Université de Montréal

Genetic predisposition to corticosteroid - related complications of childhood Acute Lymphoblastic Leukemia (cALL) treatment

par Maria Plesa

Département de Pharmacologie et Physiologie
Faculté de médecine

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RÉSUMÉ

L'ostéonécrose (ON) et les fractures (FR) sont des complications qui prennent de plus en plus place dans le traitement pédiatrique de la leucémie aiguë lymphoblastique (LAL). L'ON peut être causée par différents facteurs, dont principalement l'utilisation de glucocorticoïdes. Les glucocorticoïdes sont administrés lors du traitement de la leucémie dans le but d'initier l'apoptose des cellules malignes tout en ayant un effet anti-inflammatoire. Cependant, l'utilisation de ces corticostéroïdes comprend des effets secondaires sérieux, notamment le développement d'ostéonécrose. Des variantes génétiques peuvent mettre certains patients plus à risque que d'autres. Plusieurs gènes ont déjà été signalés comme régulés par les actions glucocorticoïdes (GC). Les variations génétiques présentes dans les régions régulatrices de ces gènes peuvent affecter leur fonctionnement normal et, en fin de compte, de déterminer un risque accru de développer l'ON associé au traitement contre la leucémie. Pour cette raison, plusieurs polymorphismes ont été identifiés et étudiés dans la cohorte QcALL de Ste-Justine, concernant les gènes suivants : *ABCB1, ACP1, BCL2L11, NFKB1, PARP1,* et *SHMT1*. Ces gènes jouent majoritairement un rôle dans les mécanismes d'action des glucocorticoïdes, mais quelques-uns ont plutôt un effet direct sur le développement d'ostéonécrose. Nos recherches ont démontré une corrélation entre ces polymorphismes et l'apparition d'ostéonécrose chez les patients de la cohorte QcALL, traités aux glucocorticoïdes. L'incidence cumulative de l'ostéonécrose a été évaluée rétrospectivement chez 305 enfants atteints de la leucémie qui ont subi un traitement à l'hôpital Ste-Justine selon les protocoles DFCI de Boston (87-01, 91-01, 95-01 et 2000-01).

Parmi les huit polymorphismes de *BCL2L11* étudiés, les 891T> G (rs2241843) et 29201C> T (rs724710) ont été significativement associés à ON ($p = 0.01$ et $p = 0.03$, respectivement). L'association du polymorphisme 891T> G a été modulée par le type de corticostéroïde (CS), l'âge, le sexe et le groupe à risque ($p \leq 0,05$). Le polymorphisme 29201C> T était particulièrement apparent chez les patients à haut risque ($p = 0,003$). La même étude était conduite en parallèle sur des patients de la cohorte DFCI de Boston (N = 192), et montrait des résultats significatifs pour les polymorphismes étudiés. En conclusion, les résultats de cette étude permettront de confirmer l'association de ces polymorphismes au développement d'ON chez les patients de LLA traités aux GC.

Mots-clés : ostéonécrose (ON), fractures (FR), dexaméthasone (DXM), prednisone (PDN), Leucémie lymphoblastique aiguë de l'enfance (cALL), SNP (Single Nucleotide Polymorphism), B-cell Chronic Lymphocytic leukemia Lymphoma like 11 gene (*BCL2L11*), Nuclear Factor of Kappa light polypeptide gene enhancer in B-cells 1 (*NFKB1*), Poly(ADP-ribose) Polymerase 1 (*PARP1*), (ATP)-Binding Cassette subfamily B member 1 (*ABCB1*), Acid Phosphatase 1 (*ACP1*), Serine Hydroxy Methyltransferase 1 (*SHMT1*), Leucémie Aiguë Lymphoblastique (LAL) au Québec (QcALL), risque élevé (RE), Institut du Cancer Dana-Farber (DFCI).

ABSTRACT

Osteonecrosis (ON) and fractures (FR) are complications that take place in the treatment of children acute lymphoblastic leukemia (cALL). They can be caused by various factors, mainly using glucocorticoids. The corticosteroids, dexamethasone (DXM) and prednisone (PDN) are administered during the treatment of leukemia to initiate apoptosis of malignant cells; while having an anti-inflammatory effect. However, the use of these corticosteroids has severe side effects, including the development of osteonecrosis. Moreover, some patients develop resistance to treatment, and are at risk of developing side effects. The genetic variants predispose some patients at higher risk than others. Several genes have been previously reported as up- or down regulated by the GCs actions. The genetic variations present in gene coding or regulatory regions can affect their function and ultimately determine an increased risk of developing ON associated to ALL therapy. Therefore, we investigated the association between several single nucleotide polymorphisms (SNPs) in six candidate genes: *BCL2L11*, *NFKB1*, *PARP1*, *ABCB1*, *ACP1*, and *SHMT1*. These genes play a role in the mechanisms of action of glucocorticoids, but some have more of a direct effect on the development of osteonecrosis. Our research has shown a correlation between these polymorphisms and the occurrence of osteonecrosis in patients in the QCALL cohort, treated with glucocorticoids. Cumulative incidence of osteonecrosis was assessed retrospectively in 305 children with ALL who underwent treatment with DFCI protocols (87-01, 91-01, 95-01 and 2000-01) in childhood ALL cohort from Quebec (QcALL). Among the eight tag *BCL2L11* polymorphisms studied the 891T>G (rs2241843) and 29201C>T (rs724710) were significantly associated with ON ($p = 0.01$ and $p = 0.03$, respectively). Association of 891T>G polymorphism was modulated by type of corticosteroid (CS), age, sex and risk group ($p \leq 0.05$ and that of 29201C>T was particularly apparent among high risk ($p = 0.003$) patients. These polymorphisms have shown significant ON association in several QcALL risk groups, mainly in corticosteroid groups, age < 10 years, and high risk (HR) group. Furthermore, the same study was conducted in parallel with patients in the replication (DFCI) cohort ($N = 192$), and we showed significant genetic association results for all studied polymorphisms. In conclusion, this study identifies that some ALL children have a high incidence of ON during the treatment that is highly associated with polymorphisms in different genes regulated by corticosteroids and ALL prognostic factors.

Keywords : Osteonecrosis (ON), Fractures (FR), dexamethasone (DXM), prednisone (PDN), childhood Acute Lymphoblastic Leukemia (cALL), single nucleotide polymorphisms (SNPs), B-cell Chronic Lymphocytic leukemia Lymphoma like 11 gene (*BCL2L11*), Nuclear Factor of Kappa light polypeptide gene enhancer in B-cells 1 (*NFKB1*), Poly(ADP-ribose) Polymerase 1 (*PARP1*), (ATP)-Binding Cassette subfamily B member 1 (*ABCB1*), Acid Phosphatase 1 (ACP1), Serine Hydroxy Methyltransferase 1 (*SHMT1*), childhood ALL cohort from Quebec (QcALL), high risk (HR), Dana-Farber Cancer Institute (DFCI) cohort.

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ABCB1: (ATP)-Binding Cassette subfamily B member 1

ACP1: Acid Phosphatase 1

ADRs: adverse drug reactions

ALL: Acute Lymphoblastic Leukemia

ATF5: Activating Transcription Factor 5

ASO: Allele-Specific Oligonucleotide

BCR-ABL1: Breakpoint Cluster Region–ABL1

Bcl-XL: B-cell lymphoma-extra large

Bcl-2: B-cell lymphoma 2

BCL2L11: B-cell Chronic Lymphocytic leukemia Lymphoma like 11 gene

BH3: Bcl-2 Homology 3

Bim: Bcl-2-like protein 11

BM: Bone Marrow

CEU: Northern Europeans from Utah

CI: Confidence Interval

CNS: Central Nervous System

COG: Children's Oncology Group

CTCAE: Common Terminology Criteria for Adverse Events

DFCI: Dana-Farber Cancer Institute

DHFR: Dihydrofolate Reductase

DXM: Dexamethasone

EFS: Event Free Survival

ETV6 RUNX1: Ets Variant Gene 6 Runt-related transcription factor 1

FR: Fractures

GCs: Glucocorticoids

GWAS: Genome Wide Association Study

HR: Hazard Ratio

HR: high risk

IC50: Half maximal inhibitory concentration

LCLs: Lymphoblastic Cell Lines

LD: Linkage Disequilibrium

MAF: minor allele frequency

MDR1: multidrug resistance protein 1

MRD: Minimal Residual Disease

MRPs: Multidrug Resistance-related Proteins

NFKB1: Nuclear Factor of Kappa light polypeptide gene enhancer in B-cells 1

ON: Osteonecrosis

OR: Odds Ratio

OS: Overall Survival

PAI-1: plasminogen activator inhibitor-1

PARP1: Poly(ADP-ribose) Polymerase 1

PCR: Polymerase Chain Reaction

PDN: Prednisone

P-gp: Permeability-glycoprotein

QcALL: Quebec childhood Acute Lymphoblastic Leukemia

SHMT1: Serine Hydroxy Methyltransferase 1

SNP: Single Nucleotide Polymorphism

SR: standard risk

TPMT: Thiopurine S-Methyl Transferase

TS: Thymidylate Synthase

3´UTR: 3´Untranslated Region

5´UTR: 5´Untranslated Region

WBC: White Blood Cells

WES: Whole-Exome Sequencing

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CHAPTER 1: INTRODUCTION

1.1 ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)

1.1.1 Introduction to ALL

Acute lymphoblastic leukemia (ALL) is seen in both children and adults, but its incidence is higher in the pediatric population, between ages 2 and 4 years. In the pediatric population, B-acute lymphoblastic leukemia (B-ALL) is the most prevalent childhood malignancy, where it accounts for 81% of childhood leukemia, and it is as well the leading cause of childhood cancer-related mortality [1]. On the contrary, T-acute lymphoblastic leukemia (T-ALL) accounts for about 15% of pediatric cases and 20% of adults' cases [2].

Leukemia is a complex disease characterized by deregulated proliferation of clonally expanded immature lymphoid or myeloid progenitor cells. As detected by conventional cytogenetic methods, patients with B-ALL have a higher percentage of abnormal clones (80%-90%) than do patients with T-lineage ALL (60%-70%) [2].

1.1.2 Diagnostic and classification of ALL

Acute lymphocytic leukemia (ALL) is a fast-growing cancer of lymphocyte-forming cells, so-called lymphoblasts. In the past, ALL has been classified according to the French-American-British (FAB) classification in three types: L1, L2 and L3. This classification, based on lymphocyte morphology is no longer used, it has now been replaced by the World Health Organization (WHO) classification. In the most recent WHO classification, the neoplasms lymphoid are assigned to two principal categories: B- and T-lineage lymphoid precursors.
Current standard cytogenetic, molecular and immunophenotyping assays using higher sensitivity and specificity tools (such as fluorescence in situ hybridization (FISH) and multi-channel flow cytometry), could help to further divide ALL in subtypes according to karyotype abnormalities and translocations [3, 4].

1.1.2.1 Diagnostic based on immunophotype of B- and T-lineage ALL

In B-lineage ALL the most important markers for differential diagnosis are CD19, CD20, CD22 (membrane and cytoplasm), CD24, and CD79a; which are useful markers used in a rapid immunological assay, for instance in monitoring minimal residual diseases (MRD), during or after ALL therapy [5]. The presence of CD10 antigen defines the "common" ALL subgroup. T-cell markers for diagnostics are CD1a, CD2, CD3 (membrane and cytoplasm), CD4, CD5, CD7 [2].

1.1.2.2 Diagnostic based on cytogenetics

Additional to immunophenotyping using membrane and cytoplasmic CD markers, cytogenetics represents an important step in ALL diagnosis.

The conventional karyotyping tests have been used for a rapid detection of numerical or structural karyotype alterations, such as hypo- and hyperdiploidy or gain or loss of chromosomal material. Modern sensitive tools, as fluorescence in situ hybridization (FISH) technology enabled the detection of genetic rearrangements and chromosomal abnormalities with high specificity, by using locus-specific probes for their detection. Many ALL subtypes are characterized by genetic alterations that perturb multiple key cellular pathways, including hematopoietic development, signalling or proliferation, and epigenetic regulation.

1.1.3 Genetic inherited factors as predictive of risk

Many factors, also called prognostic factors can affect a patient's prognosis. The prognostic factors could be related to the leukemia disease (ALL subtypes, number of chromosomes or translocations), or to the patient characteristics, such as: age, initial white blood cells (WBC), gender, race.

Two most important patients' predictors (age and initial WBC) are used in diagnostics to correct assign patients in ALL group risks. Based on these two prognostic factors, National Cancer Institute criteria (NCI) stratifies B-cell patients, in standard group, patients aged between 1 – 9 years and an initial WBC < 50,000 / μL); and in high-risk group, patients older than 10 years

and WBC >50,000 / μL. Generally, children in standard-risk group are at low-risk, they have a better prognosis than those at high-risk which are receiving intensive treatment.

Additional to age and WBC initial count, there are other high patient risk factors which are important, such as: gender, other organs involvement (CNS, lymph nodes, spleen, liver) or unfavourable cytogenetics (Hypodiploidy), t(9;22), BCR-ABL1 or MLL rearrangements [6-12]. Most frequent cytogenetic abnormalities in B-lineage are the hyperdiploidy > 50 chromosomes [Fig. A], which occurs in 25% of childhood B-cell precursor ALL and is associated with an intermediate risk [13, 14]. On the contrary, the ETV6-RUNX1 fusion [t(12;21)(p13;q22)] is also observed in 25% of childhood B-cell precursor ALL and is associated with a favourable outcome [15]. Patients with Ph-positive ALL have a translocation of chromosomes 22 and 9, denoted as t(9;22) BCR-ABL1.

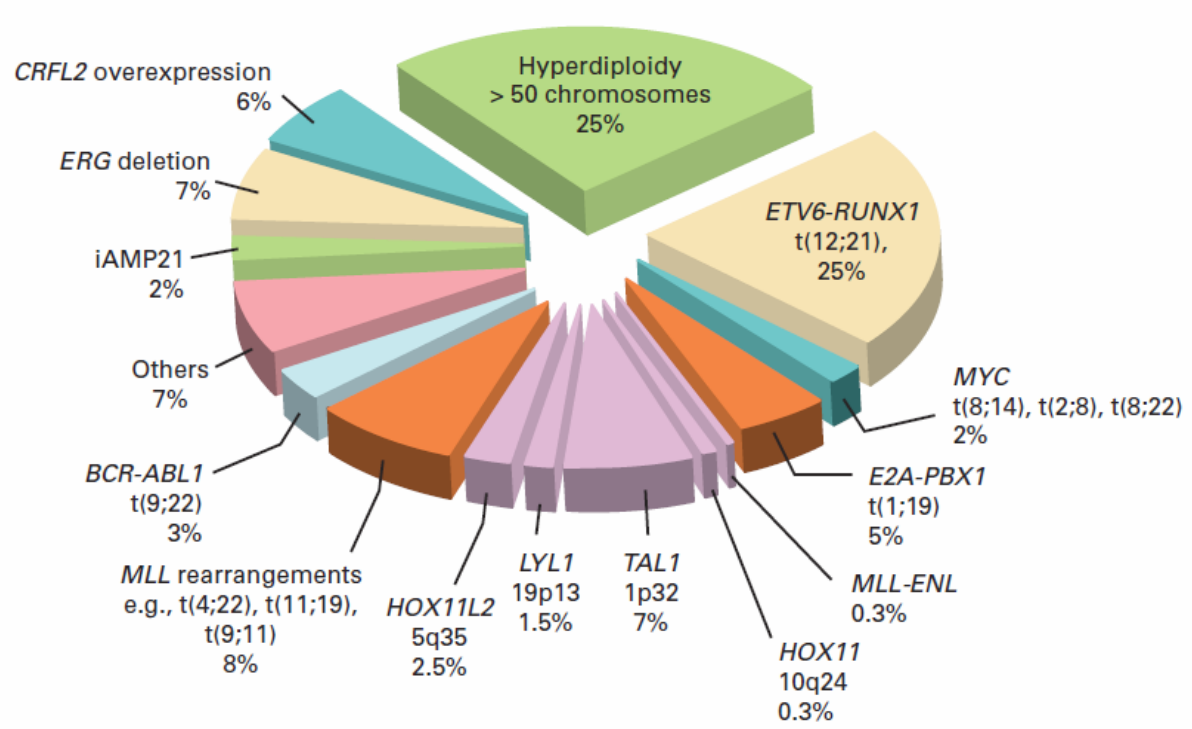

Figure A. Estimated frequency and genetic abnormalities in B-lineage and T-lineage (indicated in purple) [13].

This abnormal structure results in a gene mutation (change) called BCR-ABL in 3% of B-cell precursor ALL and is associated with unfavourable prognostic. For these patients, the reported overall EFS rate of about 25% and 10% in the case of Ph^+ ALL [8, 9, 12].

At present, there are no genetic markers in T-ALL that reliably predict treatment response or outcomes. Many of the translocations seen in T-ALL are recurrent but with a low frequency. Some genetic markers have been shown to be of clinical relevance in a small series of pediatric patients with T-ALL: $TLX1(HOX11)^+$ was associated with a favourable outcome, and TAL1+ and LYL1+ were associated with unfavourable prognosis [16].

Unlike that in B-precursor ALL, high leukocyte count does not identify high-risk T-ALL in children or adults. Also, no correlation of age with sex, initial white blood cell count, CNS disease, or early treatment response was found. Historically unfavourable outcome of patients with T-ALL has recently improved using highly effective treatment protocols. T-ALL is now treated in the same way as high-risk B-progenitor ALL. With appropriately intensive therapy, children with T-ALL have an outcome similar to that of children with B-precursor ALL, i.e., the estimated 5-year event-free survival (EFS) is 75% to 80% [7]. Nevertheless, patients with T-ALL remain at increased risk for remission induction failure, early relapse, and isolated CNS relapse [17].

1.1.4 ALL treatment

Improvements in the survival rate for ALL in children continue to be observed in the last decades. Multiple factors greatly contributed to this improvement, including a better understanding of immunobiology and cytology of ALL, the use of a combination of effective drugs at optimized doses, as well as the identification of prognostic factors with risk-adapted intensification treatment.

There are several protocols of treatments in ALL. Despite certain differences in success rates, more than 90% treated patients will enter remission and about 85% will survive free of leukemia recurrence 5-years from diagnosis [17-19]. Dana Farber Cancer Institute (DFCI) Consortium

protocols are multi institutional protocols used as research protocols in the United States and Canada, since 1981. Thereby Ste-Justine Hospital Research Center treat ALL patients in accordance with DFCI protocols. The DFCI protocols consist of five phases of therapy for a duration of two years [Fig. B], which are: investigational window (3-5 days), remission induction (4 weeks), CNS treatment (3 weeks), intensification (30 weeks), and continuation (71 weeks, depending on the risk group) phases [20-22].

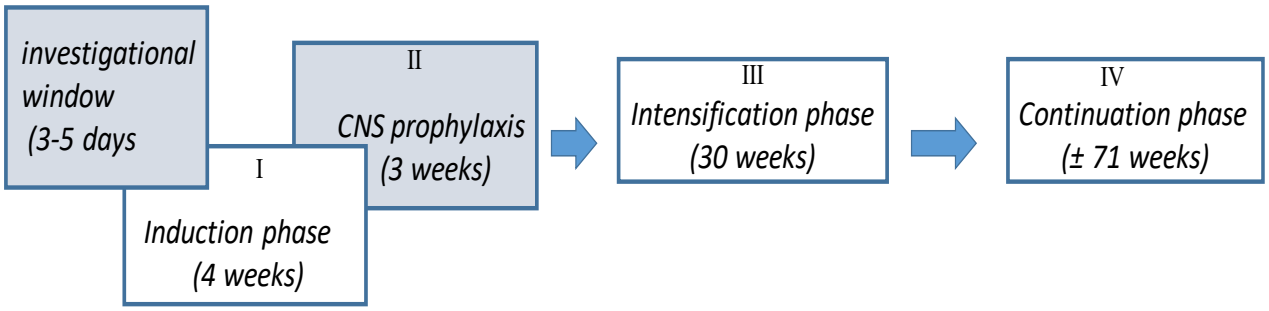

Figure B. Schema of DFCI-ALL consortium clinical research protocols

The goal of investigational window, in DFCI 91-01 protocol, was to evaluate a single therapeutic drug efficiency response (corticosteroid, PDN or DXM) prior to the induction phase. In the induction phase, a complete remission is induced. In this phase, more than 99 % of leukemia cells are killed and a normal hematopoiesis is restored. The duration of induction phase is for 4 weeks and the ALL patients receive multi-drug therapy: corticosteroids, doxorubicin, cytarabin, asparaginase, vincristine and methotrexate. During intensification and consolidation phases, the ALL patients receive similar drugs as during induction, but with differences in duration; their goals are to completely reduce the presence of lymphoblasts and maintain a complete remission.

Minimal residual disease (MRD) is used to describe residual disease after induction chemotherapy, as a number of leukemia cells that remain in the patient during the treatment which is compatible with the cure [23]. During ALL phases, a continued monitoring of MRD is performed, and it gives an indication of treatment efficacy, patient remission status, or to detect recurrence/ relapse after long-term remission. The MRD threshold is defined by a positive monoclonal antibodies reaction of a less than 1% of blast cells [24].

Corticosteroids, prednisone (PDN) and dexamethasone (DXM) are an essential component of treatment for ALL therapy. They play a crucial role to induce apoptosis in the malignant lymphoid cells. In some protocols, the corticosteroids are used as a single drug in induction phase to induce a complete remission [17]. PDN is generally used in the induction phase to induce complete remission and a poor response following a 7-day course of PDN in some ALL treatment protocol is not considered as an indicator that patients are switched to more intensive induction therapies in order to increase efficacy. Therefore, PDN response in childhood ALL is currently a prognostic factor utilized in the adaptation of chemotherapy treatment protocols. On the other hand, DXM tends to be more used in intensification and consolidation phases due to their higher anti-leukemic activity and better central nervous system (CNS) penetration than prednisone [25]. Detailed DFCI therapy, including drug dosage and drug-delivery sequence, has been reported in several publications [18-22]; therefore, only the corticosteroid-related differences (type, and daily doses) are described in Figure C. Common to all DFCI protocols, PDN has been used in the induction phase as the only corticosteroid; at a dose of 40 mg/m^2/d for the duration of 28 days.

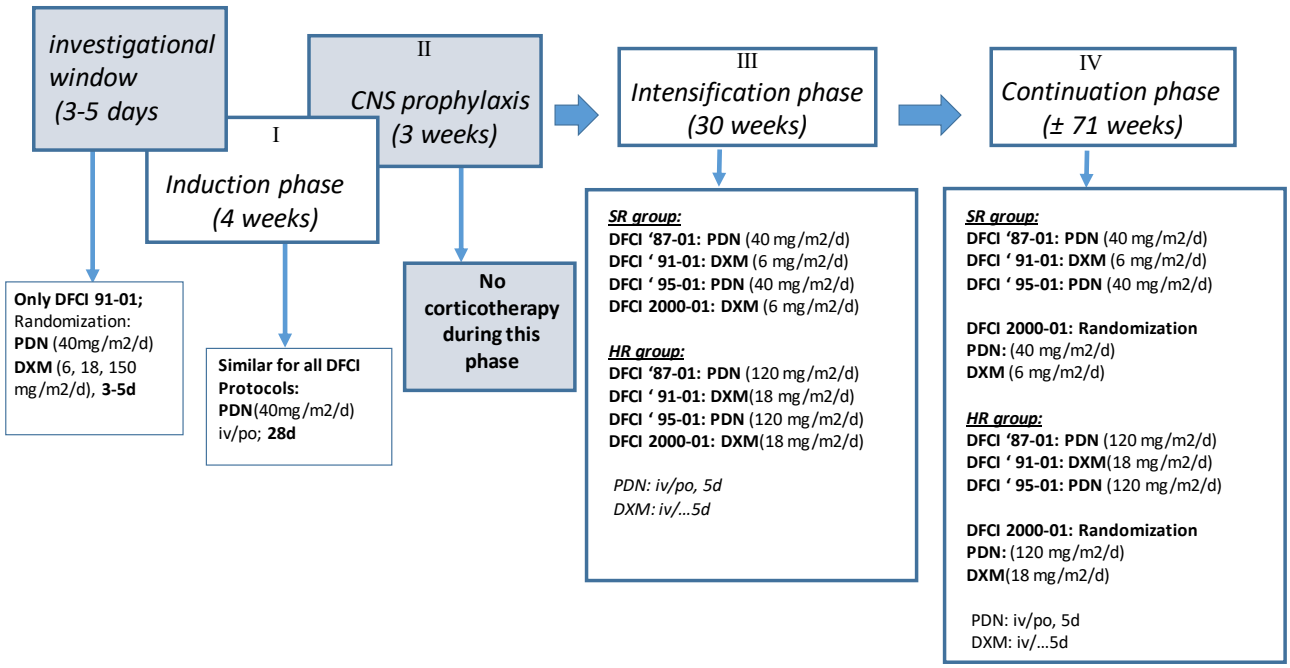

Figure C. Corticosteroid therapy in DFCI protocols [18-22].

Exceptionally, a pre-induction phase was included in protocol 91-01, also called investigational window in which patients were randomized to PDN (40 mg/m^2/d) or DXM (6, 18, 150 mg/m^2/d). Protocols 87-01 and 95–01 followed a similar plan, on both protocols, intensification therapy was characterized by the use of PDN. On the contrary, in protocols 91-01 and 2000-01, DXM was used in the intensification phase instead of PDN. In all protocols, during intensification, high risk (HR) patients received three times more corticosteroids (120 mg/m^2/d of PDN or 18 mg/m^2/d of DXM) as standard risk (SR) patients (40 mg/m^2/d of PDN or 6 mg/m^2/d of PDN). HR patients received three times as much prednisone (120 mg/m^2/d) as SR patients (40 mg/m^2/d) during intensification. Similar, the continuation therapy was used for protocols 87-01 and 95–01, with PDN at 40 mg/m^2/d (SR) or PDN at 120 mg/m^2/d (HR). In protocol 2000-01, continuation therapy was similar to protocol 91–01 except that both SR and HR patients were randomized to receive either PDN or DXM and received same doses as SR group [18-22].

1.2 GLUCOCORTICOID-INDUCED APOPTOSIS

1.2.1 Glucocorticoid receptor

The glucocorticoids exert a wide range of physiological effects, including the induction of apoptosis in lymphocytes. In glucocorticoid sensitive cells, apoptosis and other cellular effects are induced through the activation of the glucocorticoid receptor (GR). The gene encoding the human GR receptor (hGR) is located on chromosome 5 in loci 31-32 (5q31-32) [27]; it generates different splice and translation protein variants, all of which are modular proteins that contain three distinct functional regions: N-terminal trans-activation domain (NTD), the central zinc-finger DNA-binding domain (DBD) and the C-terminal ligand-binding domain, LBD [27-29, Fig. D].

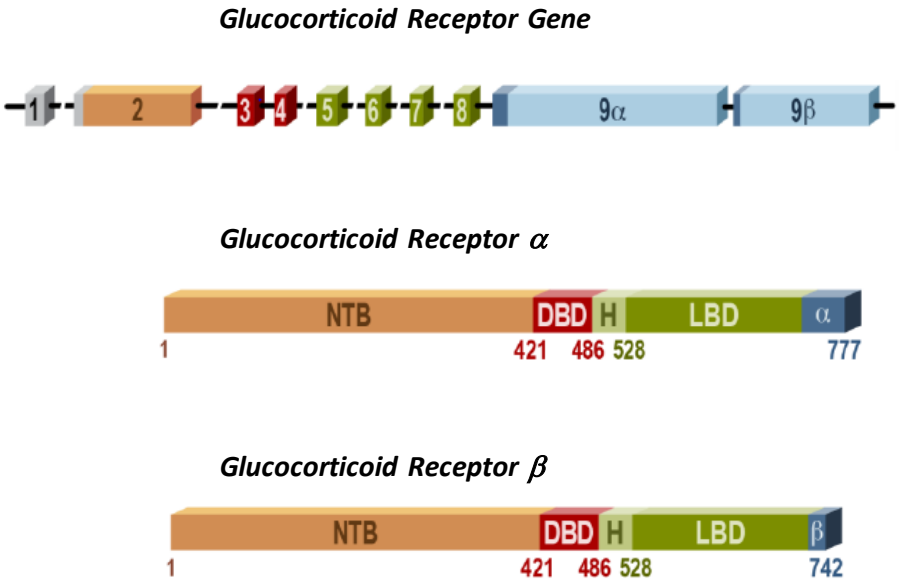

Figure D. Structure of human glucocorticoid receptor GR gene [27].

Alternative splicing of exon 9 of the hGR results in two major isoforms: hGRα and hGRβ. The hGRα isoform is highly expressed and is responsible for classical signalling and modulations of gene transcriptions. The hGRβ isoform is less expressed in corticosteroid sensitive cells, and may function as a dominant negative inhibitor of hGRα signalling, which explain in some cases resistance to CS. Furthermore, by alternative translation initiation sites in the NTD region, additional GRα-C and GRα-D isoforms are produced [27, 28]. Thereby, the NTD which is the immunogenic region, is considered the most variable in length and primary sequence among other 2 regions of the steroid hormone receptors [29]. The different translational isoforms of GRα-C (C1-C3) and GRα-D (D1-D3), respectively have been shown to induce apoptosis at different rates. The GRα-C isoform was identified as the most potent inducer of apoptosis compared to GRα-D; which can explain, in part, the broad range of responses to GC-induced apoptosis in responsive cells. [30, 31].

1.2.2 Glucocorticoid receptor signalling

GCs can exert their effects either at genomic or non-genomic level. Genomic actions of GCs are mediated by nuclear translocation of the GR. Upon GC binding, the GR dissociates from its

chaperone proteins (Hsp90) which keeps GR in inactive state [32], homodimerises via the C-terminal LDB regions [33] and translocates to the nucleus and function as a ligand-activated transcription factor. Once in the nucleus, the GR can then act as homo- or heterodimers with or without other cofactors in signalling processes; either a transcriptional activator or a repressor, depending on the gene and the cellular environment [Fig. E].

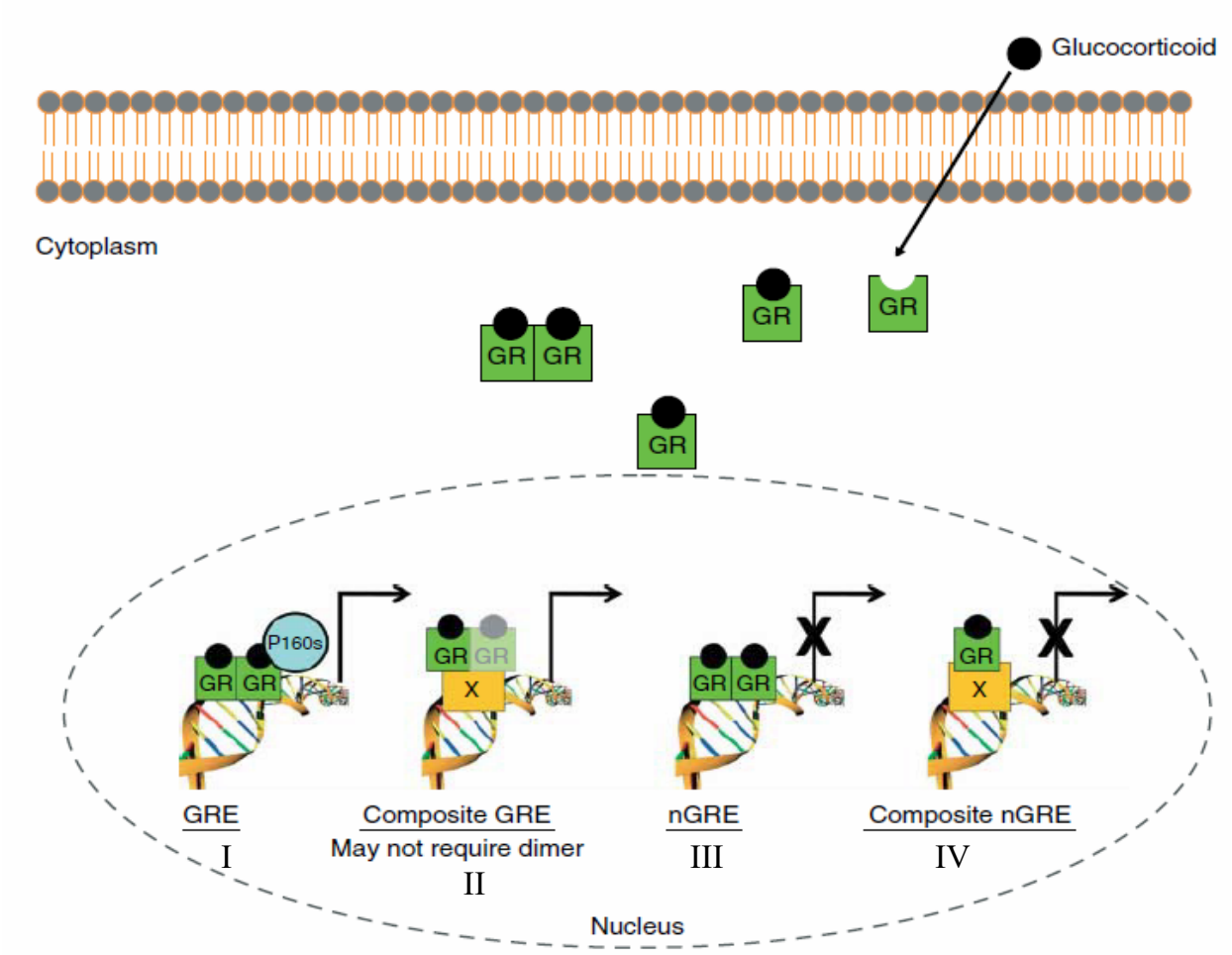

Figure E. The glucocorticoid receptor signalling [34].

GR dimers are able to bind to the GC response elements (GRE) present in the promoter regions of the target genes (Fig. E I). Once bound to the GRE, the GRs recruits co-activator proteins (p160 family proteins) and act as transcriptional activators [33].

GRs can also up-regulate gene transcription using composite GRE mechanisms (Fig. E II), in which GCs interact with DNA along with other transcription factors families, such as c-Myb, MAPK-phosphatase 1 (DUSP1) and also act as transcriptional activators [33, 35].
GC bound GR can also repress target genes via similar mechanisms to those used for activation of transcription, by direct DNA binding via negative GRE (nGREs) (Fig. E III and IV). This leads to recruitment of co-repressor proteins. Other mechanisms of repression are mediated via different DNA-bound transcription factors, such as in the repression of NF-kB action and via competition with a transcriptional activator for DNA-binding sites such as repression of FasL expression by NF-kB [36].

Non-genomic actions (also called cytoplasmic effects) of GCs by which they exert a number of rapid actions are independent of the regulation of the gene transcription at nuclear level [34 (Figure F)]. These GCs actions are exerted via membrane associated receptors and their signalling cascades. The anti-inflammatory GCs can modulate the activity and/or expression of various kinases and phosphatases, the phosphatidylinositol 3'-kinase (PI3K)/Akt pathway (caspase independent apoptosis), thus affecting the signalling efficacy toward the propagation of pro-inflammatory gene expression and mRNA stability. Conversely, phosphorylation of GR can affect GR ligand- and DNA-binding affinity, mobility, and cofactor recruitment, culminating in altered transactivation and transrepression capabilities of GR. [34, 37].

1.2.3 GC-mediated apoptosis

GCs can trigger apoptosis by an extrinsic (non-genomic actions of GCs) or intrinsic pathways (genomic actions of GCs), [Fig.F]. The extrinsic pathway is dependent on ligand (GCs) binding and subsequent activation of membrane-bound 'death signal 'receptors. Activating these receptors triggers the caspase cascade, resulting in apoptosis [38].

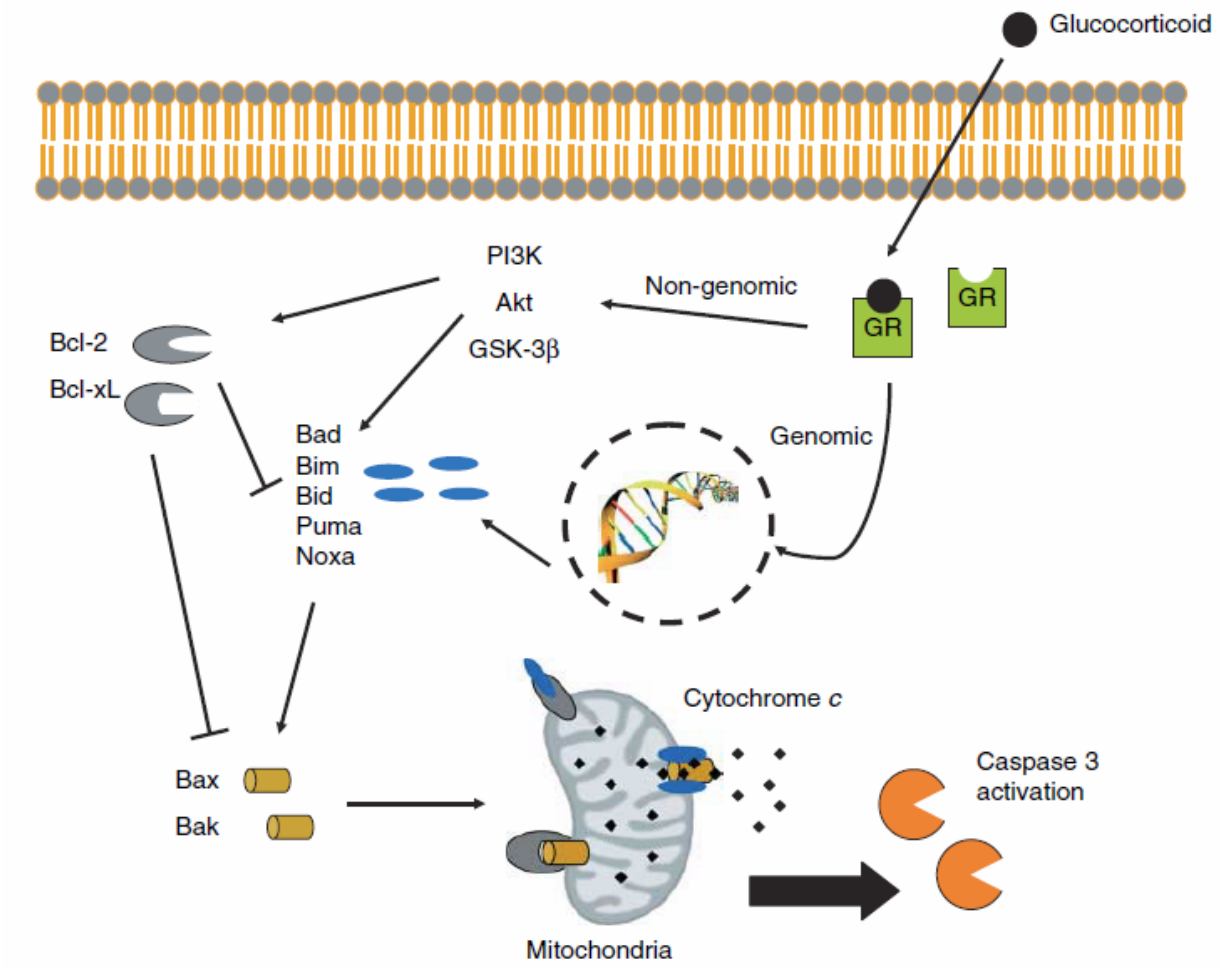

Figure F. Mechanisms of GC-mediated apoptosis [37]

The intrinsic pathway is more complex due to mitochondrial implication. This pathway is regulated by members of the Bcl-2 family consisting of pro- and anti-apoptotic proteins; they act as a "Bcl-2 rheostat" that controls many forms of apoptotic cell death. [39]. Through GC-signalling, the expression of the pro-apoptotic Bcl-2 family member Bim increases, which in cascade can activate the pro-apoptotic proteins Bax/Bak to disrupt mitochondrial membrane potential, resulting in the release of cytochrome c and other pro-apoptotic proteins. This leads to caspase activation, a family of proteins responsible for the degradation of targeted cells and apoptosis. Apoptosis can occur after the activation of the initiator caspase-8, which subsequently activates caspase 3 executors (extrinsic) or after the activation of the initiator caspase-9 (intrinsic), or through the interplay between these two pathways [40].

1.2.4 Resistance to anti-apoptotic effects of GC

Cellular drug resistance is an important cause of the poor prognosis for children with relapsed or refractory ALL, but it is uncertain to which drugs and to what extent resistance is present. Klumper and co-authors [41] determined *in vitro* resistance to 13 drugs, comparing the resistance to drugs of initial ALL cells to relapse/refractory ALL cells. They observed that relapsed cells were more resistant to glucocorticoids, L-asparaginase, anthracyclines, and thiopurines, but not vinca-alkaloids, or cytarabine. They argue that specific *in vitro* drug-resistance profiles were associated with high-risk ALL groups; and that drug resistance may explain the poor prognosis for children with relapsed/refractory ALL. Mechanisms contributing to glucocorticoid resistance include altered expression of GC receptor isoforms, altered GR expression levels, GR mutations, altered interactions with different kinases or deregulation of pro- or anti-apoptotic proteins [42, 43].

1.2.5 Corticosteroid-related complications

Given their broad physiological anti-inflammatory and immunomodulatory effects, the prolonged use of glucocorticoids in chemotherapy is complicated by numerous side effects (diabetes, obesity, increased susceptibility to bacterial and fungal infections, neuro- and bone toxicity). These GC side effects are dose and duration dependent. For example, cancer patients receiving high dose glucocorticoid therapy and prolonged therapy (>3 weeks) reported a higher incidence of toxicity compared with low doses and short duration of DXM [44, 45].

Bone toxicity, such as osteoporosis (low trauma fractures) and osteonecrosis (in situ bone death) are the major clinical consequences of ALL therapy [50]. Although glucocorticoids are known to induce apoptosis in chondrocytes, the mechanism for this effect is unknown. Inhibition of the PI3K pathway and subsequently Akt phosphorylation seem to be important mechanisms of dexamethasone-induced apoptosis. The PI3K/Akt pathway (caspase independent apoptosis) is viewed as a key player for cell survival in different cell systems [43].

1.3 PHARMACOGENOMICS

Pharmacogenomics studies the genetic variations of drug-metabolizing enzymes, receptors, transporters and how these genetic variations contributes to inter-individual differences observed in efficacy and toxicity of drugs.

1.3.1 Genetic and non-genetic source of variation

The therapeutic response to pharmacologic therapy is considerably modulated by genetic or non-genetic variations [Fig. G]. The genetic variations can be acquired in somatic cells or can be inherited as germline variations. Although, the somatic (acquired) mutations have been shown to be involved in the pathogenesis of a broad spectrum of human diseases, including cancer; the last decade has shown that inherited genetic variations (germline) are important determinants of inter-patient variability in ALL susceptibility, drug response, including efficacy and toxicity of ALL therapy.

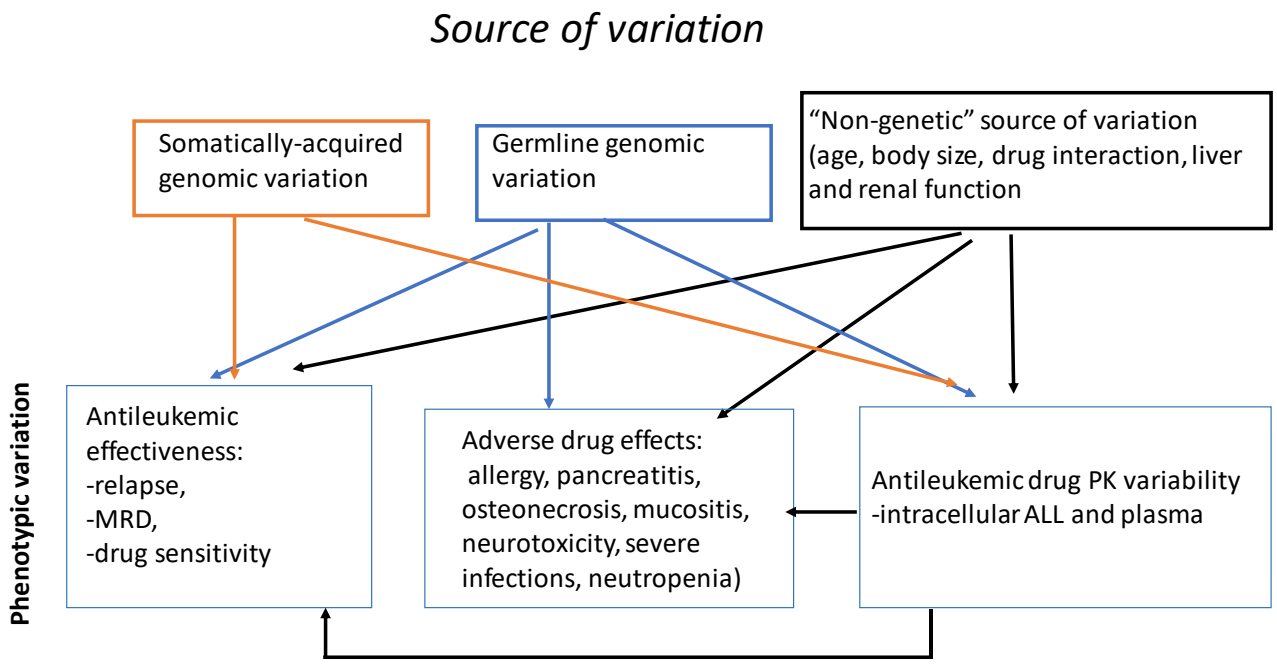

Figure G. Genomic variation and phenotypic implication in ALL response [46].

These variations can take a variety of forms and occur through different molecular mechanisms. In addition, ALL phenotypes can be affected by several non-genetic factors such as age, body size, drug interaction, organ function and they can affect intra-individual variability in therapeutic response. The non-genetic variability and inherited genetic variations can lead to adverse effects to drugs (allergy, pancreatitis, osteonecrosis, neurotoxicity) ultimately lowering antileukemic effects (relapse, MRD) [46, Fig. G].

1.3.2 Approaches for Genetic Variation Identification

Several approaches have been reported for identification of inherited genetic variations. The candidate gene approach was among the first strategies used, whereas later, progress in technology led to the development of new approaches such as genome-wide association studies (GWAS) and whole exome sequencing (WES) that allow broad multiple analysis to identify variants across the entire genome or exome.

1.3.2.1 Candidate gene approach

It relies on a prior hypothesis that the genetics variants (polymorphisms) modulating therapeutic response would be located in the gene that is relevant for the drug(s) action. The candidate gene association studies are usually based on the case-control design in which a detected genetic variation in cases is compared to controls. Cases are patients with the studied outcome and controls are patients that received the same treatment but did not suffer from the same outcome. If the frequency of a polymorphism in cases is higher than the one in controls, and statistic tests confirm association with a clinical outcome, then the variation is thought to confer a higher risk of a given outcome. Different genetic models can be considered in the analysis, such as recessive, dominant or additive, expressed in relation to derived (minor) allele. In the latter, individuals with two copies of the derived allele might be at a greater risk of studied outcome than individuals with a single copy of the variant allele. The candidate gene pharmacogenomics studies allowed identification of important genetic variants that have been reported to have clinical consequences. Genetic variants of interest were reported for thiopurine methyl transferase (TPMT) which metabolizes 6-mercaptopurine (6-MP) a key drug in ALL therapy

[47]. Patients who carry two non-functional TPMT alleles experience severe myelosuppression, if treated with standard doses of thiopurine. The TPMT-deficient patients require a reduction of 90% of the standard doses. A 50% reduction of the standard dose is required in heterozygous patients (found in 5-10% of the population) that have intermediate levels of enzyme activity. Other imported genetic variants identified through the candidate gene approach were described in genes coding for dihydrofolate reductase (DHFR) and thymidylate synthase (TS) acting in the methotrexate pathway; or in genes of vincristine and asparaginase pathways [48-50].

1.3.2.2 Genome-wide association studies (GWAS) approach

Genome-wide approaches have also been used to identify germline variations associated with ALL therapy [51-55]. GWAS, as opposed to the candidate gene approach, allows the examination of thousands of common genetic variants with no prior hypothesis of the gene involved, using commercially available SNP arrays from different ethnic populations, identifying number of variants associated with a trait. The GWAS are also case-control studies. The associated SNPs are considered to mark regions of the human genome that may influence the risk of the trait of interest. A fundamental way to report effect size of each genetic variant in case-control studies is the odds ratio (OR), the odds of disease for individuals who have a specific allele vs. the odds of disease for individuals who do not have that same allele. The statistical significance of an association is typically calculated using a chi-square test. For GWAS, a Manhattan plot is created that shows the negative logarithm of the p-value as a function of genomic location of each SNP studies. Importantly, the p-value threshold for significance is corrected for multiple testing. In general, a SNP with a p-value of 5×10^{-7} or higher is considered as significant statistical variant, suggesting its association with the disease [56]. GWA studies (and preferably all other association studies) typically perform the first analysis in a discovery cohort, followed by validation of the most significant SNPs in an independent validation cohort.

1.3.2.3 Whole exome sequencing (WES) approach

It is a method that targets only a subset of the genome, i.e. coding gene regions (representing about 2% of the genome). An advantage over whole genome sequencing approach in using this method is that beside the large number of variants identified in the coding region, it significantly reduces the sequencing space and therefore the costs. The sequencing approach may also be extended to target functional non-protein-coding elements, like microRNA, long intergenic noncoding RNA, as well as specific candidate loci [57, 58]. There are two main categories of exome capture technology: solution-based and array-based. In solution-based, DNA samples are fragmented and biotinylated oligonucleotide probes (baits) are used to selectively hybridize to target regions in the genome. Magnetic streptavidin beads are used to bind to the biotinylated probes, the non-targeted portion of the genome is washed away, and a DNA from the target region is amplified. Then, the sample is sequenced and analyses are performed filtering SNP against large public databases, as the dbSNP 1000 Genome Project [59] and HapMap (www.hapmap.org) [60]. Array-based methods are similar to solution-based ones, but less attractive because they require more input DNA [61].

1.3.3 Candidate genes studied

Recently, biological and genetic basis for ON development has been extensively investigated by several groups. They have been focused primarily on functional polymorphisms within genes involved in bone cell physiology (bone formation and resorption), vascular system (disruption of vascular supply to the bone, hypoxia and coagulation defects), oxidative stress, or on DNA oxidation injury in bone after steroids [54, 62, 63]. Several polymorphisms of candidate genes have been tested by Children's Oncology Group (COG) and they identified that children with *SERPINE1* GA/AA genotypes were significantly at risk to develop ON [63]. This gene encodes a protein known as plasminogen activator inhibitor-1 (PAI-1). Elevated PAI-1 is a risk factor for thrombosis. Another study from St Jude Children's Research Hospital showed that polymorphisms of acid phosphatase1 (ACP1) were associated with risk of Children's symptomatic ON, as well as with lower serum albumin and higher cholesterol levels [54].

It has been determined that common genetic variants, including single nucleotide polymorphisms (SNPs), influence susceptibility to ON. Therefore, in this study, we performed numerous association studies between genetic variation of common DNA polymorphisms in genes of corticosteroid pathway and ON development susceptibility. The genes of corticosteroid pathway studied in this project are presented in Table A-1, Annexe I, the role of associated gene is presented below for more details.

1.3.3.1 *BCL2L11* gene

The B-cell Chronic Lymphocytic leukemia Lymphoma like 11 gene (*BCL2L11*) is encoding Bim protein which is a member of the Bcl-2 family. Bim protein contains a BH3 homology domain required for apoptosis and a hydrophobic C-terminal domain required for its translocation to mitochondrial membrane where it may interact with other pro-apoptotic proteins [64]. *BCL2L11* has an important role in the default program of cell death by apoptosis, therefore, it may also be part of the mechanism of the osteonecrosis by increased osteoblast and osteocyte apoptosis. Many groups showed an increasing interest in *BCL2L11* polymorphisms and conducted their research to investigate whether *BCL2L11* deficiency may cause or predispose to disease development. The functional consequences of *BCL2L11*) polymorphisms are previously described, resulting in a decrease of leukemia cell apoptosis and in resistance to corticosteroid therapy [65].

1.3.3.2 *NFKB1* gene

The Nuclear Factor of Kappa light polypeptide gene enhancer in B-cells 1 (NFKB1) encodes two isoforms, a 50 kDa or 105 kDa protein, which are produced by co-translational processing. Due to the presence of the two isoforms, *NFKB1* appears to have dual function in the cells. When the 105 kDa protein is produced, it functions as a Rel protein-specific transcription inhibitor complex (RELA/p65). While, when a 50 kDa protein is produced, it functions as a DNA binding subunit of the NF-kappa-B protein (NFKB1/p50) complex [66]. NFKB1 is a transcription regulator that is activated by various intra- and extracellular stimuli such as cytokines. Activated NFKB1 translocates into the nucleus and stimulates the expression of

genes involved in a wide variety of biological functions. Inappropriate activation of NFKB1, due to alternative splicing variants, has been associated with a number of inflammatory diseases, while persistent inhibition of NFKB1 (for example by GC actions) leads to inappropriate immune cell development or delayed cell growth.

1.3.3.3 *PARP1* gene

Poly(ADP-ribose) Polymerase 1 gene (PARP1) is a well-characterized member of the PARP family of enzymes. It encodes a 116 kDa, poly (ADP-ribose) polymerase, a protein which catalyze poly (ADP-ribosyl)ation, a unique post translational modification in eukaryotic cells. By this process, the polymers of ADP-ribose (PARs) are formed and they are covalently attached via an ester linkage to glutamic acid of target proteins [67]. Poly (ADP-ribose) polymerase has three main domains: the DBD, the auto-modification domain (AMD), and the catalytic domain (NAD^+-binding domain) [68]. The central AMD encodes a 22 kDa protein C-terminus motif involved in DNA repair and gene transcriptional machinery, thus an important role maintaining genome integrity and facilitating cell survival. In addition, the poly(ADP-ribose) synthesis is important in many other cellular processes, such as chromatin replication, transcriptional regulation, and cell death; therefore, PARP-1 has a significant role in signalling mechanisms involved in inflammatory disorders.

1.3.3.4 *ABCB1* gene

The (ATP)-Binding Cassette subfamily B member 1 gene (ABCB1), also called multidrug resistance protein 1 (MDR1) gene, a member of the ATP binding cassette (ABC) family. This gene codes for the permeability glycoprotein (P-gp), which play an important role in the cell, in the efflux of exogenous (such drugs) molecules from the cell, preventing their accumulation into the cells. P-gp is therefore, an uptake-drug regulator for a large number of drugs, including GC [69], which makes this gene of interest. Many polymorphisms have been reported for this gene, rs2032582 (G2677T variant of exon 21), associated with increased risk of lung cancer, and rs1045642 (C3435T variant) a silent polymorphism involved in substrate change specificity [70, 71].

1.3.3.5 *ACP1* gene

The Acid Phosphatase 1 gene (*ACP1*), encode a product that belongs to the phosphotyrosine protein phosphatase family. It functions as an acid phosphatase and a protein tyrosine phosphatase by hydrolyzing protein tyrosine phosphate to protein tyrosine and orthophosphate. This gene is genetically polymorphic, multiple alternatively spliced transcript variants encoding distinct isoforms have been identified for this gene. The *ACP1*, rs12714403 has been reported as a polymorphism which regulates lipid levels and osteoblast differentiation. It was associated with higher risk of osteonecrosis as well as with lower albumin and higher cholesterol levels [54].

1.3.3.6 *SHMT1* gene

The Serine Hydroxy Methyltransferase 1 (*SHMT1*) encodes a protein known as the pyridoxal-5′-phosphate-dependent enzyme (PLP enzyme) that catalyzes myriad of biochemical reactions in prokaryotic and eukaryotic cells. PLP plays an important role in cellular one-carbon pathways by catalyzing the reversible, simultaneous conversions of L-serine to glycine and tetrahydrofolate (THF) to 5,10 methylenetetrahydrofolates. This reaction provides the largest part of the one-carbon units available to the cell [72].

1.3.4 Genetic association studies

Several studies of direct associations (also called candidate gene association studies) have been reported in which the target polymorphisms are themselves putative causal variants. This type of association study is the most common and powerful, but it is difficult to use for all types of variants, such as the synonymous or the non-coding variants. Therefore, indirect association studies have been developed; the polymorphism used in association is a surrogate (also called marker) for the causal locus. These types of association studies allowed the screening of a large number of SNP across the whole genome and search for disease susceptibility genes. The mapping of susceptibility genes for common complex disorders by the indirect association method depends on the existence of association, at the population level, between the causal

variants and nearby markers. Such association, because of the proximity of loci on the genome, is termed linkage disequilibrium (LD) [73].

1.3.5 Models and measures of association

The study design often used for these association studies is a population-based genetic association case-control or cohort designs. The data obtained are statistically analyzed by the presence or absence of a genetic polymorphism risk. A genetic risk can be considered in terms of either a predisposing allele or genotype to disease or of an event occurring in one group relative to another. To give an example of an SNP, if we consider a genetic marker consisting of a single biallelic locus with alleles *a* and *A*. The risk factors for the case versus control status (disease outcome) is the genotype or allele at a specific marker. The disease penetrance associated with a given genotype is the risk of disease in individuals carrying that genotype.

The association models often used for disease penetrance that implies a specific relationship between genotype and phenotype include recombinant, recessive, dominant or additive models. The recombinant model consists of the three-possible unordered genotype for the 2 alleles, such as *a/a*, *a/A* and *A/A*. Assuming a genetic penetrance parameter γ (γ > 1), a multiplicative model indicates that the risk of disease is increased γ-fold with each additional A allele. An additive model indicates that risk of disease is increased γ-fold for genotype a/A and by 2γ fold for genotype A/A. A common recessive model indicates that two copies of alleles A are required for a γ-fold increase in disease risk. A common dominant model indicates that either one or two copies of alleles A are required for a γ-fold increase in disease risk [74].

A commonly used and intuitive measure of the strength of the association is the odd ratio (OR), which compares the disease penetrance between individuals exposed to different genotype.

1.4 HYPOTHESIS AND OBJECTIVES

Corticosteroids (CS) are one of the principal components of ALL treatment and are administered, either as prednisone or dexamethasone, through all phases of treatment. They are

essential for the efficacy of the treatment, but are also associated with drug side effects, such as bone morbidity, infections or alterations in glucose and lipid metabolism. Some patients are more prone to develop such treatment complications. Patient genetic background may contribute to the variability in treatment responses.

To improve patient care and outcomes, and reduce the adverse drug reactions (ADRs), a comprehensive understanding of the contribution of genetic signatures to therapeutic responses in childhood ALL is required. Therefore, the main goal of this project was to identify polymorphisms that may contribute to toxicity related to corticosteroid treatment in childhood ALL patients, notably to corticosteroid-related symptomatic osteonecrosis.

Our strategy was to analyze the available data of candidate genes obtained either through genotyping of genes involved in CS action, or through the WES to identify germline variations associated with CS-related treatment complications. In addition, we included in our study, certain polymorphisms identified through GWAS study that were previously reported to be involved in CS-induced osteonecrosis. Moreover, revision of patients' medical files was performed to retrieve and complete the information regarding CS-related ADRs including osteonecrosis.

CHAPTER 2: PRESENTATION BY ARTICLE (submitted to Pharmacogenomic J., **Revision is invited and will be submitted by 9th of July)**

2.1 INFLUENCE OF *BCL2L11* POLYMORPHISM ON OSTEONECROSIS DURING TREATMENT OF CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

Maria Plesa[1,3], Vincent Gagné[1], Sanja Joksimovic Glisovic[1], Melissa Younan[1], Bahram Sharif-Askari[1], Caroline Laverdière[1,2], Nathalie Alos[1,2], Jean-Marie Leclerc[1,2], Stephen E Sallan[4,5], Donna Neuberg[6], Jeffery L Kutok[7], Lewis B Silverman[4,5], Daniel Sinnett[1,2] and Maja Krajinovic[1-3*]

[1] Charles-Bruneau Cancer Center, CHU Sainte-Justine Research Center, Montreal, QC,

[2] Department of Pediatrics, Faculty of Medicine, University of Montreal

[3] Department of Pharmacology, Faculty of Medicine, University of Montreal

[4] Department of Pediatric Oncology, Dana-Farber Cancer Institute, Boston, MA, USA

[5] Division of Hematology/Oncology, Children's Hospital, Boston

[6] Department of Biostatistics & Computational Biology, Dana-Farber Cancer Institute, Boston

[7] Department of Pathology, Brigham and Women's Hospital, Boston

*** To whom correspondence should be addressed:**

Maja Krajinovic, Centre de Recherche, CHU Sainte-Justine, 3175 chemin de la Côte-Ste-Catherine, Montréal, QC, H3T 1C5 Canada

E-mail: maja.krajinovic@umontreal.ca

Running title: *BCL2L11* polymorphism implication in osteonecrosis

Keywords: Acute Lymphoblastic Leukemia (ALL), Dexamethasone (DXM), Prednisone (PDN), B-cell chronic lymphocytic leukemia lymphoma like 11 gene (*BCL2L11*), Bcl-2-like protein 11 (Bim), SNP (Single Nucleotide Polymorphism), Osteonecrosis (ON), Lymphoblastic cell lines (LCLs), Quebec childhood Acute Lymploblastic Leukemia (QcALL) cohort, Dana-Farber Cancer Institute (DFCI) cohort.

Abstract

Osteonecrosis (ON) is corticosteroid-related complication, reported in children with acute lymphoblastic leukemia (ALL). We previously found that *BCL2L11* polymorphisms influence reduction of overall survival (OS) in a corticosteroid (CS) dose-dependent manner in childhood ALL patients. The same set of SNPs in *BCL2L11* gene coding for pro-apoptotic Bim protein was here investigated for an association with CS-related ON retrospectively assessed in 304 children with ALL from Quebec (QcALL cohort) who received Dana-Farber Cancer Institute (DFCI) ALL treatment protocols. Two-year cumulative incidence of symptomatic ON was 10.6%. Two *BCL2L11* polymorphisms, the 891T>G (rs2241843) in all QcALL patients and 29201C>T (rs724710) in high-risk group were significantly associated with ON, P = 0.009 and P = 0.003, respectively. The association remained significant in multivariate model (HR_{891TT} = 2.4, 95% CI 1.2 - 4.8, P = 0.01 and C>T ($HR_{29201CC}$ = 5.7, 95% CI 1.6 – 20.9, P = 0.008). Both polymorphisms influenced viability of dexamethasone treated lymphoblastoid cell lines ($P \leq 0.05$). The 891T>G influenced *Bim* gamma isoform levels (0.02) and its association with ON was also confirmed in replication DFCI cohort (N = 168, p=0.03). QcALL children had a high incidence of ON during therapy, which was highly associated with *BCL2L11* polymorphisms.

Introduction

Osteonecrosis (ON) is one of the major complications of childhood acute lymphoblastic leukemia (ALL) treatment (2-4). It can be symptomatic and cause severe pain, joint damage, articular collapse or it can remain asymptomatic and cause no disabilities. Although ON induced by chemotherapy appears to have multiple causes, the corticosteroids (CS), are considered one of the main culprits (5-9). CS, like prednisone (PDN) and dexamethasone (DXM) are widely used in ALL protocols (2, 10-12) for their capacity to induce apoptosis of leukemia cells. Beside their implication in ON, it has been demonstrated that CS are also involved in osteoporosis and vertebral fractures due to their direct action on osteoclasts/osteoblasts by change in their number or function, eventually leading to bone loss by increased bone resorption (13-15). Cellular apoptotic effect of CS is essentially mediated through glucocorticoid receptors (GRs), a ligand-activated transcription factor, ubiquitously expressed in various types of cells (16), including osteoblasts and osteocytes (17). GR-ligand complexes translocate to the nucleus and initiate a transcriptional up-regulation of pro-apoptotic genes in a cell- and gene- specific manner (18, 19). At physiological concentrations, the CS are key regulators of bone development and cell differentiation. However, CS may have an unfavorable effect when used at higher concentrations and for a prolonged time. For example, the GR is reversibly down regulated in such cases, as a part of physiological feedback loop or up-regulated as a part of a positive autoregulation mechanism (20, 21).

Other drugs used in ALL therapy may also contribute to the development of osteonecrosis. While DXM increases concentration of many coagulation proteins, asparaginase can reduce their synthesis. Therefore, unbalanced levels of coagulation proteins may also be important in the pathogenesis of ON (5). The risk of ON may be influenced by clinical prognostic factors, like age at diagnostic higher than 10 years (2), female gender (3) and higher body mass index (22). Recently, the genetic polymorphisms have been linked to the ON development (23) and have been identified through candidate gene approaches and genome-wide association studies (GWAS) (24-27).

Several genes may interact with the action of CS. Microarray profiling in CS-induced apoptosis models, has shown that CS exposure induces or represses the transcription of pro- or anti-apoptotic genes. (28). Among these, *BCL2L11* gene encoding Bim protein, was found to be up-regulated by CS. Bim protein is a member of the Bcl-2 family, it contains a BH3 homology domain required for apoptosis and a hydrophobic C-terminal domain required for its translocation to mitochondrial membrane where it may interact with other pro-apoptotic members (29). An increase in the mRNA and Bim isoforms in ALL cells pre-exposed to DXM (30) has been shown suggesting that the level of Bim expression in leukemia cells may be an important determinant of the sensitivity of ALL cells to CS-induced apoptosis. *BCL2L11* has been also described as a key regulator of osteoblast apoptosis; it is reported as induced by DXM in a dose- and time-dependent manner in murine osteoblasts, as well as in primary human bone marrow stromal cells (31).

Eight tag SNPs in *BCL2L11* were previously described by our group (1) in the context of genetic association analysis with ALL event-free survival (EFS) and overall survival (OS). Our present study aims to establish whether polymorphisms in *BCL2L11* gene may affect the risk of ON in childhood ALL. The study was conducted in 2 independent patients' cohorts. In addition, functional effect of associated polymorphisms was assessed through their impact on mRNA levels and cellular proliferation in cultured lymphoblastoid cell lines (LCLs).

Patients and Methods

***Study population*.** Discovery (QcALL) cohort consisted of 304 Caucasian children from Quebec diagnosed with ALL at the University Health Center (UHC) Sainte Justine, Montreal, Canada, for whom both genotype and clinical data were available. Patients were treated in accordance with Dana-Farber Cancer Institute ALL Consortium protocols (DFCI 87-01, 91-01, 95-01, or 00-01) between January 1987 and July 2005. The replication (DFCI) cohort consisted of 168 Caucasian patients with available clinical and genotype data who underwent treatment with DFCI 00-01 protocol in the 9 remaining DFCI Consortium Institutions. Detailed description of these cohorts and DFCI treatment regimens is provided elsewhere (1, 10-12, 32). Patient characteristics are presented in **Table 1**.

Information on symptomatic osteonecrosis (NCI grades 2-4, Table 2) was collected from patients medical files and was defined as persistent bone pain or motor function limitations associated with ON lesion confirmed by radiological examination such as X-ray radiography, computed tomography (CT) scan or magnetic resonance imaging (MRI). ON was graded in accordance with the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events, version 3.0 (33).

***Genotyping*.** The eight tag SNPs (rs2241842, rs2241843, rs73954926, rs72836346, 7582030, rs724710, rs72836345, rs6750142), previously described by our group (1), located in regulatory and coding regions of *BCL2L11* have been investigated for the association with symptomatic ON in QcALL cohort. Positively associated SNP was analyzed in replication DFCI cohort. The genotypes were either already available or obtained as previously described using PCR - coupled Allele Specific Oligonucleotide (ASO) hybridization assay (1, 34).

***Statistical analysis*.** Association of genotype with the presence of ON (at least one incident during the 2 years of therapy) was assessed by the chi-square test in all patients and in patients assigned to the high risk (HR) group due to higher CS doses administered in these patients. False discovery rate (FDR) correction was performed to adjust for multiple testing (35); significant results at FDR< 0.05 were retained. Cumulative incidence during 2-years of ALL treatment was estimated in Kaplan-Meier survival analysis framework. Hazard ratio (HR) and a 95% confidence interval (95% CI) was estimated by Cox's regression analysis in uni-variable or multi-variable model. The common prognostic risk factors, including age, sex, CS type and risk groups were analyzed beside genotype in the multi-variable models. Statistical analysis was performed by SPSS statistical package (Chicago, IL), version 22.0.

***Cellular proliferation assay and Bim expression studies*.** *In vitro* cellular viability following addition of DXM and PDN to the cultured lymphoblastic cell lines (LCLs) was previously described by Gagné V. *et al*. (1). The assay was here extended to include larger number of LCLs and it was performed in 72 and 92 LCLs exposed to DXM and PDN, respectively. The inhibitory effect on the LCLs viability was determined by the addition of different concentrations of dexamethasone (2.8 x 10^{-3} to 560 µmol/L) and prednisolone (0.75 to 750 µmol/L). Quartile

distribution of inhibitory concentrations (IC50) was used to compare the difference in cell viability between genotypes significantly associated with ON, using chi-square analysis. Expression of total Bim mRNA and its isoforms, previously obtained by quantitative and semi quantitative mRNA analysis (1) in LCLs following CS exposure, was compared between genotypes using quartile distribution and chi- square test.

Results

Thirty-two patients were diagnosed with symptomatic ON in QcALL cohort. Symptoms consisted of pain, joint rigidity and limp with the involvement of one or several sites (mainly femoral head, followed by the ankle, knee, wrist or elbow). The diagnosis was based on X-rays (n=23), CT scans (n= 4) or MRI (n=5), **Table 2.** Most patients developed ON during consolidation phase (n=23) and few patients had been diagnosed during induction (n =4) or intensification (n=5). The cumulative overall incidence of symptomatic ON during 2-years of therapy was 10.5%. Slightly higher (but not significantly different) frequency of ON was seen in patients that received DXM or were older than 10 years (Table 2).

Association analysis of eight tag SNPs with ON (**Supplemental Table 1**) revealed a significant association of two polymorphisms following multiple testing adjustment, 891T>G (rs2241843) in all patients (p=0.009, Supplemental Table 1A) and 29201C>T (rs724710) in high-risk group (p=0.003, Supplemental Table 1B). Patients with 891TT genotype had more frequently ON compared to other groups, reflected also by higher cumulative incidence of ON (HR = 2.4, 95% CI = 1.2-4.8, P = 0.01, **Figure 1A**). Similar increase in cumulative ON incidence was noted for CC genotype of 29201C>T, which was particularly apparent in patients of high-risk group (P = 0.003, HR=5.5, 95% CI =1.5-19.8, **Figure 1B**). The association of 891T>G polymorphism with ON remained significant in Cox regression multivariable model when adjusted for covariables such as age, sex, risk group or CS types (P = 0.01; HR = 2.4; 95 % CI, 1.2-4.8, **Table 3A**). In this model, the genotype was the only factor predictive of ON. The 29201C>T polymorphism remained significantly associated with ON in high-risk group multivariate model, in which CC genotype, DXM and age above 10 years were all associated with higher cumulative incidence

of ON (HR=5.7, 95% CI 1.6 – 21.9 P = 0.008, HR = 5.9; 95 % CI, 1.8-19.9, P = 0.004, HR = 3.0; 95 % CI, 1.0-9.2, P = 0.05, respectively, **Table 3B**).

We next investigated whether 891T>G and 29201C>T may affect in *vitro* sensitivity to CS or Bim mRNA levels following exposure of LCLs to CS. DXM exposed cells with 891TT genotype had lower IC50 (P = 0.05, **Figure 2A**) suggesting higher sensitivity to DXM compared to G allele carriers. Likewise, the 29201CC genotype was associated with lower IC50 values (p=0.02, **Figure 2B**). We previously reported non-significant decrease in Bim γ isoforms resulting from lower γ1 to γ2 ratio associated with 29201CC genotype (1). A similar effect was noted here when 891T>G was analyzed with Bim mRNA and its isoforms, whereby DXM pre-treated LCLs with TT genotype had significantly lower total γ mRNA levels (P =0.02, **Figure 2C**).

Two polymorphisms were further analyzed for an association with ON in the replication DFCI cohort. There was a higher frequency of 891TT genotype among patients who developed ON compared to G allele carriers (57 % vs 43 %, P = 0.03, **Figure 3**), whereas an association with 29201C>T did not reveal significant results.

Discussion

Most symptomatic ON cases reported in our study occurred in the continuation phase, but we also observed ON development during induction therapy, which is in accordance with a previous hypothesis (3, 4) that some subjects may manifest a precondition and develop early symptoms of bone morbidity.

Overall cumulative incidences of symptomatic ON related to ALL chemotherapy vary widely among the studies (3, 4, 22) ranging from 1% (4) to 11% (17). It seems dependent on other clinical risk predictors and was reported to be higher in girls, patients older than 10 years (2, 9, 36) and in those that received higher cumulative doses of corticosteroids (4, 22). We noticed an increase in frequency, although non-significant, in patients older than 10 years and those that

received DXM, whereas in high-risk patients, both factors were significant predictors of symptomatic ON in multivariate model.

In our attempt to determine the genetic component of ON, we investigated implications of the eight tag SNPs that we previously described in *BCL2L11* gene (1). *BCL2L11* is involved in the default mechanism of apoptosis in normal and malignant cells through GR-CS mediated up-regulation. Functional defects in Bim protein isoforms may have consequences at cellular level, including increased osteoblast and osteocyte apoptosis, as previously described by Espina *et al*., 2008 (31). Among SNPs investigated, two polymorphisms, 29201C>T and 891T>G, seem to influence the risk of ON development. The 891T>G is a potential regulatory SNP, since it is located in the 5'UTR of *BCL2L11* gene; The 5'UTR regions contain important cis-regulatory elements and their disruption or introduction could affect gene expression and protein abundance (37). Indeed, 891T>G is predicted to affect two transcription factor binding sites. The TT genotype was associated with increased risk of symptomatic ON; it remained significant ON predictor in multivariate model and was successfully replicated in the DFCI cohort. Furthermore, the TT genotype conferred higher sensitivity to DXM in LCLs that could explain higher incidence of adverse effect seen in patients with this genotype. Several studies have shown relationship between cellular sensitivity to DXM and Bim expression (38, 39). We did not notice the significant change in the total Bim levels in relation to 891T>G polymorphism. However, LCLs with TT genotypes had lower levels of γ isoform without apoptotic BH3 domain. Nevertheless, it is worth noting that we used one cell type and a single DXM concentration (0.28 μM) whose effect on mRNA levels was measured 48h after the DXM challenge. It is possible that the effect may vary under different conditions given that the expression of mRNA is cell-dependent and influenced by time and DXM-dose used (31, 40). In human osteoblast cells, induction of Bim expression was observed at low DXM concentration (1nM) to reach a maximum of induction at 1μM with higher doses resulting in a reduction of mRNA levels (31, 40).

The 29201C>T is a synonymous polymorphism, located in exon 8 of *BCL2L11* gene, which was predicted to affect transcriptional exonic splicing (1). Similarly, to other studies in which synonymous SNPs are reported to affect mRNA splicing, stability, and protein folding (41), we

previously reported that in DXM pretreated LCLs, the T allele affected γ isoforms formation resulting in higher γ1 to γ 2 ratio (1), which could have explained lower sensitivity of TT genotype to CS and reduced OS in ALL patients (1). In line with this observation is a recent study, which reported T allele–related decrease in basal expression of the Bim mRNA and higher T allele frequency in non-responsive chronic myeloid leukaemia patients treated with imatinib. (42). The CC genotype is in contrast expected to confer higher sensitivity to treatment, indeed shown in our study through an association with increased ON risk mainly in high risk QcALL group who received higher CS doses. This finding was further supported by higher in vitro sensitivity to DXM shown by cellular proliferation assay in LCLs. The association with ON was not, however, replicated in DFCI cohort, which might be due to lower sample size and lower incidence of ON in this cohort. DFCI cohort was composed only of patients treated with 00-01 protocol in which high-risk patients received lower CS doses during maintenance compared to earlier DFCI protocols. It is worth noting that TT891 genotype is always accompanied by CC29201 genotype, so it is possible that diplotypes with these genotype combinations are contributing to observing functional effect and higher symptomatic ON incidence, whereas CC29201 alone is not sufficient to modulate ON risk. Several limitations of our study should be nevertheless mentioned. The incidence of symptomatic ON data was collected retrospectively, based on patient medical records, and a reporting bias (under- or over-estimation of cases) is possible. Stratified analyses were underpowered due to low numbers of patients per group, which was more evident in replication cohort.

Among other *BCL2L11* polymorphisms reported in the literature, the most studied is an intronic deletion polymorphism (43-46) that affect splicing resulting in expression of Bim isoforms lacking the pro-apoptotic BH3 domain. This polymorphism is only present with high frequency in the Asian population and was reported associated with therapy resistance or early relapse in chronic myeloid leukemia or lung cancer patients treated with tyrosine kinase inhibitors (42-45). Two intronic *BCL2L11* SNPs (rs6746608 and rs1261324) were also reported as associated with decreased risk of non-Hodgkin lymphoma (46).

Candidate genes and genome-wide association studies (GWAS) identified several polymorphisms underlying ON development in ALL patients (24-27), including the plasminogen activator inhibitor-1 (*PAI-1*) rs6092 SNP (27); the rs10989692 in the glutamate receptor subunit 3A (*GRIN3A*) gene (25) and rs12714403 at the *SH3YL1*-acid phosphatase-1 (*SH3YL1-ACP1*) locus (26). Glutamate receptor variants were associated with vascular phenotypes, like cerebral ischemia, arterial embolism and thrombosis (25), whereas *ACP1* gene plays a role in lipid levels regulation and osteoblast differentiation (24). Two additional SNPs were identified though GWAS conducted in children younger than 10 years, rs75161997 that is polymorphic in non-Caucasians and located near the bone morphogenesis protein 7 (*BMP7*) gene, and rs1891059 at PROX1-antisense RNA1 (*PROX1-AS1*) locus. (24).

In conclusion, our study identified additional pharmacogenetics markers in *BCL2L11* gene contributing to ON development in childhood ALL patients. The TT891 on the background of CC29201 genotype increased the risk of ON during the treatment. The finding was further supported by increased in vitro cellular sensitivity to CS associated with these genotypes coupled with lower expression of gamma Bim mRNA isoforms, suggesting a shift toward other BH3-containing isoforms which could induce osteoblast apoptosis driving ON.

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Authorship and conflict of interest statements. Contribution: M.K. designed the study; M.P., V.G., M.Y. and B. S-A. performed experiments; M.P. and S.J-G performed medical chart reviews; C.L. JM.L., N.A, S.E.S., D.N. J.K, L.B.S and D.S. contributed to sample and clinical data collection and interpretation; M.P., V.G. and M.K. performed the data analysis; M.P. and M.K. drafted the article; All authors contributed to the interpretation of data and revised the manuscript. The authors declare no competing financial interests.

Table 1. Characteristic of ALL patients in the discovery (QcALL) and validation (DFCI) cohort

Characteristic	Number of subjects and frequency (%)	
	QcALL(n=304)	**DFCI(n=168)**
Sex		
Female	136 (44.7)	80 (47.6)
Male	168 (55.3)	88 (52.4)
Age, years		
< 10	245 (80.6)	137 (81.5)
≥ 10	59 (19.4)	31 (18.5)
Risk groups		
Standard risk (SR)	152 (50.0)	103 (61.3)
High risk (HR)	152 (50.0)	65 (38.7)
Corticosteroids		
Prednisone (PDN)	202 (66.4)	91 (54.2)
Dexamethasone (DXM)	102 (33.6)	77 (45.8)
Treatment protocol		
87-01	17 (5.6)	
91-01	55 (18.1)	
95-01	114 (37.5)	
00-01	118 (38.8)	168 (100)
Osteonecrosis		
Yes	32 (10.5)	7 (4.2)
No	272 (89.5)	161 (95.8)
Total	**304 (100.0)**	**168 (100.0)**

Table 2. Characteristics of ALL patients with or without symptomatic osteonecrosis in QcALL cohort.

Patient and treatment characteristics	No (%) of subjects	No (%) of subjects with ON
ALL treatment phase		
induction		4
intensification		5
consolidation		23
Involved site		
Single site		20
Multiple sites		12
Diagnostic study		
X-ray/spine radiographs		23
CT scan		4
MRI		5
Sex		
Female	136 (44.7)	15 (11.0)
Male	168 (55.3)	17 (10.1)
Age, years		
< 10	245 (80.6)	24 (9.8)
≥ 10	59 (19.4)	8 (13.6)
Risk groups		
Standard risk (SR)	152 (50.0)	18 (11.8)
High risk (HR)	152 (50.0)	14 (9.2)
Corticosteroids		
Prednisone (PDN)	202 (66.4)	19 (9.4)
Dexamethasone (DXM)	102 (33.6)	13 (12.7)
Treatment protocol		
87-01	17 (5.6)	0 (0.0)
91-01	55 (18.1)	7 (12.7)
95-01	114 (37.5)	10 (8.8)
2000-01	118 (38.8)	15 (12.7)
Total	**304 (100.0)**	**32 (100.0)**

Table 3. Risk of osteonecrosis in QcALL patients associated with *BCL2L11* 891T>G (A) and 29201C>T (B) polymorphism in Cox regression multivariate model.

A

QcALL cohort: all individuals			
Variable	HR	95% CI	*P*
TT891	2.4	1.2 - 4.8	0.01
Age, > 10 years	2.2	0.8 - 6.0	0.2
Corticosteroid, DXM	1.6	0.8 - 3.4	0.2
Sex, Female	1.1	0.6 - 2.3	0.7
High risk	0.5	0.2 - 1.2	0.1

B

QcALL cohort: High risk individuals			
Variable	HR	95% CI	*P*
CC29201C	5.7	1.6-20.9.0	0.008
Age, > 10 years	3.0	1.0-9.2	0.05
Corticosteroid, DXM	5.9	1.8-19.9.	0.004
Sex, Female	1.8	0.6-5.3	0.3

P value, HR (hazard ratio) and 95% CI (confidence interval).

Figure legend

Figure 1. A, Cumulative incidence of osteonecrosis in QcALL cohort in relationship to *BCL2L11* polymorphisms 891T>G in A; and 29201C>T in B

Total number of patients per genotype (number of patients with ON in brackets) is indicated next to each curve. P value obtained by the log-rank test and genotype-associated hazard ratio (HR) with 95% confidence interval (CI) are indicated.

Figure 2. Frequency of *BCL2L11* genotypes in relation to cellular viability and expression level in DXM pretreated LCL.

In vitro sensitivity to DXM in relation to 891T>G (A) and 29201C>T (B); level of gamma Bim isoform in relation to 891T>G (C). IC50 and Bimγ expression is presented as interquartile (IQR) range. The frequency and number of individuals (in brackets) presented by each genotype (black and gray part of the bar), and *p* value for the difference between groups, estimated by chi-square, is indicated on the plots.

Figure 3. The frequency of 891 T>G *BCL2L11* genotype in relation to osteonecrosis in replication cohort

The bars represent the frequency of TT genotypes versus other genotype group in patients of DFCI replication group with and without osteonecrosis (+ and -, respectively). The number of patients represented by each bar, p value for the difference between genotype groups and odds ratio (OR) with 95% confidence interval (CI) are indicated.

Figure 1. Cumulative incidence of osteonecrosis in QcALL cohort in relationship to *BCL2L11* 891T>G polymorphism (A) and 29201C>T polymorphism (B)

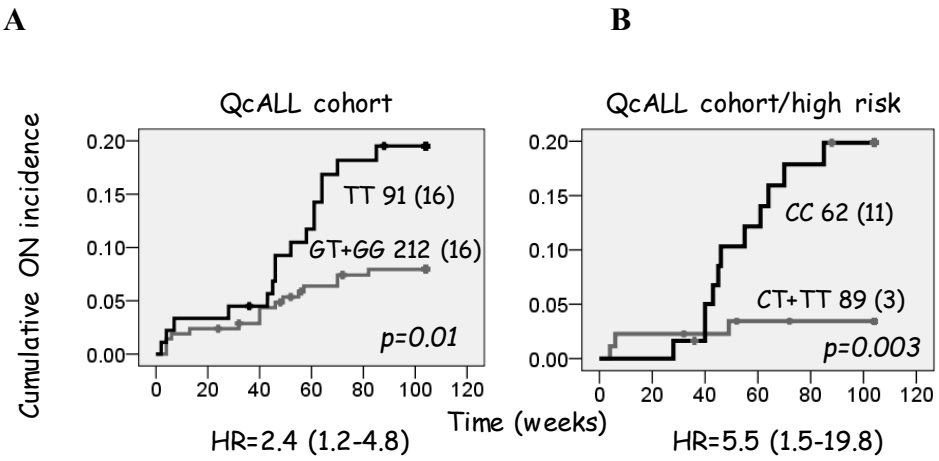
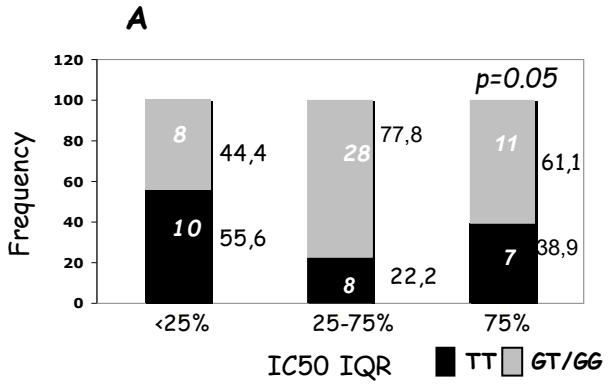
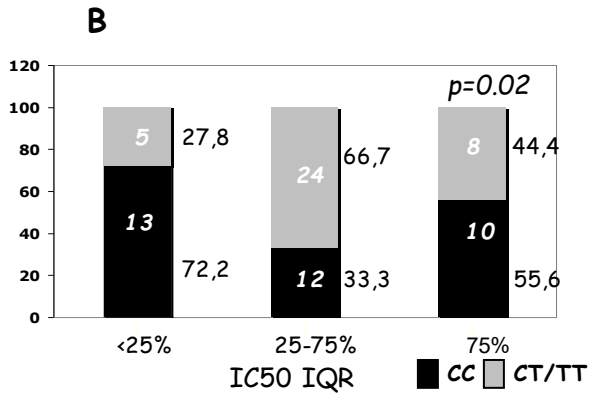


Figure 2. *In vitro* sensitivity to DXM in relation to 891T>G (A) and 29201C>T (B); level of gamma Bim isoform in relation to 891T>G (C).





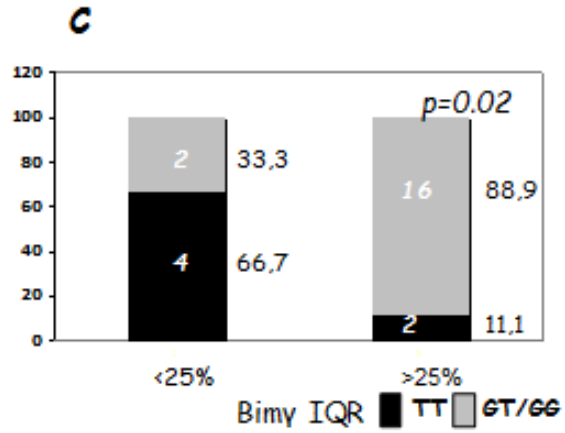
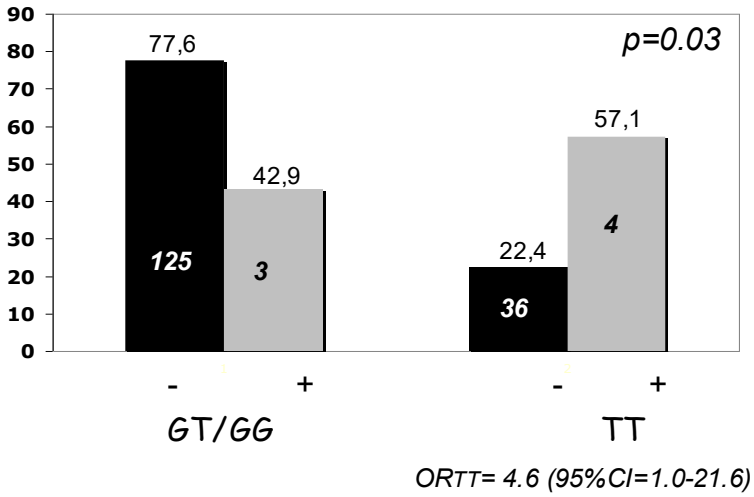


Figure 3. The frequency of 891 T>G *BCL2L11* genotype in relation to osteonecrosis in replication cohort



Supplemental Table 1A. Association of *BCL2L11* tag SNPs with osteonecrosis in all patients of QcALL cohort.

No stratification QcALL patients							
	Polymorphism			No of subjects, frequency (%)			
dbSNP	**Position**	**Genotype**	**Total**	**ON+**	**ON-**	***P***	***P1***
rs2241842	952	CC	68	12 (37.5)	56 (20.6)	0.08	0.3
T/C		CT	144	13 (40.6)	131 (48.2)		
		TT	92	7 (21.9)	85 (31.2)		
		Total	**304**	**32 (100.0)**	**272 (100.0)**		
rs2241843	**891**	GG	76	6 (18.8)	70 (25.8)	0.03	**0.009**
T/G		GT	136	10 (31.2)	126 (46.5)		
		TT	91	16 (50.0)	75 (27.7)		
		Total	**303**	**32 (100.0)**	**271 (100.0)**		
rs73954926	-1316	GG	2	0 (0.0)	2 (0.8)	0.7	0.6
T/G		GT	35	5 (15.6)	30 (11.3)		
		TT	260	27 (84.4)	233 (87.9)		
		Total	**297**	**32 (100.0)**	**265 (100.0)**		
rs72836346	-1878	CC	3	0 (0.0)	3 (1.1)	0.8	0.9
G/C		GC	35	4 (12.5)	31 (11.8)		
		GG	257	28 (87.5)	229 (87.1)		
		Total	**295**	**32 (100.0)**	**263 (100.0)**		
rs7582030	-274	AA	6	0 (0.0)	6 (2.3)	0.6	0.8
G/A		GA	71	9 (28.1)	62 (23.6)		
		GG	218	23 (71.9)	195 (74.1)		
		Total	**295**	**32 (100.0)**	**263 (100.0)**		
rs724710	29201	CC	128	19 (59.4)	109 (40.2)	0.09	0.04
C/T		CT	133	11 (34.3)	122 (45.0)		
		TT	42	2 (6.3)	40 (14.8)		
		Total	**303**	**32 (100.0)**	**271 (100.0)**		
rs72836345	-1952	CC	4	0 (0.0)	4 (1.5)	0.08	0.05
T/C		TC	52	10 (31.2)	42 (15.7)		
		TT	244	22 (68.8)	222 (82.8)		
		Total	**300**	**32 (100.0)**	**268 (100.0)**		
rs6750142	45139	AA	200	17 (53.1)	183 (67.3)	0.08	0.1
A/T		AT	94	15 (46.9)	79 (29.0)		
		TT	10	0 (0.0)	10 (3.7)		
		Total	**304**	**32 (100.0)**	**272 (100.0)**		

Supplemental Table 1B. Association of *BCL2L11* tag SNPs with osteonecrosis in patents assigned to high risk group, QcALL cohort.

High riskQcALL patients							
Polymorphism			No of subjects, frequency (%)				
dbSNP	**Position**	**Genotype**	**Total**	**ON+**	**ON-**	***P***	***P1***
rs2241842	952	CC	33	5 (35.7)	28 (20.3)	0.3	0.2
T/C		CT	75	7 (50.0)	68 (49.3)		
		TT	44	2 (14.3)	42 (30.4)		
		Total	**152**	**14 (100.0)**	**138 (100.0)**		
rs2241843	891	GG	36	2 (14.3)	34 (24.8)	0.1	0.04
T/G		GT	66	4 (28.6)	62 (45.3)		
		TT	49	8 (57.1)	41 (29.9)		
		Total	**151**	**14 (100.0)**	**137 (100.0)**		
rs73954926	-1316	GG	1	0 (0.0)	1 (0.8)	0.7	0.5
T/G		GT	21	3 (21.4)	18 (13.6)		
		TT	124	11 (78.6)	113 (85.6)		
		Total	**146**	**14 (100.0)**	**132 (100.0)**		
rs72836346	-1878	CC	0	0 (0.0)	0 (0.0)	0.9	0.9
G/C		GC	20	2 (14.3)	18 (13.6)		
		GG	126	12 (85.7)	114 (86.4)		
		Total	**146**	**14 (100.0)**	**132 (100.0)**		
rs7582030	-274	AA	4	0 (0.0)	4 (3.0)	0.4	0.4
G/A		GA	34	5 (35.7)	29 (22.0)		
		GG	108	9 (64.3)	99 (75.0)		
		Total	**146**	**14 (100.0)**	**132 (100.0)**		
rs724710	**29201**	CC	62	11 (78.6)	51 (37.2)	0.01	**0.003**
C/T		CT	68	2 (14.3)	66 (48.2)		
		TT	21	1 (7.1)	20 (14.6)		
		Total	**151**	**14 (100.0)**	**137 (100.0)**		
rs72836345	-1952	CC	2	0 (0.0)	2 (1.5)	0.5	0.3
T/C		TC	26	4 (28.6)	22 (16.1)		
		TT	122	10 (71.4)	112 (82.4)		
		Total	**150**	**14 (100.0)**	**136 (100.0)**		
rs6750142	45139	AA	103	7 (50.0)	96 (69.6)	0.1	0.1
A/T		AT	43	7 (50.0)	36 (26.1)		
		TT	6	0 (0.0)	6 (4.3)		
		Total	**152**	**14 (100.0)**	**138 (100.0)**		

SNPs id (rs number) from db SNP databases indicated. The ancestral allele is given in bold, the minor allele is underlined. *P* values derived by chi-square reflect the difference across all 3 genotypes and *P1* the difference for minor allele carriers. The genotyping was not equally efficient for SNPs and the total number of available genotype varied from 295-304. The polymorphisms retained for further analysis after multiple testing correction (FDR ≤5%) are depicted in bold.

References

1. Gagne V, Rousseau J, Labuda M, Sharif-Askari B, Brukner I, Laverdiere C, et al. Bim polymorphisms: influence on function and response to treatment in children with acute lymphoblastic leukemia. Clin Cancer Res. 2013 Sep 15;19(18):5240-9.

2. Patel B, Richards SM, Rowe JM, Goldstone AH, Fielding AK. High incidence of avascular necrosis in adolescents with acute lymphoblastic leukaemia: a UKALL XII analysis. Leukemia. 2008 Feb;22(2):308-12.

3. Mattano LA, Jr., Sather HN, Trigg ME, Nachman JB. Osteonecrosis as a complication of treating acute lymphoblastic leukemia in children: a report from the Children's Cancer Group. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2000 Sep 15;18(18):3262-72.

4. Arico M, Boccalatte MF, Silvestri D, Barisone E, Messina C, Chiesa R, et al. Osteonecrosis: An emerging complication of intensive chemotherapy for childhood acute lymphoblastic leukemia. Haematologica. 2003 Jul;88(7):747-53.

5. te Winkel ML, Appel IM, Pieters R, van den Heuvel-Eibrink MM. Impaired dexamethasone-related increase of anticoagulants is associated with the development of osteonecrosis in childhood acute lymphoblastic leukemia. Haematologica. 2008 Oct;93(10):1570-4.

6. Belgaumi AF, Al-Bakrah M, Al-Mahr M, Al-Jefri A, Al-Musa A, Saleh M, et al. Dexamethasone-associated toxicity during induction chemotherapy for childhood acute lymphoblastic leukemia is augmented by concurrent use of daunomycin. Cancer. 2003 Jun 01;97(11):2898-903.

7. Chan KL, Mok CC. Glucocorticoid-induced avascular bone necrosis: diagnosis and management. The open orthopaedics journal. 2012;6:449-57.

8. Hyakuna N, Shimomura Y, Watanabe A, Taga T, Kikuta A, Matsushita T, et al. Assessment of corticosteroid-induced osteonecrosis in children undergoing chemotherapy for acute lymphoblastic leukemia: a report from the Japanese Childhood Cancer and Leukemia Study Group. Journal of pediatric hematology/oncology. 2014 Jan;36(1):22-9.

9. Strauss AJ, Su JT, Dalton VM, Gelber RD, Sallan SE, Silverman LB. Bony morbidity in children treated for acute lymphoblastic leukemia. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2001 Jun 15;19(12):3066-72.

10. Silverman LB, Stevenson KE, O'Brien JE, Asselin BL, Barr RD, Clavell L, et al. Long-term results of Dana-Farber Cancer Institute ALL Consortium protocols for children with newly diagnosed acute lymphoblastic leukemia (1985-2000). Leukemia. 2010 Feb;24(2):320-34.

11. Silverman LB, Gelber RD, Dalton VK, Asselin BL, Barr RD, Clavell LA, et al. Improved outcome for children with acute lymphoblastic leukemia: results of Dana-Farber Consortium Protocol 91-01. Blood. 2001 Mar 01;97(5):1211-8.

12. Moghrabi A, Levy DE, Asselin B, Barr R, Clavell L, Hurwitz C, et al. Results of the Dana-Farber Cancer Institute ALL Consortium Protocol 95-01 for children with acute lymphoblastic leukemia. Blood. 2007 Feb 01;109(3):896-904.

13. Mazziotti G, Giustina A, Canalis E, Bilezikian JP. Glucocorticoid-induced osteoporosis: clinical and therapeutic aspects. Arquivos brasileiros de endocrinologia e metabologia. 2007 Nov;51(8):1404-12.

14. Alos N, Grant RM, Ramsay T, Halton J, Cummings EA, Miettunen PM, et al. High incidence of vertebral fractures in children with acute lymphoblastic leukemia 12 months after the initiation of therapy. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2012 Aug 01;30(22):2760-7.

15. Weinstein RS, Jilka RL, Parfitt AM, Manolagas SC. Inhibition of osteoblastogenesis and promotion of apoptosis of osteoblasts and osteocytes by glucocorticoids. Potential mechanisms of their deleterious effects on bone. The Journal of clinical investigation. 1998 Jul 15;102(2):274-82.

16. Bookout AL, Jeong Y, Downes M, Yu RT, Evans RM, Mangelsdorf DJ. Anatomical profiling of nuclear receptor expression reveals a hierarchical transcriptional network. Cell. 2006 Aug 25;126(4):789-99.

17. Abu EO, Horner A, Kusec V, Triffitt JT, Compston JE. The localization of the functional glucocorticoid receptor alpha in human bone. The Journal of clinical endocrinology and metabolism. 2000 Feb;85(2):883-9.

18. Biddie SC, Conway-Campbell BL, Lightman SL. Dynamic regulation of glucocorticoid signalling in health and disease. Rheumatology (Oxford, England). 2012 Mar;51(3):403-12.

19. Huang DC, Strasser A. BH3-Only proteins-essential initiators of apoptotic cell death. Cell. 2000 Dec 08;103(6):839-42.

20. Webster JC, Cidlowski JA. Downregulation of the glucocorticoid receptor. A mechanism for physiological adaptation to hormones. Annals of the New York Academy of Sciences. 1994 Nov 30;746:216-20.

21. Tonko M, Ausserlechner MJ, Bernhard D, Helmberg A, Kofler R. Gene expression profiles of proliferating vs. G1/G0 arrested human leukemia cells suggest a mechanism for glucocorticoid-induced apoptosis. FASEB journal : official publication of the Federation of American Societies for Experimental Biology. 2001 Mar;15(3):693-9.

22. Niinimaki RA, Harila-Saari AH, Jartti AE, Seuri RM, Riikonen PV, Paakko EL, et al. High body mass index increases the risk for osteonecrosis in children with acute lymphoblastic

leukemia. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2007 Apr 20;25(12):1498-504.

23. Bernbeck B, Mauz-Korholz C, Zotz RB, Gobel U. Methylenetetrahydrofolate reductase gene polymorphism and glucocorticoid intake in children with ALL and aseptic osteonecrosis. Klinische Padiatrie. 2003 Nov-Dec;215(6):327-31.

24. Karol SE, Mattano LA, Jr., Yang W, Maloney KW, Smith C, Liu C, et al. Genetic risk factors for the development of osteonecrosis in children under age 10 treated for acute lymphoblastic leukemia. Blood. 2016 Feb 4;127(5):558-64.

25. Karol SE, Yang W, Van Driest SL, Chang TY, Kaste S, Bowton E, et al. Genetics of glucocorticoid-associated osteonecrosis in children with acute lymphoblastic leukemia. Blood. 2015 Oct 8;126(15):1770-6.

26. Kawedia JD, Kaste SC, Pei D, Panetta JC, Cai X, Cheng C, et al. Pharmacokinetic, pharmacodynamic, and pharmacogenetic determinants of osteonecrosis in children with acute lymphoblastic leukemia. Blood. 2011 Feb 24;117(8):2340-7; quiz 556.

27. French D, Hamilton LH, Mattano LA, Jr., Sather HN, Devidas M, Nachman JB, et al. A PAI-1 (SERPINE1) polymorphism predicts osteonecrosis in children with acute lymphoblastic leukemia: a report from the Children's Oncology Group. Blood. 2008 May 01;111(9):4496-9.

28. Medh RD, Webb MS, Miller AL, Johnson BH, Fofanov Y, Li T, et al. Gene expression profile of human lymphoid CEM cells sensitive and resistant to glucocorticoid-evoked apoptosis. Genomics. 2003 Jun;81(6):543-55.

29. O'Connor L, Strasser A, O'Reilly LA, Hausmann G, Adams JM, Cory S, et al. Bim: a novel member of the Bcl-2 family that promotes apoptosis. The EMBO journal. 1998 Jan 15;17(2):384-95.

30. Iglesias-Serret D, de Frias M, Santidrian AF, Coll-Mulet L, Cosialls AM, Barragan M, et al. Regulation of the proapoptotic BH3-only protein BIM by glucocorticoids, survival signals and proteasome in chronic lymphocytic leukemia cells. Leukemia. 2007 Feb;21(2):281-7.

31. Espina B, Liang M, Russell RG, Hulley PA. Regulation of bim in glucocorticoid-mediated osteoblast apoptosis. Journal of cellular physiology. 2008 May;215(2):488-96.

32. Vrooman LM, Stevenson KE, Supko JG, O'Brien J, Dahlberg SE, Asselin BL, et al. Postinduction dexamethasone and individualized dosing of Escherichia Coli L-asparaginase each improve outcome of children and adolescents with newly diagnosed acute lymphoblastic leukemia: results from a randomized study--Dana-Farber Cancer Institute ALL Consortium Protocol 00-01. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2013 Mar 20;31(9):1202-10.

33. DCTD NCI. National Cancer Institute Common Terminology Criteria for Adverse Events, version 3.0. 2006 [cited; Available from: https://ctep.cancer.gov/protocoldevelopment/

34. Labuda D, Krajinovic M, Richer C, Skoll A, Sinnett H, Yotova V, et al. Rapid detection of CYP1A1, CYP2D6, and NAT variants by multiplex polymerase chain reaction and allele-specific oligonucleotide assay. Analytical biochemistry. 1999 Nov 01;275(1):84-92.

35. Storey JD TJ, Siegmund David. Strong control, conservative point estimation and simultaneous conservative consistency of false discovery rates: a unified approach. Journal of the Royal Statistical Society. 2004 22 December 2003;66(1):187-205.

36. Mattano LA, Jr., Devidas M, Nachman JB, Sather HN, Hunger SP, Steinherz PG, et al. Effect of alternate-week versus continuous dexamethasone scheduling on the risk of osteonecrosis in paediatric patients with acute lymphoblastic leukaemia: results from the CCG-1961 randomised cohort trial. The Lancet Oncology. 2012 Sep;13(9):906-15.

37. Nott A, Meislin SH, Moore MJ. A quantitative analysis of intron effects on mammalian gene expression. RNA (New York, NY). 2003 May;9(5):607-17.

38. Bachmann PS, Gorman R, Papa RA, Bardell JE, Ford J, Kees UR, et al. Divergent mechanisms of glucocorticoid resistance in experimental models of pediatric acute lymphoblastic leukemia. Cancer research. 2007 May 01;67(9):4482-90.

39. Bachmann PS, Gorman R, Mackenzie KL, Lutze-Mann L, Lock RB. Dexamethasone resistance in B-cell precursor childhood acute lymphoblastic leukemia occurs downstream of ligand-induced nuclear translocation of the glucocorticoid receptor. Blood. 2005 Mar 15;105(6):2519-26.

40. Wang Z, Malone MH, He H, McColl KS, Distelhorst CW. Microarray analysis uncovers the induction of the proapoptotic BH3-only protein Bim in multiple models of glucocorticoid-induced apoptosis. The Journal of biological chemistry. 2003 Jun 27;278(26):23861-7.

41. Hunt R, Sauna ZE, Ambudkar SV, Gottesman MM, Kimchi-Sarfaty C. Silent (synonymous) SNPs: should we care about them? Methods in molecular biology (Clifton, NJ). 2009;578:23-39.

42. Augis V, Airiau K, Josselin M, Turcq B, Mahon FX, Belloc F. A single nucleotide polymorphism in cBIM is associated with a slower achievement of major molecular response in chronic myeloid leukaemia treated with imatinib. PloS one. 2013;8(11):e78582.

43. Ng KP, Hillmer AM, Chuah CT, Juan WC, Ko TK, Teo AS, et al. A common BIM deletion polymorphism mediates intrinsic resistance and inferior responses to tyrosine kinase inhibitors in cancer. Nature medicine. 2012 Mar 18;18(4):521-8.

44. Katagiri S TT, Tadokoro K, Ohyashiki JH, Ohyashiki K. BCL2L11 (BIM) deletion polymorphism, rather than BIM SNP, is an effective predictor of early molecular relapse after

ABL tyrosine kinase inhibitor discontinuation in patients with chronic myeloid leukemia. Integr Cancer Sci Therap. 2015;2:242-4.

45. Lee JH, Lin YL, Hsu WH, Chen HY, Chang YC, Yu CJ, et al. Bcl-2-like protein 11 deletion polymorphism predicts survival in advanced non-small-cell lung cancer. Journal of thoracic oncology : official publication of the International Association for the Study of Lung Cancer. 2014 Sep;9(9):1385-92.

46. Kelly JL, Novak AJ, Fredericksen ZS, Liebow M, Ansell SM, Dogan A, et al. Germline variation in apoptosis pathway genes and risk of non-Hodgkin's lymphoma. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2010 Nov;19(11):2847-58.

CHAPTER 3: DISCUSSION

Results from several clinical studies demonstrated that osteonecrosis and fractures are debilitating complications that occur during childhood ALL treatment [49, 51]. The corticosteroids are considered as a main risk factor in the development of ON [54]. Fractures are an important manifestation of osteoporosis in children with leukemia and are frequently asymptomatic thereby they also go undetected and difficult to associate precise risk factors.

Despite clear clinical criteria for diagnosis reported cumulative incidences of ON related to ALL chemotherapy vary widely among the studies [76, 77] ranging from 9.3% to 29%. Most of these differences are related to study design, particularly the time when the information on osteonecrosis was collected, population groups, as well as chemotherapy regimens. Several studies addressed the influence of corticosteroids, including type, continuous or discontinuous administration, cumulative doses, and duration of therapy in order to derive a preventive strategy to decrease the ON occurrence [78]. Despite its higher toxicity [79], DXM remained the drug of choice in therapies due to its superior pharmacokinetic characteristics, easier penetration through the blood-brain barrier, which is reflected in superior efficacy. ON occurs less frequently with alternate weeks rather than continuous DXM administration schedule in high-risk ALL patients receiving intensified therapy [80]. These results suggest that DXM administration at higher cumulative doses and in any treatment phase highly affects the incidence of ON.

Based on patient medical records, we performed a comprehensive analysis of clinical outcomes (ON and FR) which were confirmed with radiographic imaging examinations (X-ray, MRI, or CT scan) that allowed us to unambiguously assign osteonecrosis and/or fractures for each ALL patient in QcALL cohort.

Beside *BCL2L11* gene, several other candidate genes acting in corticosteroid pathways were selected. The genotypes were obtained either by genotyping or through WES approaches. Overall, we analyzed 116 polymorphisms in 52 genes (indicated in Table A-1, Annexe I.) We

also included three GWAS polymorphisms: *ACP1*, rs1274403; *PROX1-AS1*, rs1891059 and *GRIN3A*, rs10989692. These polymorphisms have been previously identified by GWAS as associated with osteonecrosis in ALL patients [54, 55]. All selected polymorphisms were analyzed for their possible association with the increased risk of ON and/or FR in QcALL patients. The characteristics of the patients who developed or not ON are mentioned in Table 2, p. 47. Each polymorphism was analyzed for its association with osteonecrosis based on a recoded-3-genotype model, a recessive or a dominant model. In addition, we aimed to replicate significant associations in an independent cohort from DFCI, Boston. Statistically significant genetic polymorphisms in *BCL2L11*, *NFKB1*, *PARP1*, *ABCB1*, *ACP1*, and *SHMT1* genes after the adjustment for multiple comparison were obtained in QcALL cohort, as shown in Table A-2, Annexe I. The association of these polymorphisms was not statistically significant for FR or for combined phenotype (ON and FR) after multiple testing corrections.

The relationship between identified genes and ON have biological plausibility. *BCL2L11* is a gene involved in the regulation of cell cycles, and allows the initiation of apoptosis of malignant cells [62]. Therefore, *BCL2L11* may also be part of the mechanism of the osteonecrosis by increased osteoblast and osteocyte apoptosis. *ACP1* is an important gene in maintaining lipid homoeostasis [54]. *ABCB1* codes for P-gp, which acts as an efflux exporter, preventing the accumulation of GC within cells [67]. *NFKB1* has an important role in the transcription of several genes that induce the inflammatory reaction [64]. *PARP1* allows the repair and maintenance of DNA in the integrity of the genome [65]. *SHMT1* plays a role in the metabolism of folate as well as in the replication and synthesis of DNA [70]. These genes thus all have an effect either on the action of the GCs or on the development of osteonecrosis.

Among *BCL2L11* SNPs investigated, two polymorphisms, 891T>G (rs2241843) and 29201C>T (rs724710), have been indicated to influence the risk of ON development. The 891T>G is located at the 5'UTR of *BCL2L11* gene and it is a potentially regulatory SNP. The 5'UTR regions contain important cis-regulatory elements and their disruption or introduction could affect gene expression and protein abundance [82]. We found that the TT891 genotype was associated with increased risk of ON, and it remained significant predictor in Cox's regression models with the inclusion of other clinical factors. The *BCL2L11* TT891 genotype has shown

significant ON association in several groups, mainly in PDN group (p = 0.005) and female group (p = 0.007). It was also well replicated in DFCI validation cohort. The *BCL2L11* 29201C>T is a synonymous polymorphism located in exon 8 of *BCL2L11* gene. The CC29201 genotype was shown to confer increased ON risk, mainly in high risk QcALL group in both uni- and multi-variable models. Furthermore, the TT891 and CC29201genotype were also associated with higher sensitivity to DXM when testing *in vitro* in lymphoblastoid cell lines (LCLs). It has been previously demonstrated that *BCL2L11* is a key regulator of osteoblast apoptosis, and that the expression of Bim mRNA is cell-dependent, and influenced by time and DXM-dose used. In human osteoblast cells, induction of *BCL2L11* expression was observed at as low as 1nM of DXM to reach a maximum of induction at 1μM; and higher doses of DXM (> 1μM) resulted in a reduction of mRNA Bim [83]. Studies using human cells or rabbit models suggested that GC-induced ON is associated with osteoblast and osteocyte apoptosis, an ischemia condition that finally leads to ON through disruption of the osteocyte network [84, 85].

Other polymorphisms such as, *NFKB1*, *PARP1*, *ABCB1*, *ACP1*, and *SHMT1* were significantly associated with ON in QcALL cohort.

The ***NFKB1*** SNP, a +1248 G>A (rs3774932) polymorphism is located in the regulatory region (intron 1) of the gene; in which, the ancestral allele G is replaced by the derived allele A in 41% of individuals of European ancestry. Among QcALL patients who developed osteonecrosis, 21.9% individuals had GG, 40.6% had AG and 37.5 % had AA genotype. We found that the AA +1248 genotype was associated with increased risk of ON, mainly in DXM (p = 0.001) and high risk (p = 0.008) QcALL patient groups [Table A-1, Annexe I]. Based on Cox's regression model, the AA patients presented 5.5-fold higher risk of ON in DXM group (p = 0.003) [Fig, A-1, Annexe II].

PARP1 SNP, a **-219 A>T** (rs7531668) polymorphism in the promoter region of the gene; in which, the ancestral allele A is replaced by the derived allele T in 21% of individuals of European ancestry. The TT-219 genotype had a protective effect; these individuals had lower risk to develop ON compared with AA genotype (HR= .267, 95% CI = 0.278-.916), p = .36), [Fig. A-2, Annexe II].

The ***ABCB1*** SNP, a **+4196 G>A** (rs3789243) polymorphism in the coding region (exon 4) of the gene; has minor allele frequency (MAF) of 47%. Individuals who are homozygous for minor allele A presented 4.7-fold higher risk of ON in female group patients (p = 0.003) [Fig. A-3, Annexe II].

The ***ACP1*** SNP, a **380 C>T** (rs11553746) polymorphism in the coding region (missense variant of exon 3) of the gene; has MAF of 47%. This polymorphism was statistically significant in high-risk group (p = 0.003) [Table A-2, Annexe I]. with the TT380 genotype presented 4.7-fold higher risk of ON in this group of QcALL patients (p = 0.005), [Fig. A-4, Annexe II].

The ***SHMT1*** SNP, a **1420 G>A** (rs1979277) polymorphism in coding region (exon 12) of the gene; in which, the ancestral allele G is replaced by the derived allele A; MAF is 30 % This polymorphism is also involved in amino acid change, in which leucine (LEU) is changed to phenylalanine (PHE) at individuals with derived allele A. Individuals with the AA1420 genotype presented 3.5-fold higher risk of ON in females group QcALL patients [Fig. A-5, Annexe II]. Among patients of DFCI two polymorphisms were replicated. The *SHMT1* polymorphism showed an increased risk of developing osteonecrosis in female group (p = 0.02), in patients treated with PDN (p = 0.0007), standard risk patients (p = 0.0001) and in children less than 10 years old (p = 0.002), whereas APC1 polymorphism has shown significant association with the development of osteonecrosis in girls (p = 0.02). (result not shown).
The results of this study showed an association of these genes with ON reported in some ALL patients treated with GC; however, further association analysis and function related assays should be performed in order to validate these associations.

3.1 CONCLUSION

Over the last decade, many pharmacogenomic studies have been conducted to identify genetic factors contributing to treatment responses including ON or other toxicity related to ALL therapy. These studies could help identify patients who are at high risk of developing drug toxicity, patients that undergo early relapse, or who are less likely to derive full therapeutic effects from ALL medication. Specific aim of pharmacogenomics is to individualize treatment based on patients' genotype, in order to increase treatment efficacy while reducing drug side effects.

The candidate gene approach is one of the strategies that has been used by different groups to identify and associate specific genotype in response to treatment, pharmacological phenotypes, or beyond pharmacogenomics, with complex diseases or traits. To date, most association studies of ALL pharmacogenomics have been based on the candidate gene approach. Thereby in our study, using the candidate gene approach, we had the advantage to include specific genes of interest, such as genes known to be involved in metabolic pathways (methotrexate) and CS action (e.g. glucocorticoids receptor, pro- and anti-apoptotic genes). The drawback of this approach was that it evaluates only a restricted number of polymorphisms, in a preselected gene of interest. GWAS or other genome based approaches are designed to capture larger patterns of variation architecture. This process greatly increased the number of SNP that could be identified and tested for the association.

Limits of the study.

In both cohorts (QcALL and DFCI), the number of patients played an important role in analysis, mainly when stratification for prognostic factors has been performed. Moreover, knowing that ON is a multifactorial trait, other patient characteristics (for example body mass index) might be necessary to be included in the analysis.

The incidences of ON and FR were determined retrospectively, based on patient medical records, in which only symptomatic osteonecrosis was recorded. This leads us to think that some asymptomatic ON cases might have remained unreported.

The results obtained in DFCI are mostly reliable, but are limited by the number of patients and the protocol. This cohort was composed only of patients treated with the 2000-01 protocol in which high-risk patients received lower CS doses during maintenance compared to earlier DFCI protocols. In addition, data from the DFCI cohort did not allow us to analyze the results over time, with time-to-event statistics tests such Kaplan-Mayer and Cox regression.

In conclusion, our study suggests that *BCL2L11* gene together *ACP1*, and *SHMT1* are potential candidates associated with osteonecrosis in QcALL patients. The results obtained with *NFKB1*, *PARP1*, *ABCB1* require further replication studies with a larger sample size to validate these associations. On this, *BCL2L11* polymorphism comes to our attention as the most potent candidate since its implication in the treatment efficacy in ALL patients has already been reported by our group.

3.2 PERSPECTIVES

The availability of GWAS and WES approaches has made the human genetic association studies possible to identify common and rare variants underlying complex diseases on a genome-wide scale.

The next- generation sequencing coupled with the genetic association studies is expected to discover novel rare variants for a better interpretation of disease. This type of analysis uses a procedure that eliminates potential noisy SNPs having little or no contribution to a disease status, hence greatly improves the testing power for a gene- or pathway-based analysis. Much of the interest surrounding genetic association studies centers on the potential clinical application of polymorphisms that serve as markers for disease. Moreover, it has been proposed that these markers can both serve as predictors of disease and to tailor treatment of disease.

Using these approaches novel genetic variants underlying complex diseases have been identified, which gives much hope and prospects for the future of pharmacogenomics.

CHAPTER 4: REFERENCES

[1] Ries LAG, Smith MA, Linet JG, Tamra M, Young T, Bunin JL. Cancer Incidence and Survival among Children and Adolescents: United States SEER Progran 1975-1995. National Cancer Institute. Editor. Bethesda, MD: 1999.

[2] Chiaretti S and Foà R. T-cell acute lymphoblastic leukemia. Haematologica. 2009 Feb; 94(2): 160–162.

[3] Pui CH, Robison LL, Look AT. Acute lymphoblastic leukemia. Lancet. 2008 Mar 22;371(9617):1030-43.

[4] Mrózek K, Harper DP, Peter D. Cytogenetics and Molecular Genetics of Acute Lymphoblastic Leukemia. Hematol Oncol Clin North Am. 2009 Oct; 23(5): 991–999.

[5] Coustan-Smith E, Behm FG, Sanchez J, Boyett JM, Hancock ML, Raimondi SC, Rubnitz JE, et al. Immunological detection of minimal residual disease in children with acute lymphoblastic leukaemia. Lancet. 1998 Feb 21;351(9102):550-4.

[6] Silverman LB. Acute lymphoblastic leukemia in infancy. Pediatr Blood Cancer. 2007, 49 (7 Suppl): p. 1070-1073.

[7] Pullen J, Shuster JJ, Link M, Borowitz M, Amylon M, Carroll AJ, et al. Significance of commonly used prognostic factors differs for children with T cell acute lymphocytic leukemia (ALL), as compared to those with B-precursor ALL. A Pediatric Oncology Group (POG) study. Leukemia. 1999 Nov;13(11):1696-707.

[8] Frost BM, Forestier E, Gustafsson G, Nygren P, Hellebostad M, Jonmundsson G, et al. Translocation t(1;19) is related to low cellular drug resistance in childhood acute lymphoblastic leukaemia. Leukemia. 2005, 19:165–169.

[9] Czuchlewski DR, Farzanmehr H, Robinett S, Haines S, Reichard KK. t(9;22)(q34;q11.2) is a recurrent constitutional non-Robertsonian translocation and a rare cytogenetic mimic of chronic myeloid leukemia. Cancer Genet. 2011 Oct;204(10):572-6.

[10] Domer PH, Fakharzadeh SS, Chen CS, Jockel J, Johansen L, Silverman GA, et al. Acute mixed-lineage leukemia t(4;11)(q21;q23) generates an MLL-AF4 fusion product. Proc Natl Acad Sci U S A. 1993 Aug 15; 90(16): 7884–7888.

[11] Schardt C, Ottmann OG, Hoelzer D, Ganser A. Acute lymphoblastic leukemia with the (4;11) translocation: combined cytogenetic, immunological and molecular genetic analyses. Leukemia. 1992, 6:370-374.

[12] Westbrook CA, Hooberman AL, Spino C, Dodge RK, Larson RA, Davey F, et al. Clinical significance of the BCR-ABL fusion gene in adult acute lymphoblastic leukemia: a Cancer and Leukemia Group B Study (8762). Blood. 1992, 80:2983-2990.

[13] Pui C-H, Carroll WL, Meshinchi S, Robert J. Biology, Risk Stratification, and Therapy of Pediatric Acute Leukemias: An Update. Journal of Clinical Oncology. 2011, 29 (5), 551-565.

[14] Smadja NV, Bastard C, Brigaudeau C, Leroux D, Fruchart C. Hypodiploidy is a major prognostic factor in multiple myeloma. Blood. 2001, 98:2229-2238.

[15] Bhojwani D, Pei D, Sandlund JT, Jeha S, Ribeiro RC, Rubnitz JE, et al. Prognostic and oncogenic relevance of TLX1/HOX11 expression level in T-ALLs. Blood. 2007 Oct 1;110(7):2324-30.

[16] Castiel A, and Izraeli S. SIL (SCL/TAL1 interrupting locus). Atlas Genet Cytogenet Oncol Haematol. 2006,10(2):97-99.

[17] Möricke A, Zimmermann M, Reiter A, Gadner H, Odenwald E, Harbott J, et al. Prognostic impact of age in children and adolescents with acute lymphoblastic leukemia: data from the trials ALL-BFM 86, 90, and 95. Klin Padiatr. 2005 Nov-Dec;217(6):310-20.

[18] Silverman LB, Declerck L, Gelber RD, Dalton VK, Asselin BL, Barr RD, et al. Results of Dana-Farber Cancer Institute Consortium protocols for children with newly diagnosed acute lymphoblastic leukemia (1981–1995). Leukemia. 2000, 14, 2247–2256.

[19] Silverman LB, and Sallan ES. Newly diagnosed childhood acute lymphoblastic leukemia: update on pronostic factors and treatment. Curr Opin Hematol. 2003, 10: 290-296.

[20] Silverman LB, Gelber RD, Dalton VK, Asselin BL, Barr RD, Clavell LA, et al. Improved outcome for children with acute lymphoblastic leukemia: results of Dana-Farber Consortium Protocol 91-01. Blood. 2001 Mar 1;97(5):1211-8.

[21] Vrooman LM, Stevenson KE, Supko JG, O'Brien J, Dahlberg SE, Asselin BL, et al. Postinduction dexamethasone and individualized dosing of Escherichia Coli L-asparaginase each improve outcome of children and adolescents with newly diagnosed acute lymphoblastic leukemia: results from a randomized study--Dana-Farber Cancer Institute ALL Consortium Protocol 00-01. J Clin Oncol. 2013 Mar 20;31(9):1202-10.

[22] Dalle JH, Moghrabi A, Rousseau P, Leclerc JM, Barrette S, Bernstein ML et al. Second induction in pediatric patients with recurrent acute lymphoid leukemia using DFCI-ALL protocols. J Pediatr Hematol Oncol. 2005. 27(2):73-79.

[23] Paietta E. Assessing minimal residual disease (MRD) in leukemia: a changing definition and concept? Bone Marrow Transplantation. 2002 March 29(6): 459-65.

[24] Jacques J, van Dongen M, Vincent H, van der Velden J, Brüggemann M, Orfao A. Minimal residual disease diagnostics in acute lymphoblastic leukemia: need for sensitive, fast, and standardized technologies. Blood. 2015, 125:3996-4009.

[25] Dördelmann M, Reiter A, Borkhardt A, Ludwig WD, Götz N, Viehmann S, et al. Prednisone response is the strongest predictor of treatment outcome in infant acute lymphoblastic leukemia. Blood. 1999 Aug 15;94(4):1209-17.

[26] Mitchell CD, Richards SM, Kinsey SE, Lilleyman J, Vora A, Eden TO. Benefit of dexamethasone compared with prednisolone for childhood acute lymphoblastic leukaemia: results of the UK Medical Research Council ALL97 randomized trial. Br J Haematol. 2005 Jun;129(6):734-45.

[27] Lu NZ, Cidlowski JA. The origin and functions of multiple human glucocorticoid receptor isoforms. Ann N Y Acad Sci. 2004 Jun; 1024:102-23.

[28] Gruver-Yates AL and Cidlowski JA. Tissue-Specific Actions of Glucocorticoids on Apoptosis: A Double-Edged Sword. Cells. 2013, 2:202-223.

[29] Gross KL, Lu NZ, Cidlowski JA. Molecular mechanisms regulating glucocorticoid sensitivity and resistance. Mol Cell Endocrinol. 2009 Mar 5;300(1-2):7-16.

[30] Oakley RH, Sar M, Cidlowski, JA. The human glucocorticoid receptor beta isoform. Expression, biochemical properties, and putative function. J. Biol. Chem. 1996, 271, 9550-9559.

[31] Lu NZ, Collins JB, Grissom SF, Cidlowski JA. Selective regulation of bone cell apoptosis by translational isoforms of the glucocorticoid receptor. Mol. Cell Biol. 2007, 27, 7143-7160.

[32] Cadepond F, Schweizer-Groyer G, Segard-Maurel I, Jibard N, Hollenberg SM, Gigue`re V et al. Heat shock protein 90 as a critical factor in maintaining glucocorticosteroid receptor in a nonfunctional state.Journal of Biological Chemistry. 1991, 266:5834–5841.

[33] Bledsoe RK, Montana VG, Stanley TB, Delves CJ, Apolito CJ, McKee DD, et al. Crystal structure of the glucocorticoid receptor ligand binding domain reveals a novel mode of receptor dimerization and coactivator recognition. Cell. 2002, 110:93–105.

[34] Schlossmacher G, Stevens A, White A. Glucocorticoid receptor-mediated apoptosis: Mechanisms of resistance in cancer cells. J. Endocrinol. 2011, 211:17-25.

[35] Johansson-Haque K, Palanichamy E, Sam Okret. Stimulation of MAPK-phosphatase 1 gene expression by glucocorticoids occurs through a tethering mechanism involving C/EBP. J Mol Endocrinol. 2008 October 1, 41:239-249.

[36] Evans-Storms RB and Cidlowski, JA. Delineation of an antiapoptotic action of glucocorticoids in hepatoma cells: The role of nuclear factor-kappa B. Endocrinology. 2000, 141: 1854-1862.

[37] Beck IM, Vanden Berghe W, Vermeulen L, Yamamoto KR, Haegeman G, De Bosscher K. Crosstalk in inflammation: the interplay of glucocorticoid receptor-based mechanisms and kinases and phosphatases. Endocrine Reviews. 2009, 30:830–882.

[38] Elmore S. Apoptosis: A Review of Programmed Cell Death. Toxicol Pathol. 2007, 35(4): 495–516.

[39] Ploner C, Rainer J, Niederegger H, Eduardoff M, Villunger A, Geley S, et al. The BCL2 rheostat in glucocorticoid-induced apoptosis of acute lymphoblastic leukemia. Leukemia. 2008, 22:370–377.

[40] Herr I, Gassler N, Friess H, Buchler MW. Regulation of differential pro- and anti-apoptotic signaling by glucocorticoids. Apoptosis. 2007, 12:271-291.

[41] Klumper E, Pieters R, Veerman AJ, Huismans DR, Loonen AH, Hählen K, et al. *In vitro* cellular drug resistance in children with relapsed/refractory acute lymphoblastic leukemia. Blood. 1995 Nov 15;86(10):3861-8.

[42] Smith LK, Cidlowski JA. Glucocorticoid-induced apoptosis of healthy and malignant lymphocytes. Prog. Brain Res. 2010, 182, 1-30.

[43] Gross KL, Lu NZ, Cidlowski JA. Molecular mechanisms regulating glucocorticoid sensitivity and resistance. Molecular and Cellular Endocrinology. 2009, 300:7–16.

[44] Kaspers GJ, Veerman AJ, Popp-Snijders C, Lomecky M, Van Zantwijk CH, Swinkels LM, et al. Comparison of the antileukemic activity in vitro of dexamethasone and prednisolone in childhood acute lymphoblastic leukemia. Med Pediatr Oncol. 1996, 27:114–121.

[45] Strauss AJ, Su JT, Dalton VM, Gelber RD, Sallan SE, Silverman LB. Bony Morbidity in Children Treated for Acute Lymphoblastic Leukemia. J Clin Onc, 2001, 19(12): 3066-3072.

[46] Relling MV, and Ramsey MB. Pharmacogenomics of acute lymphoid leukemia: new insights into treatment toxicity and efficacy. ASH Education Book. 2013, 1:126-130.

[47] Relling MV, Pui CH, Cheng C, Evans WE. Thiopurine methyltransferase in acute lymphoblastic leukemia. Blood. 2006, 107(2):843-844.

[48] Ceppi F, Langlois-Pelletier C, Gagné V, Rousseau J, Ciolino C, De Lorenzo S, et al. Polymorphisms of the vincristine pathway and response to treatment in children with childhood acute lymphoblastic leukemia. Pharmacogenomics. 2014 Jun;15(8):1105-16.

[49] Ben Tanfous M, Sharif-Askari B, Ceppi F, Laaribi H, Gagné V, Rousseau J, et al. Polymorphisms of asparaginase pathway and asparaginase-related complications in children with acute lymphoblastic leukemia. Clin Cancer Res. 2015 Jan 15;21(2):329-34.

[50] Rousseau J, Gagné V, Labuda M, Beaubois C, Sinnett D, Laverdière C, et al. ATF5 polymorphisms influence ATF function and response to treatment in children with childhood acute lymphoblastic leukemia. Blood. 2011 Nov 24; 118(22): 5883–5890.

[51] Manolio TA. Genome wide association studies and assessment of the risk of disease. N Engl J Med. 2010, 363(2):166-176.

[52] Motsinger-Reif AA, Jorgenson E, Relling MV, Kroetz DL, Weinshilboum R, Cox NJ, Roden DM. Genome wide association studies in pharmacogenomics: successes and lessons. Pharmacogenet Genomics. 2013, 23(8):383-394.

[53] Bush WS and Moore JH. Genome-Wide Association Studies. PLoS Comput Biol. 2012 Dec; 8(12): e1002822.

[54] Kawedia JD, Kaste SC, Pei D, Panetta JC, Cai X, Cheng C, et al. Pharmacokinetic, pharmacodynamic, and pharmacogenetic determinants of osteonecrosis in children with acute lymphoblastic leukemia. Blood. 2011 Feb 24;117(8):2340-7.

[55] Karol SE, Yang W, Van Driest SL, Chang TY, Kaste S, Bowton E, et al. Genetics of glucocorticoid-associated osteonecrosis in children with acute lymphoblastic leukemia. Blood. 2015 Oct 8;126(15):1770-6.

[56] Clarke GM, Anderson CA, Pettersson FH, Cardon LR, Morris AP, Zondervan KT. Basic statistical analysis in genetic case-control studies. Nat Protoc. 2011, 6 (2):121–33.

[57] Warr A, Robert C, Hume D, Archibald A, Deeb N, Watson M. Exome Sequencing: Current and Future Perspectives. 2015 Aug; 5(8):1543–1550.

[58] Bhartiya D and Scaria V. Genomic variations in non-coding RNAs: Structure, function and regulation. Genomics. 2016, 107:59–68.

[59] Consortium GP. A map of human genome variation from population-scale sequencing. Nature. 2010, 467:1061–1073.

[60] Singleton AB. Exome sequencing: a transformative technology. Lancet Neurol. 2011, 10: 942–946.

[61] Albert TJ, Molla MN, Muzny DM, Nazareth L, Wheeler D, Song X, et al. Direct selection of human genomic loci by microarray hybridization. Nat. Methods. 2007, 4: 903–905.

[62] Relling MV, Yang W, Das S, Cook EH, Rosner GL, Neel M, et al. Pharmacogenetic risk factors for osteonecrosis of the hip among children with leukemia. J Clin Oncol. 2004;22 (19): 3930–36.

[63] French D, Hamilton LH, Mattano LA Jr, Sather HN, Devidas M, Nachman JB, et al. A PAI-1 (SERPINE1) polymorphism predicts osteonecrosis in children with acute lymphoblastic leukemia: a report from the Children's Oncology Group. Blood. 2008; 111:4496–99.

[64] O'Connor L, Strasser A, O'Reilly LA, Hausmann G, Jadams JM, Cory S, et al. Bim: a novel member of the Bcl-2 family that promotes apoptosis.EMBO J. 1998, 17:384-95.

[65] Augis V, Airiau K, Josselin M, Turcq B, Mahon FX, Belloc F. A single nucleotide polymorphism in cBIM is associated with a slower achievement of major molecular response in chronic myeloid leukaemia treated with imatinib. POS ONE. 2013; 8: e78582.

[66] Meyer R, Hatada EN, Hohmann HP, Haiker M, Bartsch C, Röthlisberger U, et al. Cloning of the DNA-binding subunit of human nuclear factor kappa B: the level of its mRNA is strongly regulated by phorbol ester or tumor necrosis factor alpha. Proc Natl Acad Sci U S A.1991, 88 (3): 966–70.

[67] Ba Xand Garg NJ. Signaling Mechanism of Poly(ADP-Ribose) Polymerase-1 (PARP-1) in Inflammatory Diseases. Am J Pathol. 2011 Mar; 178(3): 946–955.

[68] Kameshita I, Matsuda Z, Taniguchi T, Shizuta Y. Poly (ADP-ribose) synthetase: separation and identification of three proteolytic fragments as the substrate-binding domain, the DNA-binding domain, and the automodification domain. J Biol Chem. 1984, 259:4770–4776.

[69] Pariante C. The role of multi-drug resistance p-glycoprotein in glucocorticoid function: Studies in animals and relevance in humans. European Journal of Pharmacology. 2008;583(2-3):263-271.

[70] Gervasini G, Carrillo JA, Garcia M, San Jose C, Cabanillas A, Benitez J. Adenosine triphosphate-binding cassette B1 (ABCB1) (multidrug resistance 1) G2677T/A gene polymorphism is associated with high risk of lung cancer. Cancer. 2006 Dec 15;107(12):2850-57.

[71] Yi SY, Hong KS, Lim HS, Chung JY, Oh DS, Kim JR, et al. A variant 2677A allele of the MDR1 gene affects fexofenadine disposition. Clin Pharmacol Ther. 2004 Nov;76(5):418-27.

[72] Appaji Rao N, Ambili M, Jala VR, Subramanya HS, Savithri HS. Structure-function relationship in serine hydroxymethyltransferase. Biochim. Biophys. Acta. 2003, 1647(1–2):24–9.

[73] Cordell HJ and Clayton DG. Genetic association studies. Lancet. 2005 Sep 24-30;366(9491):1121-31.

[74] Clarke GM, Anderson CA, Pettersson FH, Cardon LR, Morris AP, Zondervan KT. Basic statistical analysis in genetic case-control studies. Nat Protoc. 2011 Feb; 6(2): 121–133.

[75] Alos N, Grant RM, Ramsay T, Halton J, Cummings EA, Miettunen PM, et al. High Incidence of Vertebral Fractures in Children with Acute Lymphoblastic Leukemia 12 Months After the Initiation of Therapy. J. Clin. Oncology. 2012, 30 (22):2760-67.

[76] Mattano LA Jr, Sather HN, Trigg ME, Nachman JB. Osteonecrosis as a complication of treating acute lymphoblastic leukemia in children: a report from the Children's Cancer Group J Clin Oncol. 2000;18(18):3262-72.

[77] Patel B, Richards SM, Rowe JM, Goldstone AH, Fielding AK. High incidence of avascular necrosis in adolescents with acute lymphoblastic leukaemia: a UKALL XII analysis Leukemia. 2008;22(2):308-12.

[78] Mattano LA Jr, Devidas M, Nachman JB, Sather HN, Hunger SP, Steinherz PG, et al.

Effect of alternate week versus continuous dexamethasone scheduling on the risk of osteonecrosis in paediatric patients with acute lymphoblastic leukaemia: results from the CCG-1961 randomised cohort trial. Lancet Oncol. 2012; 13(9):906-15.

[79] Belgaumi AF, Al-Bakrah M, Al-Mahr M, Al-Jefri A, Al-Musa A, Saleh M, et al. Dexamethasone-associated toxicity during induction chemotherapy for childhood acute lymphoblastic leukemia is augmented by concurrent use of daunomycin. Cancer. 2003 1;97(11):2898-903.

[80] Igarashi S, Manabe A, Ohara A, Kumagai M, Saito T, Okimoto Y, et al. No advantage of dexamethasone over prednisolone for the outcome of standard- and intermediate-risk childhood acute lymphoblastic leukemia in the Tokyo Children's Cancer Study Group L95-14 protocol. J Clin Oncol. 2005;23(27):6489-98.

[81] Labuda D, Krajinovic M, Richer C, Skoll A, Sinnett H, Yotova V, et al. Rapid detection of CYP1A1, CYP2D6, and NAT variants by multiplex polymerase chain reaction and allele-specific oligonucleotide assay. Anal Biochem. 1999 Nov 1;275(1):84-92.

[82] Barrett LW, Fletcher S, Wilton SD. Regulation of eukaryotic gene expression by the untranslated gene regions and other non-coding elements. Cell. Mol. Life Sci. 2012, 69:3613-34.

[83] Espina B, Liang M, Russell RGG, Hulley PA. Regulation of Bim in Glucocorticoid-Mediated Osteoblast Apoptosis. J Cell Physiol. 2010; 215(2): 488–96.

[84] Zalavras C, Shah S, Birnbaum MJ, Frenkel B. Role of Apoptosis in Glucocorticoid-Induced Osteoporosis and Osteonecrosis. Crit Rev Eukaryot Gene Expr. 2003; 13(2-4):221-35.

[85] Kabata T , Kubo T, Matsumoto T, Nishino M, Tomita K, Katsuda S, et al. Apoptotic Cell Death in Steroid Induced Osteonecrosis: An Experimental Study in Rabbits. J Rheumatol. 2000; 27 (9), 2166-71.

ANNEXE I

Table A-1. The characteristics of the polymorphisms used in this study.

GENE NAME	SNPs rs CANDIDATE GENE	LOCATION	REGION	REF/ALT	MAF	LD correlation (r2)
ABCB1	rs2888599	7:87229025	Intron 3	C/T	0.03	
	rs4728709	7:87233602	Intron 1	G/A	0.17	
	rs3789243	7:87220886	Intron 4	A/G	0.48	
	rs1128503	7:87179601	Exon 13	A/G	0.42	
	rs2235046	7:87174066	Intron 17	T/C	0.44	
	rs2032582	7:87160618	Exon 22	A/T/G	0.34	r2 = 0.848 (rs1128503)
	rs1045642	7:87138645	Exon 27	A/G/T	0.4	
ADPRT_PARP1	rs1341336	1:226596389	RR	A/G	0.43	
	rs7531668	1:226596019	RR	A/T	0.24	
Apaf1	rs2289315	12:99038549	5'UTR	G/A	0.05	
	rs2289317	12:99038707	5'UTR	A/C	0.12	r2= 0.963 (rs76396995)
	rs76396995	12:99038881	RR	T/A	0.08	
ATM	rs4987876	11:108092637	RR	G/T	0.08	
	rs228589	11:108093208	RR	A/T	0.47	
Bad	rs2510066	11:64052447	RR	C/T	0.26	
Bcl2	rs62098660	18:60988146	RR	G/A	0.28	
	rs2279115	18:60986837	5'UTR	G/T	0.45	
Bid	rs366542	22:18257735	RR	C/T	0.38	
	rs386333	22:18258382	RR	A/G	0.09	
Bim BCL2L11	rs2241842	2:111879415	Intron 1	A/G	0.42	
	rs2241843	2:111879381	Intron 1	C/A	0.4	r2 = .0776 (rs2241842)
	rs73954926	2:111877175	RR	T/G	0.12	
	rs72836346	2:111876613	5'UTR	G/C	0.04	
	rs7582030	2:111878227	RR	G/A	0.16	
	rs724710	2:111907691	Exon 3	T/C	0.27	
	rs72836345	2:111876539	5'UTR	T/C	0.05	
	rs6750142	2:111923629	3'UTR	A/T	0.16	
Birc4	rs5956578	X:122992696	RR	G/C		

BRCA1	rs4793204	17:41 279 298	RR	A/G	0.33	r2 = 0.963 (rs799906)
	rs799906	17:41278116	RR	T/C	0.45	
	rs8176071	17:41278005-41278006	RR	del/TGT/T	0.35	
	rs3092986	17:41277996	RR	T/C	0.03	
BRCA2	rs206114	13:32888062	RR	A/C	0.42	
	rs36221753	13:32888352	RR	TTAGC/-		
	rs206115	13:32888473	RR	C/T	0.42	r2 = 0.849 (rs206114)
	rs206116	13:32888483	RR	G/A	0.4	r2= 0.855 (rs206117)
	rs206117	13:32888709	RR	C/T	0.4	
	rs3092989	13:32889363	RR	G/A	0.18	
DDIT4	rs1053639	10:74035041	3'UTR	T/A	0.48	
	rs8316	10:74035297	3'UTR	C/T	0.39	r2=0.94 (rs1053639)
FKBP5	rs9380526	6:35658327	RR	C/T	0.34	
	rs11545925	6:35542526	3'UTR	T/A	0.05	
KIAA0101	rs11635527	15:64675313	RR	C/T	0.15	
MCL1	rs9803935	1:150552622	RR	T/G	0.4	
NFKB1	rs11940017	4:103420759	RR	T/C	0.13	
	rs28362491	4:103422155-103422158	RR	ATTG/del	0.418	
	rs3774932	4:103424193	Intron 1	A/G	0.4	
	rs1599961	4:103443569	Intron 2	G/A	0.44	
	rs3774956	4:103508526	Intron 11	C/T	0.45	
	rs1609798	4:103537442	Intron 17	C/T	0.29	
NFKB2	rs17114509	10:104154326	5'UTR (exon1)	G/del	0.35	
	rs36226954	10:104155345	Intron 1	T/C	0.02	
	rs11574849	10:104159696	Intron 13 /14	G/A	0.08	
	rs7897947	10:104157711	Intron 7/8	T/G	0.28	
NFKBIA	rs3809446	14:35875377	RR	A/G	0.21	
	rs2233409	14:35874270	RR	G/A	0.16	
	rs1957106	14:35873770	Exon 1	G/A	0.24	
	rs8904	14:35871217	3'UTR (exon6)	G/A	0.42	
NR3C1	rs10052957	5:142786701	Intron 1	G/A	0.25	
	rs10482605	5:142783521	Intron 1	A/G	0.12	r2 = 0.958 (rs6198)
	rs41423247	5:142778575	Intron 1/2	G/C	0.28	

	rs6198	5:142657621	3'UTR	T/C	0.09
NUSAP1	rs10518718	15:41624224	RR	G/A	0.1
	rs7178777	15:41634588	5'UTR	C/A	0.26
	rs10518717	15:41623368	RR	C/G	0.12
	rs7168431	15:41672384	3'UTR	A/G	0.13
SNF1LK	rs587087	21:44835301	3'UTR	G/A	0.14
	rs3366	21:44834825	3'UTR	G/T/A/C	0.21
	rs229343	21:44836213	3'UTR	C/T	0.44
	rs3746951	21:44846016	Exon 2	C/T	0.2
	rs17004546	21:44840138	Exon 8	G/A	0.03
	rs73380290	21:44835347	3'UTR	G/A	0.09
SOCS1	rs193778	16:11351211	RR	A/G	0.14
	rs243330	16:11350991	RR	C/T	0.44
TXNIP	rs7212	1:145442254	3'UTR	G/C	0.19
	rs2236566				
	rs17221744				
YWHAQ	rs10203320	2:9771620	RR	T/C	0.2
	rs2091210	2:9771200	RR	C/G/A	0.38
YWHAB	rs3092669	20:43513431	RR	C/G	0.32
ZBTB16	rs1350248	11:113928482	RR	C/A	0.14
	rs11214857	11:113929550	RR	A/G	0.34
	GWAs genes				
ACP1	rs12714403				
GRIN3A	rs10989692				
PROXI	rs1891059				
SH3YL1	rs4241316				
	Whole exome sequencing				
ABCC1	rs45511401	16: 16173232			
ABCC3	rs9890046	17: 48712711			
	rs11568591	17: 48761053			
	rs12604031	17: 48712705			
ABCC4	rs2274407	13: 95859035			
ACP1	rs11553746	2: 272203			

ADORA3	rs35511654	1: 112042787
APOB	rs676210	2: 21231524
	rs679899	2: 21250914
	rs1367117	2: 21263900
APOE	rs440446	19: 45409167
	rs7412	19: 45412079
BAD	rs2286615	11: 64039175
GSTM1	rs147668562	1: 110231746
HSP90AA1	rs8005905	14: 102568367
MTHFR	rs1801133	1: 11856378
MTRR	rs1805087	1: 237048500
	rs10380	5: 7897191
	rs1801394	5: 7870973
NCOA3	rs2230782	20: 46264888
NCOR2	rs2229840	12: 124826462
PIK3CA	rs2230461	3: 178927410
PPARA	rs1800206	22: 46614274
PPARGC1A	rs3736265	4: 23814707
SERPINA6	rs2228541	14: 94776221
SHMT1	rs1979277	17: 18232096
SLC22A1	rs12208357	6: 160543148
SLC22A2	rs316019	6: 160670282
SLC22A4	rs272893	5: 131663062
SLCO1A2	rs11568563	12: 21457434
SLC22A4	rs1050152	5: 131676320
SLCO1B1	rs4149056	12: 21331549
SLCO1B3	rs60140950	12: 21028208
SLCO1C1	rs6487138	12: 20905250

Table A-2. The polymorphisms in relation to the osteonecrosis in QcALL cohort

GENE/RS/ MODELS ON		NO FILTER	CORTICOSTEROIDS		AGE, years		SEX		RISK GROUPS	
			PDN	DXM	< 10	≥ 10	Female	Male	Standard	High
ACP1 rs11553746	REC	0.126	0.333	0.074	0.524	0.128	0.110	0.552	0.127	0.009
	RCS	0.069	0.948	0.024	0.553	0.043	0.038	0.551	0.338	0.003
	DOM	0.172	0.155	0.694	0.278	0.495	0.372	0.299	0.125	0.688
ABCB1 rs3789243	REC	0.049	0.100	0.298	0.363	0.043	0.007	0.889	0.168	0.251
	RCS	0.463	0.210	0.840	0.662	0.522	0.364	0.870	0.264	0.899
	DOM	0.014	0.040	0.188	0.156	0.012	0.002	0.628	0.064	0.122
BCL2L11 rs2241843	REC	0.033	0.194	0.104	0.046	0.642	0.056	0.354	0.230	0.117
	RCS	0.382	0.271	0.961	0.276	0.850	0.288	0.837	0.674	0.378
	DOM	0.009	0.080	0.049	0.013	0.449	0.017	0.165	0.089	0.038
BCL2L11 rs724710	REC	0.094	0.399	0.159	0.123	0.777	0.347	0.273	0.532	0.011
	RCS	0.188	0.254	0.490	0.168	0.718	0.420	0.296	0.279	0.443
	DOM	0.038	0.283	0.055	0.061	0.478	0.157	0.130	0.926	0.0027
NFKB1 rs3774932	REC	0.109	0.940	0.004	0.347	0.311	0.044	0.162	0.899	0.030
	RCS	0.036	0.941	0.001	0.148	0.127	0.042	0.300	0.647	0.008
	DOM	0.556	0.764	0.668	0.748	0.615	0.042	0.237	0.914	0.395
PARP1 rs7531668	REC	0.074	0.029	0.662	0.077	0.728	0.022	0.004	0.060	0.679
	RCS	0.101	0.026	0.584	0.166	0.433	0.974	0.001	0.180	0.384
	DOM	0.048	0.039	0.551	0.037	0.673	0.010	0.796	0.024	0.724
SHMT1 rs1979277	REC	0.412	0.814	0.156	0.673	0.313	0.013	0.144	0.414	0.255
	RCS	0.186	0.687	0.057	0.422	0.136	0.016	0.524	0.520	0.211
	DOM	0.538	0.541	0.766	0.527	0.796	0.462	0.127	0.189	0.575

Osteonecrosis (ON), prednisone (PDN), dexamethasone (DXM), Recoded (REC) as 3 genotype groups, homozygous for major allele, heterozygous and homozygous for minor allele; recessive (REC) and dominant (DOM) models

ANNEXE II

Figure A-1. Relation between +1248G>A polymorphism and risk of ON in DXM group, QcALL cohort.

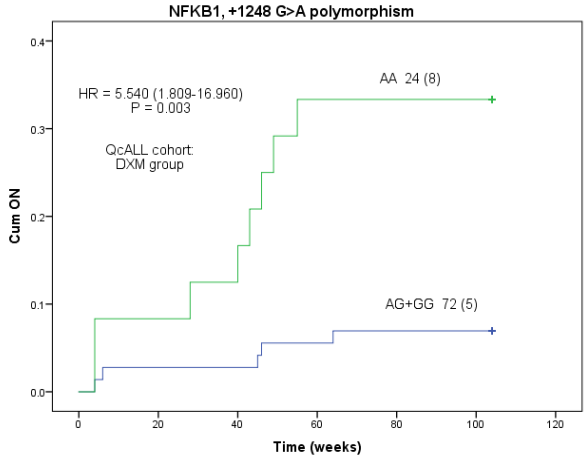


Figure A-2. Relation between -219A>T polymorphism and risk of ON, in PDN group, QcALL cohort.

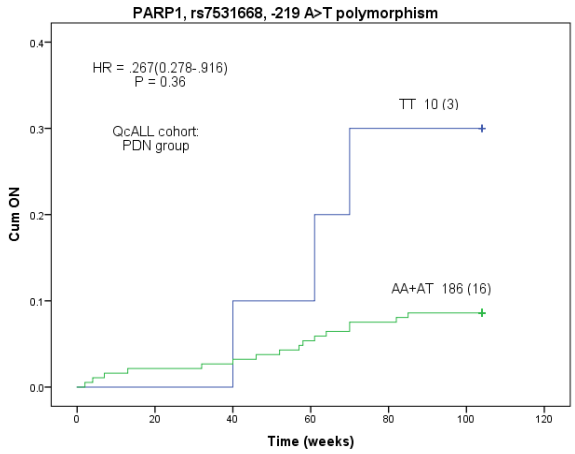


Figure A-3. Relation between +4196G>A polymorphism and risk of ON in female group, QcALL cohort.

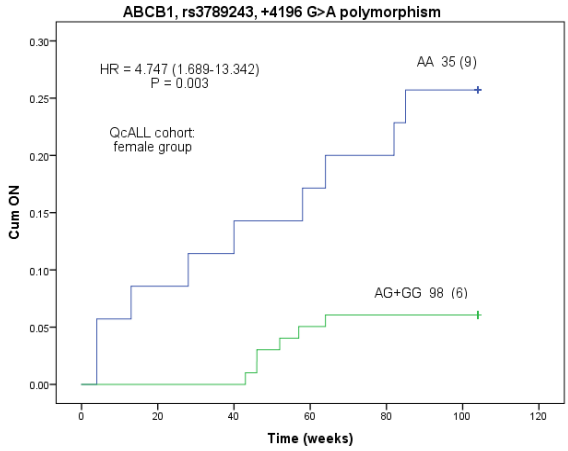


Figure A-4. Relation between 380C>T polymorphism and risk of ON in high risk group, QcALL cohort.

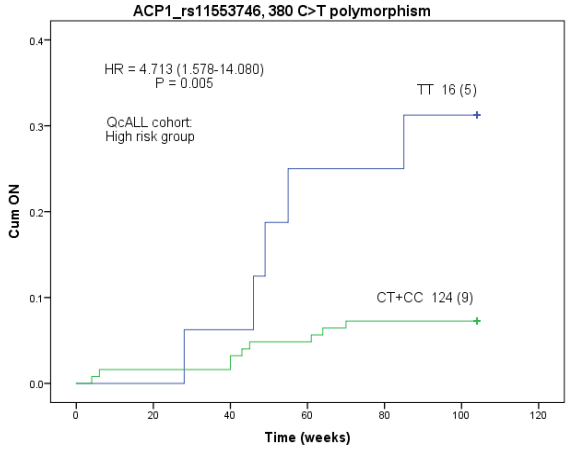


Figure A-5. Relation between 1420G>A polymorphism and risk of ON in high risk group, QcALL cohort.