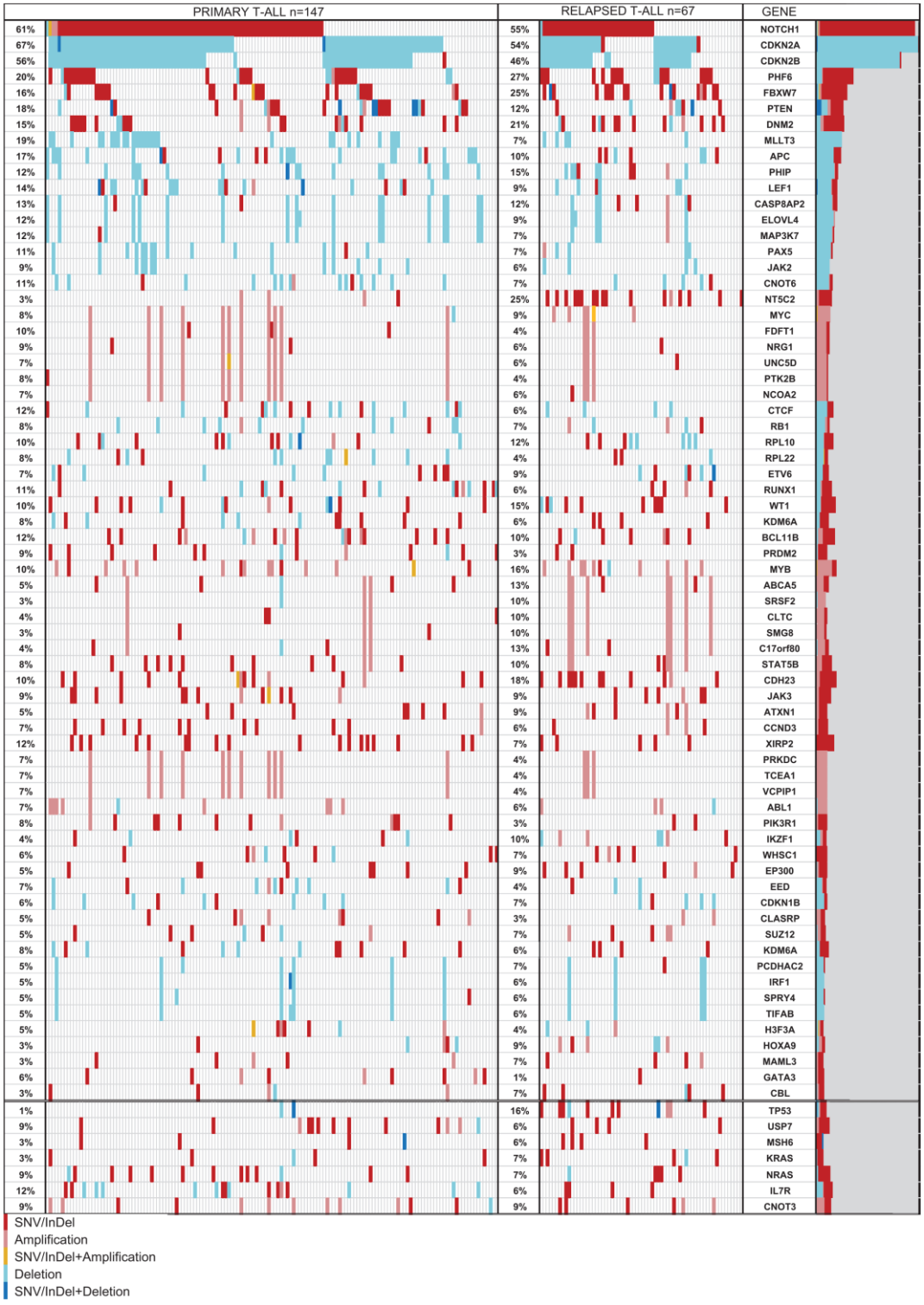

Figure 10. Identified alterations in genes in the initial diagnosis and in relapse patients. Genes with high mutation density of single-nucleotide variants, small insertions/deletions, and copy number alteration are highlighted.

Frequencies refer to all mutations (SNVs/InDels+CNAs), alterations were sorted according to their frequency and position in the genome. Red—SNV/InDel; pink—amplification; orange—SNV/InDel+amplification; pale blue—deletion; dark blue—SNV/InDel+deletion. (Richter-Pechańska *et al.*, 2017)

5. Discussion

In my research works, I aimed to evaluate the role of genetic variants in childhood primary acute lymphoblastic leukemia, its relapses, and osteosarcoma. I studied treatment-related side effects: cardio-, and neurotoxicity and the recurrence of leukemia in the CNS in the presence of heritable SNPs and the relapse of T-ALL in relation with de novo SNVs and InDels, respectively. To achieve my aim, I have searched for possible target genes in the literature and topic-related databases, studied patient's clinical records retrospectively, built the databank of our biobank, genotyped samples, prepared datasheets for statistical evaluation, contributed to statistical analysis of the data, and wrote research articles. Pharmacogenetic markers have the potential to reform the therapy of pediatric oncology. Predicting possible toxicities or underexposure related to the used chemotherapeutic drugs before their clinical manifestation with biomarkers, could increase the personalized approach and prevent treatment failures (Mlakar *et al.*, 2016). Identification of novel variations for a possible targeted therapy could increase the safety and efficacy of the treatment of ALL and OSC.

5.1. Single nucleotide polymorphisms associated with cardiotoxicity

The association of 66 SNPs and anthracycline-induced cardiotoxicity (ACT) during or after the treatment was studied in pediatric patients with acute lymphoblastic leukemia and osteosarcoma. Three patients in our cohort died of cardiac-related events (endocarditis, ventricular insufficiency, and one patient died of cardiomyopathy). SNPs in *ABCC2, NQO1, SLC22A6* genes were associated with decreased FS at the acute phase and the period of 5-10 years after the diagnosis. *CYP3A4, CYP3A5*, and *NQO1* SNPs associated with FS alteration. *ABCC2* rs3740066 was associated with decreased FS in the acute phase of treatment and after 5-10 years of the therapy. *ABCC2* is a frequently investigated gene regarding toxicities, in therapy-response or resistance, and the survival of osteosarcoma or leukemia (Andersen *et al.*, 2015, Claudia M. Hattinger *et al.*, 2016, Marsh & Hoskins, 2011, Qu *et al.*, 2012, Sun *et al.*, 2010, Vulsteke *et al.*, 2015, Zhai *et al.*, 2012). *ABCC2* was associated in adult patients with NHL with acute and chronic ACT. (Wojnowski *et al.*, 2005). Higher risk for ACT was registered with another *ABCC2* SNP, rs8187710 SNP, which is near rs3740066 (Leong *et al.*, 2017). *ABCC2* rs8187710 was over-represented in HSCT patients with anthracycline-related congestive heart

failure (Armenian *et al.*, 2013). *ABCC2* rs3740066 associated with MTX toxicity (Lopez-Lopez *et al.*, 2013). MTX AUC_{0-48} (area under the concentration–time curve) associated significantly with rs3740066 (Hegyi *et al.*, 2017). Overall these findings are in accordance with the results reported by us. *NQO1* rs1043470 was connected with reduced cardiac function rates during the treatment and between the 5-10 years after the therapy in our analysis. *NQO1* prevents oxidative stress and defends against pro-oxidant drugs like anthracyclines (Blanco *et al.*, 2008). This result ties well with previous studies wherein pediatric patients with ALL experienced dismal prognosis with an *NQO1* variant (M Krajinovic *et al.*, 2001). Worst survival associated with a nearby SNP, rs1800566 (NQO1*2) of patients with breast cancer treated with anthracyclines (Siegel *et al.*, 2012). In rats, doxorubicin-induced the down-regulation of *Nqo1* and increased ROS level (Lagoa *et al.*, 2014). Although, our study focused on a different phenotype than these previous reports. *SLC22A6* rs6591722 was associated with lower FS values 5-10 years after the diagnosis. In rats, renal *Slc22a6* was down-regulated after MTX administration (Shibayama *et al.*, 2006). *Slc22a6* was studied with cardiac hypertrophy and cardio-renal fibrosis in cell lines and rats (Savira *et al.*, 2017). This was the first time to detect *SLC22A6* in association with cardiotoxicity in human samples. *CYP3A4* rs3735451 and *CYP3A5* rs776746 were associated with FS alterations in patients with ALL in our cohort. *CYP3A4* rs3735451 was protective against ischemic stroke in adult patients (N. Gao *et al.*, 2020). *CYP3A5* rs776746 was associated with cardiac toxicity in patients with lymphoma (Rossi *et al.*, 2009). In pediatric patients with ALL, rs776746 was associated with lower enzyme activity, with the mRNA expression, daunorubicin plasma concentration, and adverse drug reactions (Huang *et al.*, 2017). We have verified that these SNPs have a role in cardiac function which is in agreement with former results regarding adverse events.

5.2. Single nucleotide polymorphisms associated with central nervous system toxicity

Testing new, somatic mutations at the diagnosis of pediatric ALL is the backbone of the treatment strategy, however, the management of ALL still lacks inherited germline variations, although their role is undoubted in interindividual variability of treatment response and toxicities. The clinical relevance is uncertain how to predict which patients

are at risk for CNS complications. To support the therapeutic plan, we hypothesized that SNPs of the blood-brain barrier are associating factors with the prevalence and survival of CNS first relapse and toxicity. We studied the association of 60 SNPs of chemotherapeutic drug-metabolizing and transporting genes with acute CNS toxicity and CNS first relapse episodes in patients with childhood acute lymphoblastic leukemia.

In our study on the Hungarian cohort, *ABCB1* rs1045642, rs1128503, and rs2032582, the combination of *ABCB1* rs1045642 with *ABCG2* rs2231142, and *GSTP1* rs1695 associated with adverse acute CNS toxicities. On the international (Austrian-Czech-NOPHO) cohort, we could not validate these results, *GSTP1* rs1695 was in an opposite association with the adverse reaction, ATE. When analyzing the total Combined cohort, *ABCB1* rs1045642 protected against toxic seizure, even in the Induction-like phase. Rs1128503 and rs2032582 were associated with ATE in the Hungarian cohort and with toxic seizure on the Combined cohort, respectively. Interestingly, they were in an opposite direction association with CNS-relapse. Studying the survival of Hungarian patients with ALL, we have found worse survival of patients in associations with *CYP3A4* rs3735451 and *CYP3A5* rs4646450 with in the AE and ATE cohorts. In the Combined ATE cohort, *GSTP1* rs1695 was in association with survival. The genes and their drug targets studied in any of the analyses on the Combined cohort are shown in Figure 11.

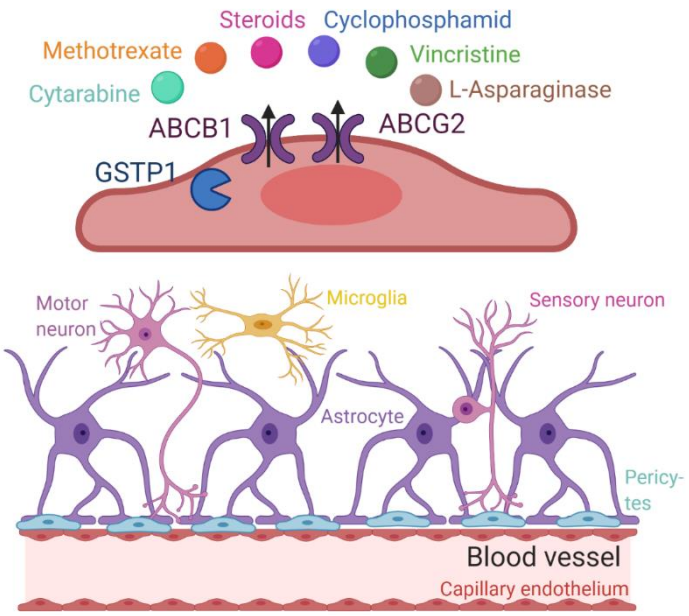

Figure 11. Capillary endothelial cell in the brain tissue (Created with BioRender.com)

Capillary endothelial cells providing the blood-brain barrier function together with neurons (pericytes, astrocytes, microglia) to protect the CNS against harmful agents.

Abbreviations: ABCB1: ATP Binding Cassette Subfamily B Member 1, ABCG2: ATP Binding Cassette Subfamily G Member 2, GSTP1: Glutathione S-Transferase Pi 1

ABC transporters play an important role in the pharmacokinetics of chemotherapeutic drugs in ALL. They can contribute to the resistance against methotrexate, cytarabine, vincristine, anthracyclines, and dexamethasone, influence the response to treatment and survival (Aberuyi *et al.*, 2020, Olarte Carrillo *et al.*, 2017, Ramos-Peñafiel *et al.*, 2020). SNPs in *ABCB1* were already analyzed in hematological malignancies, but are not in use in clinical practice so far (Ankathil, 2017). *ABCB1* rs1045642 associated with higher 24-hour plasma MTX concentration and with relapse of ALL and influenced the cerebrospinal fluid (CSF) concentration of MTX (Ma *et al.*, 2015, Ramírez-Pacheco *et al.*, 2016, Y. X. Zhang *et al.*, 2020). Rs1045642 variant carriers had a higher chance to experience toxicity during induction therapy in ALL including prednisolone and vincristine (Gregers *et al.*, 2015). Rs1128503, rs2032582, rs1045642 CGT haplotype associated with more blasts using glucocorticoids in initial remission induction of ALL therapy (Gasic *et al.*, 2018). Oral medications in ALL, like steroids, are substrates of ABCB1 and can activate its expression. As it is expressed more on the surface of enterocytes, T-genotype was associated with lower drug levels in serum (Nakamura *et al.*, 2002). Variant alleles of rs2032582 increased vincristine transport *in vitro* (Schaefer *et al.*, 2006). Vincristine elimination was influenced by rs2032582 and rs1045642 analyzing patients with pediatric ALL (Plasschaert *et al.*, 2004). Other SNPs in *ABCB1* like rs4728709 protected against associated with vincristine-related neurotoxicity (Ceppi *et al.*, 2014, Lopez-Lopez *et al.*, 2016). These previous results lead to similar conclusion together with our findings. They confirm that *ABCB1* SNPs in linkage disequilibrium are a good choice for further validation in the individualization of ALL treatment. ABCG2 is an important mediator of drug-drug interactions. ABCB1 and ABCG2 work as efflux transporters in blood-brain barrier to eliminate xenobiotics reaching the CNS. An interplay was proven between them in blood-brain barrier. Inhibiting ABCB1, in presence of the *ABCG2* rs2231142 variant, an increased dual substrate-level was measured in the brain (Bauer *et al.*, 2016). However, we could not validate the association of adverse neurologic toxicity with the *ABCB1* and *ABCG2* combined genotype on the international cohort, which is most probably due to the low frequency of *ABCG2* variant allele in the European population, our result is in line with the referred study and can be concluded that these genes are worth to be analyzed together.

There might be also other important differences between patients regarding the genetic makeup. It is assumed that a more active transporter function of variant rs2032582 and a less active function with rs1045642 and rs1128503 is present in leukemia. Impaired drug efflux can explain the susceptibility to toxicity (Kulma *et al.*, 2019). Rs1045642 seems to be the determinant of the mRNA level in these combinations. In haplotype-constellations ABCB1 function varies further (Gurney *et al.*, 2007). Regarding therapy-response, the *ABCB1* rs1045642 variant allele could act oppositely regarding survival in the same tumor type with the same drug-treatment in early or advanced-stage cancer. It is proof of the importance of tumor tissue genetics, which could influence clinical observations, too (Kulma *et al.*, 2019). The role of *ABCB1* with its SNPs in survival probably more complex than just a drug-transporter. The less effective function of ABCB1 caused by polymorphisms can result in an increased carcinogen level in the cell, thus causing susceptibility to ALL or relapse. The resistance against apoptosis through ABCB1 in lymphocytes was also described (Gollapudi & Gupta, 2001). This can explain the therapy resistance in lymphoblast, too. ABCB1 can influence the cytokine-transit through membranes, so the cell's immune response and outcome of the patient. An observed genotype-phenotype association often caused no change in the Pgp-activity which can make the interpretation of results even more complex (D. H. Kim *et al.*, 2006). *GSTP1* rs1695 associated with CNS toxicity, attention deficit among ALL survivors, ALL susceptibility and relapse, and influenced the risk for CNS relapse (Anderer *et al.*, 2000, B. Leonardi *et al.*, 2017, Kishi *et al.*, 2007, M Krajinovic *et al.*, 2001, Maja Krajinovic *et al.*, 2002, Krull *et al.*, 2013, Stanulla *et al.*, 2005). These are all important findings to understand the role of rs1695 in ALL.

Patients with AE had worse survival among Hungarian patients, however, the death ratio related to neurotoxicity was inconsequential. Reasons behind dismal survival could be the treatment delays, dose-reductions or omissions of chemotherapy, or the administration of enzyme inducer antiepileptic drugs which resolved the effect of chemotherapy due to the faster metabolism (Baker *et al.*, 1992, R. E. Hough *et al.*, 2019, Schrøder & østergaard, 1994, Watanabe *et al.*, 2018). Delays in the intrathecal treatment related to neurotoxicity associated with a higher risk for CNS relapse (Mateos *et al.*, 2021). CYP enzymes are important in the drug-metabolism also in the brain (Ferguson & Tyndale, 2011). The prevalence of vincristine neurotoxicity was found to be influenced

by *CYP3A5* (Egbelakin *et al.*, 2011). The regulation of these enzymes in the brain is different from the circumstances in the liver and so their influence on drug response and adverse effects (Ghosh *et al.*, 2016, Kuban & Daniel, 2021, McMillan & Tyndale, 2018). The significant role of *CYP3A4* and *CYP3A5* were already proven to influence the survival of pediatric ALL (Borst *et al.*, 2011, Gézsi *et al.*, 2015). Our results further highlight the role of CYP enzymes in this regard.

5.3. Inverse association between CNS toxicity and relapse

In the Hungarian cohort, *ABCC1, BCL2, CEP72, CYP3A4, CYP3A5* and *SLC22A7* associated with the prevalence of relapse or with the survival of the studied cohort. In the Combined cohort, *ABCB1* rs2032582, and rs1128503 were in association with CNS relapse.

This was the first time to show the association of *CEP72* with relapse of ALL. The expression of *ABCC1* was already shown to associate with the risk of relapse in pediatric ALL (Mehrvar *et al.*, 2019). *BAX/BCL-2* ratio at diagnosis showed association with survival in children with ALL (Kaparou *et al.*, 2013). BCL2 inhibitor venetoclax is a promising agent for the combination with chemotherapy in pediatric and adult patients with relapsed T-ALL (McMahon & Luger, 2019, Richard-Carpentier *et al.*, 2020). *SLC22A7* was hypo-methylated among non-responders in relapsed patients with ALL, showing possible clinical benefit (Burke *et al.*, 2014). Even though we could not replicate these associations on the Combined cohort between the survival and SNPs as presented in the Hungarian CNS relapse cohort, the results suggest the repetition of the validation in an international, more representative study population. The lack of association between survival and SNPs could be caused by the matched case-control selection method in the extended Combined cohort in our analysis.

ABCB1 expression was higher among high-risk patients with ALL and associated with lower 40 months survival rate (Olarte Carrillo *et al.*, 2017). *ABCB1* rs1045642, rs2032582, and rs1128503 were associated with worse EFS, with ALL susceptibility and increased the risk of relapse among children with ALL (Ceppi *et al.*, 2014, Hattori *et al.*, 2007, Talaat *et al.*, 2018, Urayama *et al.*, 2007, Yang *et al.*, 2010, Zaruma-Torres *et al.*, 2016, Zhai *et al.*, 2012). Rs1045642 associated with the risk of CNS relapse in childhood ALL (Stanulla *et al.*, 2005). Our findings are consistent for rs2032582, and rs1128503

rare alleles with previous survival results in ALL. We have proved the first time the inverse function of these SNPs in association with CNS toxicity and CNS relapse among pediatric patients with ALL.

5.4. Genetic alteration associated with risk of T-ALL relapse and survival

Relapse-specific alterations were found in *NT5C2* in this study. In *NT5C2* known mutations were found in activating hotspots, missense exchanges, or a non-frameshift deletion. These alterations gave a gain of function for the nucleotidase function. This is important regarding the therapy: inactivation of nucleoside analogs applied in the therapy can cause resistance against these drugs. *NT5C2* mutations were associated with early first relapse. Activating mutations of *NT5C2* were already described in relapse and being responsible for chemotherapy resistance (6-mercaptopurine, 6-thioguanine) in ALL relapse (148, 222). The impact of *NT5C2* gene mutations on survival is controversial. According to our results mutations had no prognostic impact. In contrast, Barz et al. found that *NT5C2* mutations are associated with poor outcomes after relapse, however, they studied pediatric B-cell ALL (Barz *et al.*, 2020). The importance of *NT5C2* in relapse was verified in some recent publications. Analysis of matched diagnostic and relapse samples of teenage and young adult patients with T-ALL supported the hypothesis that relapse is driven by the evolution of sub-clones associated with drug resistance e.g.: *NT5C2* mutations (Mansur *et al.*, 2021). Mutations in *NT5C2* as subclonal events were frequent in both B- and T-ALL at relapse (Waanders *et al.*, 2020). Clonal evolution of *NT5C2* mutations is known for relapsed acute lymphoblastic leukemia (Tzoneva *et al.*, 2018). *NT5C2* was associated with resistance to 6-mercaptopurine and relapse in acute lymphoblastic leukemia, where germline and acquired mutations play a role, too (227, 228). Targeted therapy for *NT5C2* may inhibit treatment resistance and relapse in ALL (Dieck & Ferrando, 2019).

5.5. Limitations of studies conducted in Hungary

The challenges we face in this research area are the small patient populations; in collaborations, the divergent protocols used in onco-haematology, or the different phenotype categories used in clinical practice. The diagnosis of CNS leukemia is still a major hurdle: there is a need for more precise diagnostic approaches (86, 230, 231).

Limitations of the studies conducted in Hungary were the retrospective data collection; the survival analysis of a retrospectively selected, matched cohort resulted in a skewed population. Patients who died before the period of sample collection were underrepresented in our cohort. Furthermore, there are possible explanations why the genotype-toxicity associations could not be fully validated. Some neurotoxic drugs differ among treatment protocols. E.g. the NOPHO protocols use a lot more vincristine than the BFM based protocols, their very high rate of PRES may also relate to this. Recently, some important non-genetic factors of methotrexate neurotoxicity were suggested, like N_2O use for anesthesia, B12 vitamin, or zinc deficiency (Forster *et al.*, 2016). We think there were major differences among national groups in these regards. To compensate for the possible diversity of the study, we enlarged the size of the populations; we collected international uniformed cohorts where demographic data of the patients matched. Identification of the studied phenotypes was based on the comprehensive definitions of the Delphi consensus to get more representative associations (Schmiegelow *et al.*, 2016).

6. Conclusions

1. Chemotherapeutic drug-metabolizing enzyme and transporter SNPs have a possible role in the prevalence of treatment-related cardiotoxicity in ALL (T-, and B-cell subtype) or OSC. Potential biomarkers can be the genetic variations in *ABCC2, NQO1, SLC22A6* predisposing for decreasing FS values. SNPs in *CYP3A4, CYP3A5* could be indicators for further FS changes.

2. Chemotherapeutic drug-metabolizing enzyme and transporter SNPs have a possible role in the prevalence of treatment-related neurological complications in ALL (T-, and B-cell subtype): including toxicity or CNS relapse. Significantly associated SNPs in carrier genes were *ABCB1, GSTP1* in association with neurotoxic events, AE, or ATE. *CYP3A4, CYP3A5, GSTP1* influenced prognosis in these populations.

3. Chemotherapeutic drug-metabolizing enzyme and transporter SNPs were studied with CNS relapse in *ABCB1, ABCC1, BCL2, CEP72, CYP3A4, CYP3A5, SLC22A7*. They influenced the appearance of CNS relapse or survival of the studied groups. Inverse association between the function of SNPs predisposing for toxicity or relapse in ALL (T-, and B-cell subtype) was proved through two *ABCB1* SNPs.

4. *NT5C2* most probably contributes to T-ALL relapse.

7. Summary

The risk-directed treatment improved the survival of pediatric malignancies by around 80%, however, acute and late adverse effects or therapy failures still influence the quality of life and survival (Butler *et al.*, 2021). In our research, my aim was to investigate the role of heritable SNPs and novel mutations related to these adverse events in ALL or OSC.

We have collected retrospectively data from clinical records and patient samples from Hungarian Pediatric Oncohaematology Biobank in Hungary and received from European collaborations, respectively. We searched for relevant candidate genes of drug-metabolizing enzymes and transporters for analyzing the association between germline SNPs and the studied phenotypes: cardiotoxicity, neurotoxicity, and CNS relapse. We also looked for acquired mutations in driver and passenger genes in the evolution of T-ALL relapse.

Among patients with ALL or OSC, *ABCC2* rs3740066, *NQO1* rs1043470, and *SLC22A6* rs6591722 were associated with worse FS in the acute phase of the treatment or at 5–10 years after the diagnosis. Only in the ALL group, *CYP3A4* rs3735451, *CYP3A5* rs776746, and *NQO1* rs1043470 were in association with the change in FS values studying periods with different cut-points of therapy. *ABCB1* rs1045642 associated with toxic seizure. *ABCB1* rs1128503 or rs2032582 were in association with adverse toxic seizure and CNS relapse, on the Combined cohorts, however, in an inverse manner: higher risk for toxicity and lower risk for CNS relapse and vice-versa. *CYP3A4* rs3735451 and *CYP3A5* rs4646450, rs776746 showed worse OS and EFS for AE, ATE, or CNS relapse cohorts. Inactivating mutations of *NT5C2* was associated with early T-ALL relapses, but not with survival.

If these SNPs and mutation will be part of the screening process in the clinical practice after validation steps in the future, our findings may contribute to the improvement of drug safety and efficacy and provide novel targets for precision medicine in the treatment of pediatric patients with malignancies.

8. Összefoglalás

A kockázat-alapú kezelés körülbelül 80%-kal javította a gyermekkori rosszindulatú daganatok túlélését. Azonban az akut és a késői mellékhatások, illetve a terápia sikertelensége továbbra is rontják az életminőséget és a túlélést (Butler *et al.*, 2021). Munkám során az öröklődő SNP-k és az új mutációk szerepét vizsgáltam a mellékhatások, ill. a relapszus kialakulásában, ALL és OSC betegpopulációkban.

A retrospektív vizsgálatokhoz a mintákat és a betegadatokat részben a magyarországi Gyermekgyógyászati Onkohematológiai Biobank, részben az európai együttműködések révén szereztük be. Célkitűzésünk volt a vizsgált fenotípusok (kardiotoxicitás, neurotoxicitás és CNS relapszus) és a releváns génekben (gyógyszermetabolizáló enzimek és transzporterek) jelen lévő csíravonal SNP-k közti összefüggések vizsgálata. Továbbá T-ALL betegcsoportban a relapszus kialakulásában releváns gének szerzett mutációit is kerestük.

ALL-ben ill. OSC-ben szenvedő betegek esetén az *ABCC2* rs3740066, *NQO1* rs1043470, és az *SLC22A6* rs6591722 rosszabb lineáris ejekciós frakcióval (linEF) asszociált a kezelés akut fázisában valamint a diagnózis után 5–10 évvel is. Az ALL csoportban a *CYP3A4* rs3735451, a *CYP3A5* rs776746 és az *NQO1* rs1043470 összefüggést mutatott a linEF értékek változásával a terápia különböző pontjai közötti időszakokat vizsgálva. Az *ABCB1* rs1045642 toxikus görcsroham előfordulásával asszociált. Ugyanakkor az *ABCB1* rs1128503 és az rs2032582 a toxikus görcs rohammal és a központi idegrendszeri (KIR-i) relapszussal ellentétesen függött össze a teljes betegcsoportban: magasabb toxicitási, és alacsonyabb KIR-i relapszusra való kockázattal asszociáltak és fordítva. A *CYP3A4* rs3735451 és a *CYP3A5* rs4646450, az rs776746 SNP-k jelenléte rosszabb teljes (OS) ill. eseménymentes túlélést (EFS) jelentettek az AE, az ATE és a KIR-i relabált kohorszokban. Az *NT5C2* inaktiváló mutációi korai T-ALL relapszussal társultak, de a túlélést nem befolyásolták.

A jövőben, amennyiben ezeket az SNP-ket és mutációt validálások után alkalmasnak találják arra, hogy a klinikai gyakorlatban szűrésre kerüljenek, eredményeink hozzájárulhatnak a gyógyszerek biztonságosságának és hatékonyságának javításához és a rosszindulatú daganatos gyermekgyógyászati betegek precíziós kezelésében új gyógyszercélpontokat tárhatnak fel.

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10. Bibliography of the candidate's publications

10.1. Publications related to the thesis

Sági, J.C.; Gézsi, A.; Egyed, B.; Jakab, Z.; Benedek, N.; Attarbaschi, A.; Köhrer, S.; Sipek, J.; Winkowska, L.; Zaliova, M.; et al. Pharmacogenetics of the central nervous system—toxicity and relapse affecting the cns in pediatric acute lymphoblastic leukemia. Cancers (Basel). 2021, 13, doi:10.3390/cancers13102333.
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10.2. Publications not related to the thesis

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