Omics-based insights into therapy failure of pediatric B-lineage acute lymphoblastic leukemia

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Abstract

B-lineage acute lymphoblastic leukemia (B-ALL) is the most common type of cancer seen in children and is characterized by a variable clinical course. Although there have been remarkable improvements in the therapy outcomes of pediatric B-ALL, treatment failure remains the leading-cause of death in 18% of the afflicted patients during the first 5 years after diagnosis. Molecular heterogeneities of pediatric B-ALL play important roles as determinants of the therapy response. Therefore, many of these molecular abnormalities have an established prognostic value in the disease. The present review discusses the omics-based revelations from epigenomics, genomics, transcriptomics and proteomics about treatment failure in pediatric B-ALL. Next it highlights the promise of the molecular aberration-targeted therapy to improve the treatment outcomes.

Introduction

Pediatric B-lineage acute lymphoblastic leukemia (B-ALL) is a malignant disease that afflicts B-lymphoblast causing an accumulation of the leukemic blasts in the bone marrow, lymph nodes and peripheral blood.[1] B-ALL is the most common type of pediatric cancer; in the USA acute lymphoblastic leukemia represents 25.4% of all types of cancer affecting children aged between 0-14 years.[2] Similarly, based on the Saudi Cancer Incidence Report (CIR) of the year 2015, which was published on Sep 2018, ALL was the predominant type of cancer in Saudi patients younger than

14 years; amongst 727 children diagnosed with cancer 176 (24.2%) were reported with ALL.[3] Although some hereditary factors have been described to elevate the risk of developing pediatric B-ALL, the main cause of the disease is still unclear.[4] While there have been remarkable developments in the treatment of pediatric B-ALL, chemotherapy-resistance and relapse remain the leading-cause of death in 13-18% of children with B-ALL.[5,6]

In contrast to studying molecules of interest (genes, transcripts including miRs or proteins) individually using single-target assays,[7] omics approaches allow the characterization of a large number of molecules in a single experiment.[8] Therefore, omics techniques provide a valuable opportunity to almost comprehensively study the molecular biology of malignant cells.[9] The knowledge of the molecular basis of cancer can be then used to develop markers for diagnosis/prognosis and targets for therapy.[10] The omics-findings reported by epigenomics, genomics, transcriptomics and proteomics about unsuccessful treatment of childhood B-ALL provided useful clues about the molecular defects that underpin the chemotherapy-resistance and relapse. Furthermore, these findings identified candidates that may serve as predictive biomarkers of the treatment outcomes and/or therapeutic targets for improved treatment in children with B-ALL. The present review familiarizes the reader about the clinical outcomes of childhood B-ALL and the impact of the molecular aberrations on the prognosis of the disease. Next, it discusses the major omics-based findings about the poor therapy outcomes of the disease. Finally, it highlights the potential of novel treatment modalities that target the molecular drivers of drug-resistance and relapse in order to improve the therapy outcomes in patients with childhood B-ALL.

Clinical outcomes of pediatric B-ALL

The clinical outcomes of pediatric B-ALL improved significantly in the last two decades. A study based on 498 young patients with B-ALL enrolled between 1996 and 2000 reported that the estimated event free survival for 5 years of follow up (5-years EFS) was 82%.[11] A later report showed 79.6% 6-years EFS for 2169 children diagnosed with B-ALL from 1995 until 2000.[12] A comprehensive study conducted on 5060 pediatric B-ALL patients from 15 countries who were diagnosed in the period between 2002 and 2007 reported 75% 5-years FES and 82% 5-years overall survival (5-years OS).[6] In Saudi Arabia, the clinical data are comparable to the mentioned above; a report based on 596 children with B-ALL from eight health-care centers in three major cities enrolled between 2004 and 2008 showed that the 5-EFS was 73.3% and the 5-years OS was 86.9%.[5]

Pediatric B-ALL patients exhibit heterogeneous clinical outcomes (prognosis). Based on the 5-years EFS and the 5-years OS the disease is divided into three risk groups; standard-risk (SR)

group, intermediate-risk (IR) group and high-risk (HR) group.[6] A study conducted on 5060 children from 15 countries with B-ALL reported that the 5-years EFS and the 5-years OS were 81% and 90% for the SR group (1564 patients), 75% and 83% for the IR group (2650 patients) and 55% and 62% for the HR group (846 patients), respectively.[6]

Molecular heterogeneities and prognosis of pediatric B-ALL

The prognosis of pediatric B-ALL is highly influenced by the molecular variabilities of the disease. Various genetic abnormalities are evident in at least 75% of pediatric B-ALL patients.[13] Depending on the impact of such abnormalities on the pathology of the disease, they may contribute to either good or poor prognosis. As a result, different molecular alterations were considered prognostic markers of pediatric B-ALL and have been employed for the risk stratification of the patients.[14]

Molecular aberrations associated with good prognosis

Different molecular abnormalities were reported to confer good prognosis in pediatric B-ALL patients. Hyperdiploidy is observed in 30% of pediatric B-ALL patients and is associated with 80% 5-years EFS and 90% 5-years OS.[15] In addition, the chromosomal translocation t(12;21) (p13;q22) that results in the production of *ETV6-RUNX1* fused genes is present in 25.2% of childhood B-ALL cases (171 patients) and patients harboring such aberration have 75% 10-years EFS and 88% 10-years OS.[16] Trisomies 4 and 10 are found in 25% of pediatric B-ALL patients and are combined with 86.2% 5-years EFS and 95.9% 5-years OS.[17] Another good prognostic marker, yet rare in young patient with B-ALL (2%) is the chromosomal translocation t(8;14) (q23;q32.3), which is associated with 85% 5-years EFS.[18] The fused genes *TCF3-PBX1*, which is a consequence of the chromosomal translocation t(1;19) (q23;p13), is observed in 5.6% of pediatric B-ALL patients and is accompanied with 85.9% 5-years EFS.[19,20]

Molecular alterations associated with poor prognosis

As discussed earlier, while some chromosomal abnormalities confer good prognosis, others are characteristics of poor prognosis in childhood B-ALL. Hypodiploidy is found in 8% of pediatric B-ALL cases and the afflicted patients show 38% 8-years EFS and 50% 8-years OS.[21] Philadelphia chromosome t(9;22) (q34;q11), which results in the fusion of two distinct genes *BCR-ABL*, is not common in pediatric B-ALL (4.7%) and is associated with 44% 5-year EFS (40% 10-year EFS) and 53% 10-years OS.[16] In addition, the fused genes MLL-AF4 resulted from t(4;11) (q21;q23) is very rare in pediatric B-ALL (2%) and confers 40% 5-years EFS.[1] Similarly, intra-chromosomal amplification of chromosome 21 is evident in 2% of children with B-ALL and is associated with 40% 5-year EFS.[22] Other gene mutations or deregulations have an established prognostic value in pediatric B-ALL. Mutations and deletions in *IKZF1* are common in pediatric B-ALL (29%) and patients with such mutations have 55% 5-yaers EFS.[21] Mutated

JAK is present in 11% of pediatric B-ALL patients and is combined with 65% 5-year EFS.[23] In addition, *BCR-ABL* like cases, which exhibits gene expression profile very similar to the transcriptomic signature of *BCR-ABL* positive cases, is evident in 16% of children with B-ALL and is associated with 32% 5-years cumulative incidence of relapse (CIR).[24]

Omics-based insights into therapy failure in patients with pediatric B-ALL

Epigenomics

Aberrant epigenome has been implicated in the undesired treatment outcomes of children with B-ALL.[25,26] A study conducted on 33 pairs of samples, which were collected at diagnosis and relapse, found that DNA hyper-methylation was a hallmark of relapse samples; 905 genes were hyper-methylated in relapsed pediatric B-ALL.[27] The same study showed that different regulators of Wnt pathway and tumor suppressor genes were amongst the hyper-methylated genes. Further investigations on the hyper-methylated genes showed reduced transcript expression of adenomatous polyposis coli (*APC*), a tumor suppressor gene and negative regulator of Wnt pathway, protein tyrosine phosphatase receptor type O (*PTPRO*), negative regulator of Wnt pathway, and cyclin-dependent kinase inhibitor 2A (*CDKN2A*), a tumor suppressor gene, in relapsed pediatric B-ALL.[27] These findings indicate that epigenetics-based silencing of tumor suppressor genes and regulators of cancer-related pathways, such as Wnt, is a mechanism through which the leukemic blasts resist the apoptotic effect of chemotherapy. The involvement of reduced expression of tumor suppressor genes, such *CDKN2A,* in relapsed pediatric B-ALL is supported by the significant increase of relapse rate in pediatric B-ALL patients with biallelic loss of *CDKN2A*.[28] In consistent with the view that deregulated Wnt pathway contributes to poor therapy outcomes, Dandekar *et al*.[29] reported a significant improvement in the chemosensitivity of B-ALL cells from relapsed young patients upon the inhibition of Wnt pathway. This proposes Wnt pathway as a good therapeutic target in pediatric B-ALL patients who are at high-risk of relapse. *Bim* is a pro-apoptotic gene known to be upregulated in childhood B-ALL blasts in response to the treatment with glucocorticoids.[30] Interestingly, *Bim* is epigenetically silenced in dexamethasone-resistant pediatric B-ALL, indicating that suppressed expression of drug-responsive genes is another mechanism utilized by the drug-resistant blasts to stop chemotherapy-induced death.[30] Acquired defects in epigenetics regulators have been accused of driving the rise of a leukemic clone that causes the disease return. Mar *et al*.[31] studied matched diagnosis and relapsed pediatric B-ALL samples and reported gained mutations in histone-lysine N-methyltransferase (*SETD2*), Lysine-specific demethylase 6A (*KDM6A),* histone-lysine N-methyltransferase 2D (*MLL2*) and CREB-binding protein (*CREBBP*) in relapsed samples. As relapsed pediatric B-ALL characterized by less accessible DNA for gene transcription, inhibiting histone deacetylastion and DNA methylation seems an attractive strategy to restore less compact chromatin in drug-resistant childhood B-ALL. Vorinostat, a histone deacetylese inhibitor (HDI), was shown to reverse the relapse-specific gene expression signature and to sensitize drug-resistant leukemic blast to chemotherapy.[32] The same study also showed that the demethylating agent decitabine restored the expression of genes known to be epigenetically silenced in relapsed pediatric B-ALL, such as *CDKN2A* and *PTPRO;* and restored chemotsensitivity. Bachmann *et al*.[30] who reported epige-

netics silencing of *BIM* in dexamethasone-resistant blasts, showed that vorinostat restored *BIM* expression and chemosensitivity to dexamethasone. These findings have paved the way for vorinostat and decitabine as possible choices of therapy to overcome chemoresistance-driven by aberrant epigenomics. In a case report, decitabine with dexamethasone induced a complete remission in a young patient in her third relapse, indicating the usefulness of decitabine for re-induction therapy.[33] In a phase 2 trial, 50% of relapse/refractory patients (mix of pediatric and adult ALL) were shown to achieve complete remission upon receiving decitabine and vorinostat with standard chemotherapy.[34]

Genomics

Genetic lesions have been historically described in pediatric B-ALL, of which some are characteristics of drug-resistance and relapse in children with B-ALL.[13] Next generation sequencing of 300 genes in matched diagnosis and relapse samples from 264 patients revealed mutations in 32 genes, such as the transcriptional coactivators *CREBBP* and *NCOR1* (nuclear receptor corepressor 1); and the transcription factors *ERG* (transcriptional regulator ERG), *SPI1* (transcription factor PU.1), *TCF4* (transcription factor 4) and *TCF7L2* (transcription factor 7-like 2), in the relapse samples.[35] This study provided an evidence of the involvement of altered transcription machinery in the unsuccessful therapy of pediatric B-ALL patients. Interestingly, the same study reported an impaired transcriptional regulation of glucocorticoide-responsive genes due to *CREBBP* mutations, re-highlighting the link between the altered expression of drug-responsive genes and treatment failure in children with B-ALL.[35] In another study, mutations in *CREBBP* and *KRAS* were also implicated in the mechanism through which the disease returns; aberrations in *CREBBP* and *KRAS* were found in a leukemic clone at diagnosis that also persisted at relapse, indicating that a clone harboring such abnormalities were responsible for the disease recurrence.[36] Another piece of evidence of the key role of impaired drug-responsive genes in the therapy failure of pediatric B-ALL came from a study that reported a significant association of deleted genes known to function in glucocorticoid signaling, such as *BTG1* (B-cell translocation protein 1), *NR3C1* (glucocorticoid receptor) and *TBL1XR1* (transducin beta-like 1X-linked receptor 1), with relapsed cases of pediatric B-ALL.[27] Characterization of genome-wide copy number alterations (CANs) in matched diagnosis and relapse samples from 20 patients revealed that the relapse samples were associated with deletions of genes linked to the development and differentiation of B-cells. At the top of these genes were *PAX-5*, *EBF1* and *IKZF1* which encode for paired box protein pax-5, early B-cell factor 1 and IKAROS family zinc finger 1, respectively.[37] In line with these data, lost function of *EBF1* was recently implicated in the pathology of undesired treatment outcomes of pediatric B-ALL.[38] In addition, deletion of *BTG1*, which is a drug-responsive gene, was found to significantly associate with lost function of *IKZF1*.[39] Moreover, the prognostic value of *IKZF1* aberration as a molecular marker of poor treatment outcomes was significantly increased when combined with the deletion of *BTG1*.[39] These data imply that mutations in genes implicated in B-cell development and differentiation; and defects of drug-responsive genes are synergetic drivers of treatment failure in pediatric B-ALL patients. In a cohort consisting of 2535 patients, single nucleotide polymorphisms (SNPs) were identified in 25 genes like glycogen phosphorylase (*PYGL*) and phosphodiesterase 4B (*PDE4B*) and were reported to significantly associate with relapsed cases.[40] *PYGL* is a target of adenosine monophosphate (AMP) and is implicated in the response of anti-leukemic drugs; increased expression of *PYGL* was significantly associated with *in vitro* response of pediatric B-ALL blasts

from 174 patients to prednisolone.[41] Further investigations on *PDE4B*, which is a major regulator of cyclic AMP, in pediatric B-ALL cohort consisting of 191 patients showed a strong correlation between the gene expression and poor response to remission induction therapy.[42] This gave a rationale for targeting *PDE4B* that resulted in an improved sensitivity of pediatric B-ALL blasts to prednisolone, proposing PDE4B as a therapeutic target for better outcomes of remission induction therapy.[42]

Transcriptomics

Transcriptomics have been very useful to explore the altered gene expression in leukemia.[43-45] Holleman *et al*.[41] used DNA microarray to identify transcriptomic signatures of *in vitro* resistance to individual anti-leukemia drugs including prednisolone, vincristine, asparaginase and daunorubicin in B-ALL samples from 173 children. The study reported differentially expressed genes in resistant compared with sensitive pediatric B-ALL samples for prednisolone (33 genes), vincristine (40 genes), asparaginase (35 genes) and daunorubicin (20 genes). The authors demonstrated that in order to overcome the cytotoxicity of asparaginase, which inhibits the synthesis of ribosomal proteins at the transcription level, asparaginase-resistant B-ALL exhibited an increased expression of genes responsible for the synthesis of ribosomal proteins. Similarly, vincristine-resistant B-ALL showed an up-regulation of genes involved in actin cytoskeleton in order to reduce the effect of vincristine that targets the microtubules. These data indicated that unlike drug-sensitive B-ALL, undergoing gene expression changes to stop or delay the chemotherapy effects is a feature of drug-resistant pediatric B-ALL.[41]

In an attempt to identify genes underlying *in vivo* resistance to chemotherapy, gene expression profiling was performed on pediatric B-ALL samples collected at diagnosis from patients who appeared later to have high MRD (21 patients) or negative MRD (30 patients).[46] The study reported 51 genes with differential expression and that the resistant samples were associated with low expression of apoptotic genes, such as *SIVA* (SIVA apoptosis-inducing factor), *TNF* (tumor necrosis factor) and *GRIM-19* (cell death regulatory protein GRIM-19); and decreased expression of proliferative genes like *CDCA1* (also known as NUF2; kinetochore protein Nuf2), *CDCA4* (cell division cycle-associated protein 4), *E2F1* (E2F transcription factor 1), *E2F6* (transcription factor E2F6), *MCM5* (DNA replication licensing factor MCM5), *MYBL2* (Myb-related protein B), *PARD3* (partitioning defective 3 homolog) and *TTK* (TTK protein kinase). This report gave an evidence for the contribution of defective apoptosis and impaired proliferation to therapy failure in pediatric B-ALL patients. In line with this view, reduced expression of transcription factor (E2F3a), which regulate cell cycle progression, was shown to significantly predict poor prognosis and high-risk of relapse in pediatric B-ALL patients.[47] Similarly, Cui *et al*.[48] demonstrated that serrate RNA effector molecule homolog (ARS2), which functions in the progression of cell cycle, was significantly down-regulated in pediatric B-ALL patients at high-risk of relapse. An additional piece of evidence of the involvement of impaired proliferation in drug-resistance of B-ALL came from Flotho *et al*.[49] who compared the transcriptome of MRD positive pediatric B-ALL (n=109 samples) with that of MRD negative (n=78 samples). The study reported reduced expression of proliferative genes including cyclin B2 (*CCNB2*), cell division cycle 2 kinase (*CDC2*), CDC28 protein kinase regulatory subunit 1B (*CKS1B*) and topoisomerase II alpha (*TOP2A*) in MRD positive samples.[49] In agreement with the view that drug-resistant pediatric B-ALL is associated with aberrant apoptosis system, a transcriptomic study conducted on 189 pediatric B-ALL patients reported decreased expression of the pro-

apoptotic gene *CASP8AP2* which encodes for caspase 8 associated protein 2 in MRD positive samples.[50] Validation of this result at the protein level using flow-cytometry in an independent patients' cohort (n=99) concluded a strong association between the low expression of CASP8AP2 and relapsed childhood B-ALL cases.[50] A recent study reported similar findings where the decreased expression of CASP8AP2 increases the risk of relapse in pediatric B-ALL patients.[51] Another transcriptomics study focused on 70 apoptotic genes reported a significant association of reduced expression of the pro-apoptotic gene *HRK*, which encodes for harakiri BCL2 interacting protein, with asparaginase-resistant pediatric B-ALL and high expression of the anti-apoptotic gene *MCL-1*, which encodes for myeloid cell leukemia 1 protein, with prednisolone-resistant pediatric B-ALL.[52] Collectively, these studies showed that while drug-resistant pediatric B-ALL is less promoted to undergoing cellular proliferation, it manages to escape drug-induced death by deregulating apoptosis.

Proteomics

Proteomics is a useful approach to identify aberrant protein expression associated with cancer.[53,54] Different proteomics studies have been conducted on pediatric B-ALL, from which only three studies attempted to address the issue of chemotherapy resistance. Verrills *et al.*[55] used NOD/SCID mouse xenograft model of pediatric B-ALL and two dimension deference electrophoresis (2D-DIGE) coupled with mass spectrometry to study proteins associated with *in vivo* resistance to vincristine, which targets microtubules. The authors reported 19 proteins with differential expression in the vincristine-resistant B-ALL xenografts compared with the vincristine-sensitive B-ALL xenografts. From the 19 proteins, elsolin, ezrin, actin regulatory protein CAP-G, heat shock cognate 71 kDa protein and T-complex protein 1 subunits beta and epsilon, which were linked to actin cytoskeleton, were found to be overexpressed in the vincristine-resistant pediatric B-ALL xenografts. In consistence with the transcriptomics study reported by Holleman *et al.*,[41] these findings indicated that enhancing actin cytoskeleton network is a mechanism used by the leukemic blasts to overcome the cellular injury caused by vincristine. A later study used two dimensional gel electrophoresis (2DE) and mass spectrometry to identify proteins exhibiting differential expression in B-ALL cell lines following *in vitro* treatment with prednisone.[56] Four B-ALL cell lines were used (REH, 697, Sup-B15 and RS4;11), of which REH appeared to be resistant to the drug. Following the treatment with prednisone, 17 proteins and 16 proteins displayed altered expression in the resistant cells and sensitive cells, respectively. Interestingly, the resistant cells up-regulated the expression of proteins with known anti-apoptotic activity like non-metastatic cells 2 and cofilin 1. In contrast, the sensitive cells showed elevated expression of voltage-dependent anion-selective channel protein 1 and ER-60 protease, which are pro-

Table 1. Summary of the molecular aberrations shown by omics studies to be associated with chemotherapy-resistance or relapse in children with B-ALL.

Gene or protein	Function	Abnormality in pediatric B-ALL	Reference
APC, PTPRO and *CDKN2A*	Tumor suppression and/or negative regulation of Wnt pathway	Hyper-methylated in relapse	27
SETD2, KDM6A, MLL2, CREBBP and *NCOR1*	Regulation of epigenetics and/or coactivation of transcription	Mutated in relapse	31,35
ERG, SPI1, TCF4 and *TCF7L2*	Transcription factors	Mutated in relapse	35
KRAS	Cellular proliferation and survival	Mutated in relapse	36
BTG1, NR3C1 and *TBL1XR*1	Glucocorticoid signaling	Deleted in relapse	27
EBF1 and *IKZF1*	Development and differentiation of B-cells	Deleted in relapse	37
PYGL	Drug response	SNP in relapse	40
PDE4B	Regulation of cyclic AMP	SNP in relapse	40
CDCA1, CDCA4, E2F1, E2F6, MCM5, MYBL2, PARD3 and *TTK*	Proliferation / Apoptosis	Reduced expression in high-MRD / Reduced expression in high-MRD	46 / 46
SIVA, TNF and *GRIM-19* *CCNB2, CDC2, CKS1B* and *TOP2A*	Proliferation	Low-expression in high-MRD	49
CASP8AP2	Apoptosis	Low-expression in high-MRD	50
HRK	Apoptosis	Decreased expression in asparaginase-resistant B-ALL	52
MCL-1	Cell survival	Increased expression in prednisolone-resistant B-ALL	52
Elsolin, ezrin, actin regulatory protein CAP-G, heat shock cognate 71 kDa protein a and T-complex protein 1 subunits beta and epsilon	Actin cytoskeleton	Overexpressed in vincristine-resistant B-ALL xenografts	55
Non-metastatic cells 2 and cofilin 1	Cell survival	High-expression in drug-resistant B-ALL in response to the treatment with prednisone	56
Voltage-dependent anion-selective channel protein 1 and ER-60 protease	Apoptosis	High-expression in drug-sensitive B-ALL in response to the treatment with prednisone	56
Galactin and high mobility group protein B2	Cellular survival	Low-expression in drug-sensitive B-ALL in response to the treatment with prednisone	56

apoptotic proteins, and decreased the expression of galactin and high mobility group protein B2, which enhance cellular survival. This study further stresses on the importance of apoptosis-related proteins as key determinants of treatment outcomes in pediatric B-ALL.[56] Recently, Guzmán-Ortiz et al.[57] gradually exposed pediatric B-ALL cell line (CCRF-SB) to vincristine until the malignant cells became resistant to the drug at concentration of 6 nM. Next, nano-liquid chromatography and tandem mass spectrometry were utilized to study the proteomes of the vincristine-resistant CCRF-SB cells and control CCRF-SB cells. The authors reported 135 proteins to be exclusively evident in the proteome of the resistant cells. Following functional enrichment analysis of the 135 proteins, they were found to enrich for Ras pathway, cytokine-mediated signaling and oxidative phosphorylation. These findings are in consistence with other reports, where active Ras pathway was shown to increase the risk of early relapse in pediatric B-ALL patients; and up-regulation of genes implicated in oxidative phosphorylation was found in glucocorticoid-resistant childhood B-ALL samples compared with those sensitive to the drug.[58,59] This suggests that such pathways are important for acquiring resistance to chemotherapy; hence they may represent good therapeutic targets in pediatric B-ALL. Of notice, amongst the proteins that were evident only in the proteome of the resistant blast, was microtubule-associated protein 4 (MAP4), which promotes the assembly of microtubules that are targeted by vincristine. This agrees with the pervious findings reported by Holleman et al.[41] and Verrills et al.;[55] and provides further support to the conception that changes in gene and protein expression in order to overcome the cytotoixic effects of chemotherapy is a feature of drug-resistant pediatric B-ALL.

Advances in pediatric B-ALL therapy

Gaining new insights into the molecular biology of unsuccessful treatment of pediatric B-ALL patients highlights novel therapeutic target for desired treatment outcomes. Tyrosine kinase inhibitors (TKIs) have been an attractive option in this context. An impressive improvement was reported in the treatment outcomes of patients with *BCR-ABL* abnormality using imatinib; the 5-years EFS was reported to be 80% compared 40% using the conventional chemotherapy.[60] A later study incorporated imatinib and dasatinib, another form of TKIs, in the remission induction therapy of patients with Philadelphia chromosome and reported 69% 5-years EFS compared with 32% 5-years EFS in those who did not receive TKIs.[61] Furthermore, only 18% of the TKIs treated group underwent hematopoietic stem cell transplantation compared with 79% of the TKIs non-treated group.[61] Dasatinib was also tested for imatinib *BCR-ABL* resistant subgroup; a study reported a complete remission using dasatinib in patients who exhibited persistent MDR positivity using amatinib.[62] *BCR-ABL* like patients, who are known to respond poorly to standard chemotherapy (32% 5-years CIR), were reported to profit from treatment using TKIs; dasatinib induces a complete remission in refractory patients with ph-like abnormality.[24,63,64] mTOR kinase inhibitors (MLN0128 or AZD2014) were reported *in vivo* to improve the efficacy of dasatinib and imatinib against ph-like childhood B-ALL.[65] Pediatric B-ALL cells with *JAK* mutation, which confers poor prognosis (5-years EFS =65%), were reported *in vivo* to better respond to ruxolitinib (*JAK* inhibitor).[66] A trail is currently held to evaluate the efficacy of ruxolitinib in childhood B-ALL with *JAK* mutations.[67] TKIs are promising agents that can be directed to target molecular abnormalities that drive resistance of the leukemic blasts to the conventional treatment. Therefore, the prior knowledge of such molecular aberrations is essential for a proper selection of TKIs. Another seemingly promising approach of treatment is by the use of nanoparticles such as silver nanoparticles (Ag NPs) and gold nanoparticles (Au NPs).[68-71]

Conclusions

The molecular heterogeneities of pediatric B-ALL play important roles as determinants of the treatment outcomes. The present review discussed the omics-based studies that attempted to address the issue of therapy failure in pediatric B-ALL patients at the levels of epigenetics, genomics, transcriptomics and proteomics. The findings discussed here may provide a better understanding about the molecular landscape associated with the unsuccessful treatment of pediatric B-ALL; and unveil potential drivers of chemotherapy-resistance and relapse in pediatric B-ALL patients (Table 1). The major next task is to validate the promising findings in a larger patients' cohort to confirm their utility in the prognosis and/or therapy of the disease.

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