Promising genes and variants to reduce chemotherapy adverse effects in acute lymphoblastic leukemia

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A B S T R A C T

Almost two decades ago, the sequencing of the human genome and high throughput technologies came to revolutionize the clinical and therapeutic approaches of patients with complex human diseases. In acute lymphoblastic leukemia (ALL), the most frequent childhood malignancy, these technologies have enabled to characterize the genomic landscape of the disease and have significantly improved the survival rates of ALL patients. Despite this, adverse reactions from treatment such as toxicity, drug resistance and secondary tumors formation are still serious consequences of chemotherapy, and the main obstacles to reduce ALL-related mortality. It is well known that germline variants and somatic mutations in genes involved in drug metabolism impact the efficacy of drugs used in oncological diseases therapy. So far, a broader spectrum of clinically actionable alterations that seems to be crucial for the progression and treatment response have been identified. Although these results are promising, it is necessary to put this knowledge into the clinics to help physician make medical decisions and generate an impact in patients' health. This review summarizes the gene variants and clinically actionable mutations that modify the efficacy of antileukemic drugs. Therefore, knowing their genetic status before treatment is critical to reduce severe adverse effects, toxicities and life-threatening consequences in ALL patients.

Introduction

Acute lymphoblastic leukemia (ALL) is the commonest childhood cancer worldwide, accounting for approximately 80% of all childhood leukemias [1,2] and more than 349,000 newly cases were diagnosed in 2018 over the world [3]. ALL is a serious problem for developing countries, especially in Latin America, where the incidence of lymphoid leukemias is among the highest in the world and overall survival (OS) rates are lower compared to high-income countries (HIC) [4–7]. Currently, with risk-adapted stratification of patients, the development of targeted therapies in combination with conventional chemotherapy and the identification of novel molecular subtypes, OS in childhood ALL from HIC exceeds 90% at 5-years of follow-up but it is significantly lower in developing countries [5,8]. Furthermore, some patients relapse during early phases of therapy or develop resistance to treatment (either intrinsically or acquired resistance), and even those cases who are considered cured could suffer lifelong sequelae due to chemotherapy [9,10]. These survival rates disparities between racial groups are also evident in developed countries, as it has been observed in Hispanics in the United States, where high-risk clinical features and lower outcomes are common [11,12]. It is well known that social and demographical aspects like low quality of care due to deficiencies in health infrastructure have a direct impact in mortality rates. Nonetheless, biological factors such as genetic variants and epigenetic processes are also determinant in the disease progression and in treatment response [7]. In fact, these aspects can also explain inter-individual and inter-population variations in the antileukemic drugs metabolism [11]. Identifying genes involved in drug response variability, also known as pharmacogenes, and detecting their genetic variation that influence both pharmacological efficacy and treatment toxicity, represents a big challenge [13].

The development of high throughput technologies in the last decade such as microarrays and next generation sequencing have allowed us to increase our knowledge about the genetic alterations that modulate drugs efficacy for many human diseases, particularly in cancer [14,15]. Due to their biological impact in the protein activity, studies of common germline variants as single nucleotide polymorphisms (SNPs) and copy number variants (CNVs) in pharmacogenes, in addition to somatic
mutations (mainly point mutations, gene deletions and gene fusions) have been the major targets of pharmacogenomics studies. Thiopurine S-methyltransferase (TPMT), nudix hydrolase 15 (NUDT15), solute carrier organic anion transporter family member 1B1 (SLCO1B1), ATP Binding Cassette Subfamily B Member 1 (ABCB1), retinoid acid receptor gamma (RARG), solute carrier family 28 member 3 (SLC28A3), and UDP glucuronosyltransferase family 1 member A6 (UGT1A6^4) are some examples of the potential useful pharmacogenes. Incorporating other relevant genes like 5’-Nucleotidase, Cytosolic II (NT5C2), phosphoribosyl pyrophosphate synthetase 1 (PRPS1) and Nuclear Receptor Subfamily 3 Group C Member 1 (NR3C1) into the ALL treatment is still a pending task, as these genes are frequently mutated in relapsed ALL and there is evidence that highlights their usefulness in clinical applications. This review summarizes the current understanding and emerging insights of genes and treatment in ALL.

Diagnosis, risk classification and treatment

ALL is a fast-growing malignancy originated from lymphoid progenitor cells in the bone marrow and comprises a highly clinical and genetically heterogeneous disease. Presenting symptoms are non-specific; however, fever, bruising, skin pallor, petechia, bone pain, weakness, night sweats, and weight loss are common in ALL pediatric patients. More than a half of the cases have hepatosplenomegaly, mental changes and oliguria; testicular enlargement and mediastinal mass could be also present [16]. Diagnosis of ALL is established by morphological and immunophenotypical features of the bone marrow cells aspirate. A leukemic blasts percentage ≥25% confirms ALL disease, while flow cytometry identifies precursor B-cell, T-cell, and mature B-cell immunophenotypes, in 80%, 15%, and 5% of the pediatric patients, respectively [17].

Since decades ago, diverse protocols have been designed to treat leukemia, but the treatment based on the patients’ risk for relapse gives the best OS rates. Based on clinical features (age and leukocyte count), immunophenotypical and genetic characteristics of the leukemic cells, current treatment regimens stratify childhood ALL patients in risk groups (as example, St Jude protocol identifies low/ standard risk, high risk and very high risk groups) to reduce toxicity. The standard treatment based on chemotherapy is indicated for the low risk-group. Meanwhile, in addition to chemotherapy, protocols that include radiation therapy, hematopoietic stem cell transplant (HSCT), targeted therapy (like tyrosine kinase inhibitors or TKIs) and chimeric antigen receptor (CAR) T-cell therapy are suitable for high risk and very high risk groups [5,18]. All protocols comprise three phases: a) remission induction (0–28 days) to restore normal hematopoiesis, b) consolidation (29–60 days) and c) maintenance (2 < 36 months), whose purpose is to eradicate residual leukemic cells. Patients who do not reach remission during the first month or relapse after the completion of primary treatment, undergo a second remission induction phase. In all cases, drugs such as vincristine, asparagine, daunorubicin, glucocorticoids, cyclophosphamide, cytarabine and methotrexate are administered during the first two phases of therapy, and nucleoside analog drugs as thiourines (mercapto-purine and thioguanine) are central agents in the maintenance phase (Fig. 1) [10,19].

Germline variants, mutations and treatment response

The current OS among ALL cases has dramatically improved with the increasing knowledge of the genetic background of ALL patients and its association with the treatment response. Novel information about the genomic features of leukemic cells allowed us to identify specific molecular targets. It is well known that variants and mutations in genes influencing the drug absorption, distribution, metabolism and excretion explain the differences in drug response between patients. In this regard, BCR-ABL, TPMT, NUDT15, among others, have been recognized as relevant contributors to improve clinical management of ALL cases (Fig. 2).

TPMT

Thiopurines as 6-thioguanine (6-TG), 6-mercaptopurine (6-MP) and azathioprine are commonly used in the treatment of many types of cancer, autoimmune diseases, and critical in the maintenance phase for ALL patients. Thiopurines are prodrugs metabolized into thioguanine nucleotides (TGN), which are purine analogs used to arrest the cell cycle and to induce apoptosis by TG incorporation into the DNA [20]. One of the key genes involved in thiopurine metabolism is TPMT, which encodes for the enzyme that catalyzes the S-methylation of thiopurine drugs. Over 37 SNPs have been associated with decreased enzyme activity compared to the wild-type gene (TPMT^1) and variable number of tandem repeats (VNTRs) in the promotor region have been found as correlated with TPMT low expression [20,21]. The rs1800462 (p.Ala80Pro), rs1800460 (p.Ala154Thr) and rs1142345 (p.Tyr240Cys) SNPs are the most common variants worldwide and the mutant alleles TPMT^2 (rs1800462), TPMT^3A (rs1800460 and rs1142345), TPMT^3B (rs1800460) and TPMT^3C (rs1142345) have significant evidence on their clinical utility [22,23]. Patients who are homozygous or compound heterozygous for mutant TPMT alleles display a nonfunctional TPMT enzyme, which leads to high levels of TGN and risk to develop severe myelotoxicity during treatment with standard doses of thiourines. Meanwhile, patients with only one non-functional allele could likely experience toxicity, thus, the Food and Drug Administration (FDA) strongly recommends a reduction from 30 –70% and up to 10-fold reduction of daily doses in patients with two nonfunctional alleles [24]. Besides the mutant alleles of TPMT, it has been reported that homozygous of VNTR 5’a/5’a show high expression levels of the enzyme, while those carrying VNTR 7th allele had lower TPMT expression [20]. This could have an impact by compensating TPMT in patients who are intermediate metabolizers by modulating gene expression levels based on VNTR architecture. TPMT genotypes have also been associated with event-free survival (EFS), as carriers of one TPMT mutant allele (heterozygous) have better EFS than the TPMT^2 patients. It is possible that high intracellular accumulation of TGNs in heterozygous patients could influence the high efficacy of the treatment of these cases [25]. Otherwise, evidences suggest that low or absent TPMT activity increase the risk to develop secondary tumors but reduce the risk to relapse [26].

Despite the importance of TPMT genotyping and knowing that certain risk alleles are well established as markers of potential thiourine toxicity, patients homozygous for the WT allele might have adverse effects too. The importance of other genes involved in thiopurine metabolism like NUDT15 has been addressed, which also has a determinant role in the metabolism of thiopurine drugs.

NUDT15

NUDT15 gene encodes for a member of the nudix hydrolase enzyme family that converts thioguanine triphosphate (TGTTP) to thioguanine monophosphate (TGMPP) by negatively regulating the activation of thiopurines. Genetic variants in NUDT15 like rs116855232 (p.Arg139Cys), rs147390019 (p.Arg139His), rs186364861 (p.Val18Ile), rs869320766 (p.Val18_Val19insGlyVal), p.Lys356_Cys (no rsID), rs746607156 (p.G17_V18del) and the recently described rs766023281 (p.Arg34Thr) have been associated with a deficient activity of the enzyme, which causes poor metabolism of thiouropurines, elevated levels of toxic metabolites and myelosuppression. These variants either affect the protein stability or their interactions with its substrate, resulting in a reduction from 75 to 100% of NUDT15 enzymatic activity [27,28]. As example, Moriyama et al., observed that p.G17_V18del variant derives in a protein with low thermostability displaying total loss of its catalytic activity [29]. The p.Arg139Cys also seems to relate with the stability of the protein, particularly in an a-helix at the thiopurine binding pocket.
Fig. 1. Chemotherapeutic treatment schedule for pediatric acute lymphoblastic leukemia. Different chemotherapeutic agents in different doses are administered depending on patient’s classification risk in each of the treatment’s phases: remission induction (0–28 days), consolidation (29–60 days) and maintenance (>2 - 36 months). The selection of these agents is also defined by patient’s response and acute lymphoblastic leukemia evolution in which mutation of specific genes are known to be involved.

site [30]. NUDT15 polymorphisms also show differential distribution among populations [28,31,32]. For instance, the p.Arg139Cys variant (T risk allele), significantly associated with 6-MP-induced leukopenia, has been found in 9.8% in East Asians, 3.9% Hispanic populations, but very rarely in Europeans and non-existent in African populations [32]. Meanwhile, the p.Arg34Thr, p.Lys35Glu, and p.G17/V18del have been detected only in European and African patients [29]. Although their effect along with SNP in other genes involved in thioguanine metabolic pathways remains poorly studied, it is suggested that NUDT15 genotyping is useful to standardize the tolerated dose of 6-MP in children with ALL [33]. It is recommended a reduction of 50% of the normal dose in those cases with at least one NUDT15 deficient allele [31–34].

RARG

Retinoic acid receptors (RAR) bind to DNA regulatory sequences termed retinoic acid response elements (RARE) and transcriptionally co-regulate downstream gene expression in response to their agonist all-trans retinoic acid (ATRA) [35]. RARgamma (RARG), also known as NR1B3, encodes a nuclear receptor that can both activate and repress transcription in response to ATRA [36]. This gene belongs to the nuclear receptor superfamily and shares 90% homology with the other two members, RARA and RARB [35]. RARG plays a central role in maintaining the balance between the self-renewal state of hematopoietic stem cells (HSCs) and their differentiation [37]. Anthracyclines are used in most of ALL treatment protocols and cumulative dose has been described as one of the causes of cardiac dysfunction and congestive heart failure in pediatric patients [38,39]. Aminkeng et al., reported that the nonsynonymous variant rs2229774 (p.Ser427Leu) in RARG was significantly associated with anthracycline-induced cardiotoxicity (ACT) in childhood cancer patients [40]. Although rs2229774 in RARG was more frequent in cases with European ancestry, this association was also replicated in non-Europeans [40]. This variant alters RARG function leading to derepression of the key ACT genetic determinant Topoisomerase II beta (Top2b) [40]. Anthracyclines exert their anticancer activity by binding and inhibiting Topoisomerase II [41]. The Ser427Leu allele does not repress Top2b expression as effectively as wild type RARG. In vitro studies showed that even though rs2229774 causes a well-tolerated amino acid substitution, still results in a significant reduction in transcriptional activation from RARE elements [40,42].

SLC28A3

The SLC28 family has three members of sodium-dependent, concentrative nucleoside transporters: concentrative nucleoside transporter (CNT) 1, CNT2, and CNT3 (SLC28A1, SLC28A2, and SLC28A3, respectively). These proteins are responsible for high-affinity transport of both naturally occurring nucleosides and synthetic nucleoside analogs used in the treatment of cancer and viral drugs. SLC28A3 transports both pyrimidine and purine nucleosides into cells in a sodium-dependent manner. High levels of SLC28A3 mRNA transcripts are found in the pancreas, trachea, bone marrow, and mammary gland [43]. Moreover, it has been observed that SLC28A3 can transport several anthracyclines drugs including doxorubicin into cells, providing a potential mechanism by which these variants could affect ACT. Studies have provided evidence for the association among of rs7857758 (p.Leu461Leu) and rs885004 (Leu248Leu) with ACT [40,44]. The most consistent results were found between the minor allele rs7857758 461Leu with ACT [44–46], which is also associated and with reduced SLC28A3 mRNA expression in monocytes [47]. Although scarce evidences regarding the role of this SNP in
ALL, there is a recommendation for genetic testing to all patients under anthracyclines treatment to reduce the incidence of ACT [40, 46].

**UGT1A6**

Glucuronidation is an important pathway in the detoxification and elimination of many drugs and is catalyzed by the UDP-glucuronosyltransferases (UGTs) superfamily of enzymes [48]. UGT1A6 is a phenol-specific enzyme that catalyzes glucuronidation and subsequent elimination of a diverse range of drugs, toxic xenobiotics and endogenous substrates [49]. The UGT1A family is encoded by a single nested gene locus spanning 200 kb on chromosome 2q37 [50]. The synonymous variant p.Val209Val (rs17863783) has been associated with an increased risk of ACT in pediatric patient with ALL from several populations [45, 46]. As well, rs17863783 is a tag marker of the UGT1A6 *4* haplotype, which has been reported to cause a 30–100% reduction in enzyme activity [49, 51]. Since UGT1A6 plays a determinant role in glucuronidation-mediated drug detoxification, patients carrying UGT1A6 *4* haplotype could have an increased ACT risk due to accumulation of toxic metabolites when treated with anthracyclines [42]. Recently, the Canadian Pharmacogenomics Network for Drug Safety (CPNDS) Clinical Practice Recommendations Group found that UGT1A6 *4* rs17863783, RARG rs2229774 and SLC28A3 rs7853758 variants have strong evidence to define them as pharmacogenomic markers for ACT. Therefore, their pharmacogenomic testing is strongly recommended in all childhood cancer patients with an indication for doxorubicin or daunorubicin therapy [52].

**SLCO1B1**

Membrane transporters are essential in the influx and efflux of wide variety of compounds, nutrients, small molecules and ions. In cancer treatment, they play a key role in maintenance of chemotherapy drugs inside the cell or exporting them out of the cell. Among the family of transporters, solute carrier (SLC) family transporters have gained interest not only as regulators of drug transport, but as nutrient uptake mediators that promote growth and survival of cancer cells. The SLCO1B1 gene is the most explored in leukemia. SLC01B1 encodes for a liver-specific transmembrane sodium-independent transporter protein involved in the active influx of many endogenous compounds like bilirubin, hormones and drugs for their removal from the body [53, 54].

Evidences suggest that polymorphisms in SLC01B1 may impair its transporter activity for chemotherapy agents, including methotrexate (MTX) [55]. It has been reported that genetic variants in this gene substantially influence MTX transport and toxicity risk in childhood ALL [56]. Of these variants, the rs4149056 (p.Val174Ala) has been associated with SLC01B1 loss-of-function and with reduced MTX clearance [57, 58]. The rs4149056 (Val174Ala) has been significantly associated with lower event-free survival rates and persistence of minimal residual disease (MRD), and the rs10841753 with lower plasma MTX levels in pediatric ALL patients [59]. Due to most of chemotherapy protocols for childhood ALL includes MTX and it is well known that this drug may cause potentially severe adverse effects and toxicities that can be life-threatening, it is suggested the SLCO1B1 genotyping before treatments based on MTX [59, 60].
NR3C1

Glucocorticoids (GC), such as prednisone, prednisolone and dexamethasone are considered the backbone of chemotherapy regimen, and they were some of the first drugs used in ALL treatment. Given their immunosuppressor effects, they are also used to treat autoimmune-mediated diseases such as asthma, rheumatoid arthritis and lupus, showing their relevance in the pharmacogenomics field [61]. The knowledge about GC as cytotoxic agents derives mainly from the treatment of childhood leukemia [62]. These drugs exert their cytotoxic function by binding to the glucocorticoid receptor (GR), a nuclear receptor ligand-activated transcription factor encoded by the NR3C1 gene. GR dimerizes and translocates to the nucleus, leading to the expression of genes involved in cell survival, proliferation and glucose metabolism, among others biological processes [63,64]. Patients that display GC resistance during the treatment have an increased risk of relapse, making GC response as one of the most useful prognostic factors in ALL [65]. The mechanism underlying lymphocytes GC cell death induction is not well understood; however, evidences show that SNPs and somatic mutations in NR3C1 could contribute to GC resistance in ALL patients. GR polymorphisms rs56149945 (p.Asn363Ser and rs41423247 have been associated with corticosteroid response and high sensitivity to methylprednisolone in human lymphocytes in vitro and prednisone response [66–68], but also with side effects derived (as hepatotoxicity) from GC-based treatment and disease outcome in childhood ALL [68–71]. Somatic NR3C1 gene have been detected at diagnosis and conserved at relapse in some cases. In fact, 10% of ETV6-RUNX1 children with poor response to induction treatment have deletions in NR3C1 [72]. Additionally, NR3C1 is found frequently mutated in relapsed ALL and all mutations identified so far are truncated mutations (e.g., p.D144Efs x11) inducing haploinsufficiency of the NR3C1 protein [73]. Experimental evidences showed that GCR defects could be decisive for glucocorticoid resistance, and that ectopic expression of GCR abates GC resistance in ALL cell lines and xenograft models with NR3C1 haploinsufficiency [74,75]. It has been suggested that the Bcl-2 blocking to induce GC response in NR3C1 mutated ALL cells, as Bcl-2 is the major negative regulator of NR3C1 [75].

ABCB1

ATP Binding Cassette Subfamily B Member 1 (ABCB1) encodes for the transporter protein MDR1 (multidrug resistance 1), also called P-glycoprotein (P-gp). It is responsible of xenobiotics transport, acting as an ATP-dependent drug efflux pump and therefore contributing to chemoresistance by the active transport of drugs outside of the cell, decreasing the intracellular levels of chemotherapy agents. Among the 50 SNPs identified in this gene; the rs1045642 (C3435T), a synonymous variant at codon 1142 (Ile1142Ile) is the most widely studied [76–78]. Minor allele 3435T has been associated with decreased mRNA expression, altered substrate specificity and low protein stability [79–81], ultimately causing a reduced transporting activity [82]. Although there is differential distribution among populations, studies in ALL patients have reported that carriers of the 3435T allele have higher risk of ALL development, bone marrow toxicity and lower EFS after doxorubicin, vincristine and prednisolone administration in comparison with homozygotes to the wild type allele [77]. Otherwise, patients with homozygote CC genotypes have higher risk to liver toxicity after high doses of MTX treatment [77,83–85]. Other variants like the rs128503 (C1236T) and rs2229109A (1199G>A) have been associated with high risk to develop ALL and relapsed ALL, respectively [77,85]. Low survival of ALL patients has been also observed in cases with high ABCB1 gene expression; however, it has been reported that these patients have better outcome after using methotrin alongside chemotherapy [86].

BCR-ABL1-Positive and Ph-like phenotype

The treatment of BCR-ABL1-Positive and Ph-like is one of the most successful examples of genetic and genomic characterization of novel subtypes and their molecular features in ALL. The use of tyrosine kinase inhibitors (TKIs) as first-generation TKI like imatinib, second- and third-generation TKIs (dasatinib, nilotinib, bosutinib and ponatinib) and other kinase inhibitors (ruoxlitinib for JAK-driven alterations) in combination with cytotoxic chemotherapy have substantially increased OS and reduced the need of HSCT during the first remission in BCR-ABL1 (Ph+ ALL) patients [87,88]. TKIs are also beneficial to the Ph-like subtype, a recently described molecular subtype of B-ALL that exhibits a gene expression profile similar to BCR-ABL1+ patients but lacks this recurrent fusion gene. Concomitant loss-of-function mutations in IKZF1 and CDKN2A/B are frequent in Ph-like cases, but mutations that activate cytokine receptors (CRLF2, PDGFRB, CSFIR) and kinase signaling pathways (Janus kinases, ABL1, ABL2) are the hallmark of this subtype, which is also associated with poor prognosis and Hispanic ethnicity [89]. Ph-like phenotype shows different frequencies among age and risk groups, being less common in children (standard risk: 10% and high risk: 15%) than adolescents (21%), young adults (>27%), and older adults (>40 years old: 10–20%) [89–91]. Although Ph-like is a clinically aggressive subtype (hyperleukocytosis at diagnosis and persistent MRD), these patients also benefit with TKIs treatment [90,92]. Resistance to TKIs during the first-line or subsequent-line therapies has been documented, albeit the patterns of kinase domain (KD) mutations in Ph-positive pediatric ALL are still unknown. BCR-ABL adults with chronic leukemia and ALL have acquired point mutations (T315I, G250E, Q252H, Y253H/F, E255K/V) that induce TKI resistance [93,94]. KD mutations are also associated with shorter OS; therefore, the identification of BCR-ABL1 subclones could improve the therapeutic management based on TKIs in BCR-ABL1+ patients.

Pharmacogenes associated with ALL relapse

Although major improvements in ALL treatment have been achieved, relapsed ALL occurs in around 15–20% of childhood patients around the world. In fact, if considered as a separate entity, relapsed ALL represents the fourth most common childhood cancer and one of the leading causes of cancer-related deaths in children [95]. Leukemic blasts at relapse are usually more resistant to chemotherapy than blasts at diagnosis, and clones could be either selected by the treatment (present as minor subpopulations at diagnosis) or arise from different subclones with chemotherapy-acquired mutations [96]. Relapse-specific mutations have been described in over 12 genes, 46% of them in NTSC2, PRPS1, NR3C1, and TP53 [97,98]. Notably, NTSC2 and PRPS1 are involved in thiopurines metabolism and NR3C1 in glucocorticoids drug response (described above), showing that relapse could be induced by additional mutations acquired during chemotherapy treatment [98]. Relapsed ALL prognosis is dismal and developing strategies to treat these patients is of utmost importance. In this regard, NTSC2 and PRPS1 rise as promising therapeutic targets [97,100].

NTSC2

NTSC2 gene encodes a 5′-nucleotidase enzyme that catalyzes the 5′-dephosphorylation of purine nucleotides and is involved in the intracellular nucleoside pool homeostasis by facilitating the exportation of purine nucleotides out of the cell [101]. NTSC2 also metabolizes nucleoside-analog drugs including 6-MP and 6-TG, which constitute the backbone of maintenance phase of chemotherapy. NTSC2 mutant proteins show increased nucleotidase activity in vitro, inducing depletion of the purine-nucleotide pool inside the cell and resistance to nucleoside analogs drugs [102,103]. Over 32 mutations in NTSC2 have been described and at least 25 different mutation specifically in relapsed ALL [104]. Gain-of-function mutations are common events at
relapse in both B-ALL and T-ALL, especially in early relapses (from 3 to 45% and 19 to 38% for relapsed B-ALL and T-ALL cases, respectively) [97,98,102,103,105,106]. Although a wide array of NTSC2 mutations is known, their mechanisms of activation differ between them. Three classes of mutations are proposed, the most frequent being mutations that disrupt the switch-off mechanism of NTSC2 [107]. Structural and biochemical studies showed that leukemic cells carrying the most common mutation, p.R367Q (c.1100G>A) display excessive exportation of purines and causes loss-of-function phenotype, cell growth impairment and leukemia-initiating cell activity [106–109]. Other mutations like p.K359Q act in a constitutively active state, leading to excessive nucleotidase activity and poor prognosis [107,109]. As well, it has been reported that 90% of mutations described in leukemia are located into 3 hot spots (residues 232–242, 355–365, and 400–418) and in silico analysis suggests that these mutations drive chemoresistance and relapsed in leukemia [104]. Evidence proposes that NTSC2 mutations driving chemotherapy resistance arise during the late events of the clonal evolution of relapsed ALL, pointing out the NTSC2 inhibitors as promising targets to switch the thiopurine resistance due to gain-of-function NTSC2 mutations [107,109,110].

PRPS1

The ribose-phosphate diphosphokinase 1 encoded by PRPS1 gene is a rate-limiting purine biosynthesis enzyme, crucial component of the de novo synthesis of purine and pyrimidine nucleotides but also essential in the purine salvage pathway, which recycles the breakdown of nucleic acids for their synthesis. More than 25 pathogenic mutations in PRPS1 have been found in human diseases. In leukemia, these mutations lead to constitutive purine biosynthesis, accumulation of purine nucleotides inside the cells and drug resistance. Experimental studies showed that PRPS1-mutant cells have an increased synthesis of inosine monophosphate metabolites that competitively inhibit the conversion of thiopurines into 6-MP (active metabolites) [111,112]. Gain-of-function mutations and increased levels of PRPS1 could also facilitate the conversion of 5-Fluorouracil (5-FU) (a pyrimidine analog used for several types of cancers) to their active metabolites (fluorodeoxyuridine monophosphate, fluorodeoxyuridine triphosphate and fluorouridine triphosphate), subsequently triggering genomic damage and apoptosis [113]. Therefore, ALL patients who carry activating mutations could benefit from 5-FU administration during therapy [100]. Whole exome sequencing analysis revealed PRPS1 mutations in 2.7% and 13% of relapsed B-ALL cases from Germany and China, respectively, being p.Ala190Thr and p.Ser103Thr the most common. All patients carrying PRPS1 mutations had early relapse during treatment and poor prognosis [111]. Furthermore, increased expression of PRPS1 has been associated with high white blood cell count, positive MRD and higher risk for relapse [99]. These studies underline the importance of PRPS1 as a novel therapeutic target in ALL treatment.

Targeted B-lineage receptor genes in relapsed/refractory ALL

The lineage-specific surface molecules like CD19, CD20 and CD22 are several targets of the current approaches for relapsed/refractory ALL [114]. These cell surface molecules are found in the majority of B-lineage leukemias and lymphomas [115–117]. Along with CD19 and prior to the expression of CD20, CD22 is detected at B-cell differentiation earliest stages [118]. Today, these molecules are considered potential targets of pharmaceutical companies, research organizations and clinical trials to develop new therapies that target tumor cells at both the cellular and genetic levels. Two examples of these therapies are the antibody-based drugs and chimeric antigen receptor T-cells (CAR T cells) therapy, which are opening a new era of hematological cancers treatment, especially for NCI high-risk groups, adolescents and young adults (AYAs) ALL [119–121].

CD19

The CD19 antigen is the most ubiquitously expressed marker in B-cells. CD19 expression initiates during B-lineage commitment from hematopoietic stem cells, continuing along the B-cell differentiation process until its down-regulation in the plasma cell stage [122]. Due to their differentiation arrest, CD19 expression is maintained, and commonly up-regulated, in B-lymphoid leukemias and lymphomas [123]. Based on this knowledge, treatment targeting CD19 have been developed and two of them are approved by the FDA, blinatumomab and tisagenlecleucel (CAR T-cell therapy) (Fig. 3) [120,121].

Blinatumomab is a type of immunotherapy treatment for relapsed/refractory and MRD+ precursor B-cell ALL. It consists in a chimeric T-cell-engaging (BITE) antibody designed to redirect cytotoxic T-cells for lysis of the target cell [124,125]. Blinatumomab has two antibody arms, one anti-CD19 directed to B-lymphoblasts and one anti-CD3 that engages the T-cells, leading to activation and proliferation of T-cells and inducing apoptosis of B-lymphoblasts [126,127]. Blinatumomab has proven efficacy in adults, and in recent years it has also been evaluated in children and AYAs [120,128–133]. As many immunotherapy approaches, a downside of blinatumomab is the selection of CD19-negative leukemic clones and therapy resistance as a result. Combination of blinatumomab with second- or third-generation TKIs can overcome resistance by targeting CD19-negative BCR-ABL1+ clones [134,135]. These results look promising so far, but larger-scale clinical trials are needed to test the incorporation of blinatumomab in children with relapse/refractory ALL, children with persistent MRD and for patients who do not tolerate well the cytotoxic therapies [136,137].

Tisagenlecleucel (CTL019), also targeting CD19, is a CAR T-cells therapy approved by FDA in 2017 to treat diffuse large B-cell lymphoma, and refractory, second and later relapsed ALL (Fig. 3). Tisagenlecleucel induces a cytotoxic response of T-cells and complete remission rates as high as 80% can be achieved in children and AYAs B-ALL [120,121,127]. In fact, patients with unfavorable genetic features have reached good remission rates when treated with tisagenlecleucel, and these remission rates are even comparable to cases with good-prognosis genetics [138]. The main obstacles of CAR-T cells therapy include the development of cytokine release syndrome, infections, neurological toxicities, and its high cost of manufacture since it is individualized for each patient [139].

CD22

Most immunotherapy drugs were designed for patients with persistent disease, either as MRD positivity, intolerance to cytotoxic chemotherapy and unsuccessful remission. Inotuzumab/ozogamicin (InO) is a monoclonal anti-CD22 antibody linked to calicheamicin approved for adults with relapse/refractory B-ALL [140,141]. InO efficacy in adults is proven, but studies in childhood and AYAs ALL are still lacking. The first study in children showed complete remission rates in 67% of patients, irrespective of genetic subtype or prior treatment [142]. A recent phase II trial from Children’s Oncology Group Protocol (AALL1621) reported a complete response in 58% of children and AYAs with relapse/refractory CD22+ B-ALL, and 65.4% of InO responders achieved MRD levels below 0.01% [143]. InO is generally well tolerated at the same doses as adults and clinical outcome has been outstanding so far, even for children with multiple relapses who did not respond to other therapeutic approaches [144].

Other pharmacogenes as future possibilities in the field of ALL treatment

The complex genomic and epigenomic background of ALL and the numerous factors involved in leukemogenesis and relapse exhibit the difficult pathways towards the identification of new therapeutic targets for ALL. Advances in treatment of other hematological diseases (as myeloid leukemia and lymphomas) and solid tumors have increased our
possibilities to treat ALL. As example, it has been reported that certain mutations in the granulocyte colony stimulating factor (G-CSF) gene significantly reduce the anti-leukemic potential after ibritinib treatment, a Bruton’s tyrosine kinase (BTK) inhibitor (FDA-approved) [145–147]. BTK has been found abnormally expressed in ALL [148,149] and pre-B cells have shown high sensitivity to ibritinib in preclinical models of B-ALL [148]. Other example is survivin, an apoptosis inhibitor and cell cycle regulator that is expressed in many types of tumors (including ALL) and is associated with chemoresistance [150,151]. Several clinical trials have found survivin as a therapeutic target to ALL [152–154]. In the same way, the BCL-2 gene is another example of a potential pharmacogen in ALL. This gene was initially discovered as relevant in the pathogenesis of follicular lymphoma, and as a contributor to the pathophysiology of diverse hematological malignancies. Based on observations derived from acute myeloid leukemia and chronic lymphocytic leukemia with 17p deletions and p53 mutations [155], the use of inhibitors of the anti-apoptotic protein BCL-2 (Venetoclax) to treat ALL subtypes carrying p53 deletions (as hypodiploid ALL that has a dismal prognosis) is currently undergoing [156–158]. Venetoclax plus chemotherapy is exhibiting good efficacy in adult refractory/relapsed T-ALL [159]. Additionally, preclinical studies have shown remarkable effectivity in childhood B-ALL [160] and the first phase 1 trials are currently developing, with encouraging results (NCT03181126) [161].

Available genetic tests and drugs to optimize therapy in ALL

Before the 1960s, monochemotherapy protocols were used for all patients with ALL, but it was an incurable disease. Aminopterin (1948) followed by pituitary adrenocorticotropic hormone (1950), prednisone (1950), and mercaptopurine (1953) were the first chemotherapy agents used in ALL treatment, however the mortality rate was 100% [162–164]. During the 60’s, the effectiveness of a combination of anti-leukemic agents (including vincristine, asparaginase, daunorubicin, cytarabine, and etoposide) in the induction and maintenance of remission in children with acute leukemias was demonstrated. Treatment based on a combination of these drugs reached 50% of remission; nevertheless, all patients still relapsed [165,166]. After that, the implementation of risk-adapted therapies, which included agents FDA-approved and that were developed during the 50’s and 60’s, improved considerably the remission and OS (approx. 50%) rates [167].

Precision or personalized medicine, which has shown an outstanding development after the human genome sequencing, is considered a therapeutic approach that takes into consideration the individual needs of patients based on their genetic, biomarker, phenotype, or psychosocial characteristics. This kind of medicine distinguishes each individual from other patients with similar clinical presentations, in order to provide therapy that is best suited for each patients’ condition [168]. Precision medicine also identifies genetic variants or mutations that could specifically influence treatment response either to increase their OS and to minimize the negative effects during treatment [169]. Studies conducted in ALL to identify useful genetic variants to optimize drug therapy have provided enough evidence on their clinical utility for BCR-ABL, TPMT, NUDT15, RARG, SLC28A3, and UGT1A1*4 [170]. In fact, FDA has approved their genotyping as routine clinical analysis before specific drugs administration [42,45,49,170,171]. Table 1 enlists FDA approved test in ALL and Fig. 3 displays a timeline of the FDA-approved drugs where a genetic testing is required before its prescription [172].

Conclusions

Genomic approaches in cancer research were a turning point in precision medicine. Not only it gave us a greater insight of the biological mechanisms involved in cancer development, progression and recurrence, but also we have been able to find new ways to treat ALL patients, to develop targeted therapies and to design a more efficient classification system. Nevertheless, ALL still represents a challenge in low- and middle-income countries, where high rates of relapse and death have been reported. The promise of precision medicine to assign a treatment based on the genetic background of each patient at most, or for specific populations at least, is still a challenging task. Great progress has been made, nevertheless, precision medicine is not yet part of the daily care for patient. Therefore, it is necessary to bring this knowledge from the bench to the clinic and translate the newest findings in health improvement of ALL patients.

Declaration of Competing Interest

The authors declare no conflict of interest.

CRediT authorship contribution statement

Diego Alberto Bárcenas-López: Conceptualization, Data curation, Writing - original draft, Writing - review & editing. Diana Karen Mendiola-Soto: Data curation, Writing - original draft. Juan Carlos Núñez-Enríquez: Writing - review & editing. Juan Manuel Mejía-Arangüe: Writing - review & editing. Alfredo Hidalgo-Miranda: Writing - review & editing. Silvia Jiménez-Morales: Conceptualization, Writing - original draft, Writing - review & editing, Supervision, Funding acquisition.
Table 1

| Gene      | Variant | Biological effect                                      | Drug                       | Clinical Effect | PGx Label                                      |
|-----------|---------|-------------------------------------------------------|----------------------------|-----------------|------------------------------------------------|
| BCR-ABL1  |         | Constitutively activated tyrosine kinase. Causes abnormal activation of intracellular signal transduction pathways, leading to an unstable genome and abnormal cellular proliferation, and amplification of leukemia clones. | Tyrosine kinase inhibitors (imatinib, dasatinib, nilotinib, bosutinib, ponatinib) | Treatment response | Testing for ALL, as well to AML and CML cases at diagnosis and in positive patients under relapse. |
| RAG      | rs229774 | Reduced repression of the key ACT enzymatic activity. | Anthracyclines             | Cardiotoxicity risk (ACT) | Testing should be performed in ALL children treated with doxorubicin or daunorubicin. |
| SLC28A3  | rs785375 | Reduced SLC28A3 mRNA expression in monocytes.         |                            |                  |                                                |
| UGT1A6*4 |         | 20-100% reduction in enzyme activity.                 |                            |                  |                                                |
| TPMT      | rs1800462| Low enzyme activity.                                   | Thiopurines (Thioguanine)  | Myelosuppression high risk | Patients treated with Thiopurines               |
| NUDT15    | rs116855232, rs147390019 | Loss-of-function of NUDT15 and reduced degradation of active thiopurine nucleotide metabolites. |                            |                  |                                                |

CML: Chronic myeloid leukemia, AML: Acute myeloid leukemia, ALL: Acute Lymphoblastic Leukemia, ACT: Anthracycline-induced cardiotoxicity; PGx: pharmacogenomics/pharmacogenetics.

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