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AI-Guided Repurposing of FDA-Approved Anti-Leukemic Molecularly Targeted Therapies for Fatal Blast Crisis Chronic Myeloid Leukemia: Integrative Genomics for Precision Medicine of Relapsed/Refractory Cancers in the Post-Pandemic Era

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Abstract

Background: The COVID-19 pandemic accelerated the paradigm of drug repurposing, leading to the rapid identification of new uses for existing therapeutics under urgent clinical need. This success story has ignited a broader movement towards leveraging repurposing strategies for relapsed, refractory, and traditionally difficult-to-treat diseases, specifically cancers. Chronic myeloid leukemia (CML), although treatable in the chronic phase (CP), is usually fatal in the blast crisis phase (BC-CML), exemplifying a pressing challenge to oncology, where standard tyrosine kinase inhibitor (TKI) therapies often fail, resulting in poor survival outcomes.

Methods and Results: In a multi-institutional cohort of 141 CML patients, we performed WES across disease phases (123 CP-CML, 6 AP-CML, 12 BC-CML). Mutational landscapes were interrogated using the AI-driven PanDrugs2 platform to identify druggable targets and repurposable FDA-approved anti-leukemic therapies. A 54% surge in mutational burden was observed transitioning from AP-CML to BC-CM, revealing 67 recurrent pan-leukemic gene mutations. Notably, actionable alterations were found in *NPM1, DNMT3A, PML, AKT1, CBL, JAK2, TET2, IDH1, and BCL2*, with therapeutic opportunities using existing agents such as venetoclax, ivosidenib, decitabine, and azacitidine. Emerging vulnerabilities, including *RPTOR* and *BCR* mutations, suggest further avenues for mTOR and BTK inhibitor applications beyond traditional TKI paradigms.

Conclusions: Our integrated genomic and AI-guided approach demonstrates the transformative potential of drug repurposing for BC-CML, highlighting immediate actionable options where conventional therapies fail. This strategy not only offers hope for patients with BC-CML but also paves a visionary path toward precision medicine frameworks for relapsed, refractory, and otherwise intractable cancers in the post-pandemic clinical era. Prospective multi-omics studies and tailored clinical trials are urgently warranted to expand these opportunities across the oncology landscape.(**International Journal of Biomedicine. 2025;15(3):469-482.**)

Keywords: chronic myeloid leukemia • blast crisis • mutational profiling • drug repurposing • personalized treatment

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Abbreviations

AP, accelerated phase: **AML**, acute myeloid leukemia; **AL**L, acute lymphoid leukemia; **BC**, blast crisis; **CML**, chronic myeloid leukemia; **CP**, chronic phase; **CLL**, chronic lymphocytic leukemia; **HMAs**, hypomethylating agents; **IM**, imatinib mesylate; **TKIs**, tyrosine kinase inhibitors; **WES**, whole exome sequencing.

Introduction

The COVID-19 pandemic accelerated global drug repurposing efforts, with anticancer agents such as imatinib investigated for antiviral efficacy.[1] TKIs, the cornerstone of CML therapy, demonstrated antiviral potential against SARS-CoV-2.[2] Furthermore, the management of CML patients during the pandemic highlighted the critical need for flexible, rapid therapeutic strategies.[3]

Chronic myeloid leukemia (CML) is a malignancy of myeloid lineage in hematopoiesis.[1] It occurs when clonal hematopoietic stem cells multiply abnormally, resulting in the overproduction of non-functional cells, or blasts, especially granulocytes, in the bone marrow and peripheral circulation.[2] The Philadelphia chromosome, resulting from a reciprocal translocation between the *BCR* gene on chromosome 22 and the *ABL* gene on chromosome 9, is a defining characteristic of this disease and was discovered in 1959 by David A. Hungerford and Peter C. Nowell, the Nobel Prize winners for this discovery. This genetic abnormality leads to the creation of BCR-ABL fusion oncogenes that result in the constitutive expression of the tyrosine kinase BCR-ABL on hematopoietic stem cells, which culminates in the continued proliferation and development of leukemic stem cells.[2] CML occurs in 1 to 2 of every 100,000 individuals around the world, making up around 15% of new adult leukemia cases.[3] CML is a triphasic disease that begins with the indolent chronic phase (CP-CML). This phase is characterized by a significant increase in myeloid precursors and mature cells. The overall survival rate of CP-CML patients results in life expectancy comparable to that of the general population, at least in technologically advanced countries, due to the introduction of tyrosine kinase inhibitors (TKIs) and bcr-abl fusion oncoprotein inhibitors in the last three decades.[4] However, a fraction of CML patients progress to the accelerated phase (AP) and finally the blast crisis (BC), the latter of which has an overall survival rate of 3 to 18 months.[3] The BC-CML is characterized by an acute and rapid increase in the number of primitive hematopoietic cells in the bone marrow and blood, as well as treatment failure, anti-leukemic therapy resistance, relapses, and eventually death within a few months.[5]

This makes treating BC-CML one of the most challenging tasks in cancer medicine in the 21st century. As a result, it is extremely important to discover new pharmacological targets and treatments for BC-CML.[1,5]

BC-CML usually looks like either acute myeloid leukemia (AML) or acute lymphoid leukemia (ALL), which is called the myeloid blast crisis (M-BC) or lymphoid blast crisis (L-BC), respectively.[6] Targeted therapies, including drugs and inhibitors for specific gene mutations in AML and ALL, have been developed and approved by the FDA, and are now used in routine clinical practice for personalized treatment of patients with these genetic alterations.[7,8] However, it is still unclear whether the same genes linked with AML and ALL are responsible for myeloid and lymphoid blast crises, respectively.[4,5] If this is the case, it would provide the chance to treat MBC-CML and LBC-CML patients with FDA-approved medications developed against AML-/ALL-lineage genes. Therefore, the goal of this study was to investigate pan-leukemia gene mutations, particularly AML and ALL lineage, in patients with BC-CML and to create a database of FDA-approved treatments effective against these AML- and ALL-specific gene mutations. This database would include drugs that have the potential to be used for treating BC-CML.

Materials and Methods

Study Populations

This study recruited 141 CML patients from two centers: Hayatabad Medical Complex (HMC), Peshawar, Pakistan, and King Abdulaziz Hospital Al-Ahsa, Saudi Arabia (from January 2012 to June 2022). Imatinib mesylate (IM) was administered to all patients as the first-line treatment. Those who developed resistance to IM were then treated with second-generation TKIs. Of 141 participants, 123 age/gender-matched CP-CML patients served as controls, and 18 AP-CML patients served as the Experimental Group 1. Also, 12 of 18 AP-CML patients who progressed to BC-CML during the study were included in Experimental Group 2. The European Leukemia Net (ELN) recommendations from 2013 and later ELN-2020 were used for all clinical classification and treatment response criteria.[9-11] Regarding categorization of CML patients into CP-, AP- and BC-CML, CML patients were classified as CP if the percentage of blast cells in their blood was less than 5%, the percentage of basophils was between 15% and 19%, the percentage of blasts and promyelocytes was less than 30%, and there were no blast cells in extramedullary locations.[9] For AP, the percentage of blasts was taken as 15%-29%, the presence of promyelocytes and blasts as more than 30% in the blood or the bone marrow, the platelet count low (100×10^9/L), the presence of basophils 20% or greater, and Ph+ cells with chromosomal anomalies.[9] To categorize a patient as BC-CML, the presence of 30% or more of blasts in either the blood or the bone marrow and in extramedullary locations was considered a basic criterion.[9-11] Standards for evaluating treatment response in CML were adopted from ELN-2013 and ELN-2020.[9,11,12] Adverse events were classified using standard nomenclature version 4.03.[13]

Sample Handling

Peripheral blood samples (3-5 mL) were obtained from study participants in the BD Vacutainer® EDTA Tube (Becton Dickinson, USA). The samples were collected during the biweekly visits of the CML patients to the outpatient department of the medical oncology units of participating centers. These clinical specimens were preserved at −70°C for subsequent analysis. Before the extraction of DNA, both the patient samples and the reagents utilized in the extraction process were acclimatized to room temperature, specifically within the range of 15 to 25 °C. The process of DNA extraction encompassed multiple stages. The QIAamp DNA blood mini kit was (Qiagen, Hilden, Germany) utilized for DNA extraction, according to the manufacturer's instructions. The procedure entailed the amalgamation of 200 µL of the blood specimen, 200 µL of Buffer AL, and 20 µL of QIAGEN Protease within a tube. After being vortexed, the resulting mixture underwent incubation for 10 minutes at a temperature of 56°C. Two hundred microliters of ethanol were introduced, followed by a subsequent vortexing of the mix. The resulting solution was then carefully transferred to a QIAamp Mini spin column, from which the extract was extracted post-centrifugation. Subsequently, the flow-through was eliminated after incorporating Buffer AW1 and subsequent centrifugation. This procedure was reiterated with Buffer AW2 to guarantee the complete removal of all buffers, after which the column underwent centrifugation. The DNA was eluted by applying distilled water or incorporating Buffer AE, followed by incubation and centrifugation processes. Absorbance was assessed at 260 nm to ascertain the concentration of DNA. A concentration exhibiting a ratio of 1.7 to 1.9 at A260/A280 was deemed to possess purity. The DNA was quantified by utilizing a Nanodrop Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). The DNA was diluted to 70-80 ng/µL concentrations and 40 ng/µL for use in WES and Sanger Sequencing, respectively. Until additional testing was conducted, the DNA was preserved at −80°C.

Genetic Analysis

Next-Generation Sequencing (NGS) was conducted on experimental (AP- & BC-) CML samples alongside an equivalent number of CP-CML samples serving as controls. After DNA extraction, exome-capturing arrays were employed to capture the extracted material.[14] The SureSelectXT V6-Post Capture Exome kit (©Agilent Technologies, Santa Clara, California, USA) was used to construct the library and enhance the exomes after DNA capture. The SureSelect kit included approximately 99% of the reference sequences database and comprised 214,405 exons with splice sites.[15] Subsequent to DNA fragmentation, the tagmentation of the resulting fragments was conducted, accompanied by amplification and purification processes, which were facilitated through magnetic beads. Oligonucleotides were employed to delineate the specified regions. The enriched libraries were amplified through PCR, and subsequently quantified with a Qubit fluorometer. The 2100 Agilent Bioanalyzer (©Agilent Technologies, Santa Clara, California, USA) was configured to ascertain the dispersion of library sizes. The Illumina

NextSeq500 sequencer (Illumina Inc., San Diego, CA, USA) facilitated the process of cluster generation and comprehensive exome sequencing by depositing quantifiable DNA libraries into the flow cell.[15]

The BCL2FASTQ (Illumina Inc., San Diego, CA, USA) was employed to convert the BCL files into FASTQ files. The BWA Aligner (Illumina Inc., San Diego, CA, USA) was utilized to align the FASTQ data to the human genome using the BWA-MEM algorithm. The annotation and filtration of genomic variants identified by the Genome Analysis Toolkit (GATK) were conducted utilizing Illumina Variant Studio (Illumina Inc., San Diego, CA, USA).[15]

Validation

To ascertain a common biomarker associated with the proliferation of CML, an analysis of mutated genes was conducted across all patients in the advanced phase of the disease. Strategies for filtration that focused on identifying rare variants while omitting intron and synonymous variants were employed to refine the Excel file containing NGS data. Moreover, all variants with established predictions were excluded, whether classified as benign (B) or tolerant (T). Certain variants were designated as B when they exhibited a frequency of 70% or greater for B, whereas others were categorized as T when T's frequency reached 70% or more.[16] Likewise, variables exhibiting a population frequency exceeding 0.005 in the dbSNP and Exome Sequencing Project (ESP) database were discarded.

Consequently, the variant calling process was confined to variants exhibiting intermediate to high protein effects and splice variants, culminating in approximately 124 rare variants. Furthermore, in-depth data analysis was conducted to explore unique gene mutations found in AP-CML patients that is absent in CP-CML patients and healthy controls, indicating the potential involvement of these mutations in disease progression.[15,16] Data generated through NGS can be accessed via the NCBI repository, where it has been submitted under the SRA accession number PRJNA734750, available at https://www.ncbi.nlm.nih.gov/sra/PRJNA734750. For identifying druggable mutations within pan-leukemic genes associated with myeloid and lymphoid lineages, particularly those related to AML and ALL, we meticulously filtered for genes that have been previously documented as mutated in AML and ALL, as well as other types of leukemias, lymphomas, and hematological malignancies. To achieve this objective, we compiled a comprehensive list of genes that exhibit mutations in AML and ALL, drawing upon the extensive literature available in PubMed.[17-19] The genes identified in this compilation, which are uniquely mutated in AP-CML and BC-CML while absent in CP-CML or healthy controls (sourced from genomic databases), were meticulously sorted and prepared for subsequent analysis.

PanDrug Platform

To explore the druggability of pan-leukemic genes associated with BC-CML, online artificial intelligence (AI) tools were employed. We utilized genes uniquely mutated in AP-CML and BC-CML, while not mutated in CP-CML or healthy controls. To achieve this objective, we used an online resource called PanDrugs2 (www.pandrugs.com).[19] The druggability assessment was conducted by inquiring about the gene names within the PanDrugs2 database. A gene was deemed druggable if an FDA-approved medication existed targeting it for treating AML, ALL, or other hematological malignancies.

Statistical analysis

We employed the methods reported previously by our group.[20] In accordance with the normality assessment, absolute values and percentages were presented for categorical variables, while the mean and a suitable measure of variation were provided for continuous variables. The chi-square test or Fisher's exact test was employed for categorical data to compare two groups. For continuous data, a two-sample independent test or Mann–Whitney U test was utilized. The analysis of variance for groups of three or more was conducted using either ANOVA or the Kruskal–Wallis test. To evaluate the survival outcome, Kaplan-Meier survival analysis curves were constructed.[20] A log-rank test was employed to conduct the group comparison. The data analysis was conducted utilizing SAS/STAT software version 9.4, developed by SAS Institute Inc., located in Cary, NC, USA. The R package was used for statistical computing.[16,17,21]

Results

Clinical Features of CML patients

Of the 141 patients diagnosed with CML, 4.3% (n=6) were AP-CML, and 8.5% (n=12) were BC-CML. The study subjects comprised 86 (61.0%) males and 55(39.0%) females, with a male-to-female ratio of 1.6:1. The mean age of all patients was 36.4, ranging from 9 to 67 years. Laboratory results demonstrated that 79.4% (n=112) of patients developed anemia, whereas 56.0% (n=79) developed leukocytosis (>50×10^9/L). Splenomegaly was seen in 71.6% (n=101) of patients. Furthermore, a comparison between different phases of CML patients showed that leukocytosis was significantly increased in the advanced phase of CML compared to CP-CML cases (P=0.0184) (Table 1). Regarding clinical assessment, a significant relationship was seen between hepatomegaly and advanced-phase CML patients (P=0.0014). Although splenomegaly was observed in all phases, it was significantly associated with the advanced-phase CML, as all AP-CML and BC-CML patients developed splenomegaly, compared to only 67.5% (n=83) of CP-CML patients (P=0.0014). Furthermore, the size of the spleen significantly increased with the disease's progression.

Clinical Outcome of CML Patients in Three Phases of the Disease

After using IM as the first-line treatment for all patients, 20(14.2%) patients were either intolerant or did not respond to the drug, even after increasing the dose. These 20 patients were treated with nilotinib (NI) as second-line therapy, 12 (60%) of whom were CP-CML, 2 (10%) were AP-CML, and 6 (30%) were BC-CML patients. Of the BC-CML patients receiving

NI (n=6), 4 (66.7%) were non-responders to the second-line treatment. All BC-CML patients were treated as Philadelphia-positive acute leukemia patients. There was a significantly high mortality rate of 75% (9/12) for BC-CML patients compared to CP-CML patients, who had a comparatively very low mortality rate of 8.1% (10/123), with one death not related to cancer. In addition, the overall survival rates for CP-CML and AP-CML patients were 91.9% (113/123) and 100% (18/18), respectively, whereas BC-CML patients had a survival rate of 25% (3/12) (Table 1). Our data shows the limited effectiveness of current treatment modalities to treat BC-CML, which thus necessitates a hunt for new drug targets and novel treatments. It also highlights the need to find early biomarkers for CML progression that could be targeted to prevent progression.

Table 1.

Comparison between demographics and clinical characteristics in different phases of CML.

Characteristics	Patient groups			*P*-value
	CP-CML n (%)	AP-CML n (%)	BC-CML n (%)	
No. of patients	123 (87.2)	18* (4.3)	12* (8.5)	
Mean (range)	35.5 (9-67)	35.6 (27-43)	38.1 (29-50)	
Male	74 (60.2)	12 (66.67)	8 (66.7)	0.5985
Female	49 (39.8)	6 (33.33)	4 (33.3)	
Ratio: Male: Female	1.5-1	2: 1	2: 1	
Hemoglobin: (g/dL)				
<12	69 (56.1)	15 (83.3)	9 (75)	0.0642
>12	14 (11.4)	3 (16.7)	3 (25)	0.2609
WBC count ($\times10^9$/L), Mean	313.7	315	325	
<50	20 (16.3)	3 (20)	2 (16.7)	0.8276
≥50	64 (52.0)	15 (80)	10 (83.3)	0.0184
Platelets ($\times10^9$/L)				
<450	75 (61.0)	12 (66.7)	10(83.3)	0.2528
≥450	33 (26.8)	6 (33.3)	2 (16.7)	0.8722
Imatinib				
Yes	82 (66.7)	12 (66.7)	7 (58.3)	0.7260
Nilotinib as 2nd Line				
Yes	41 (33.3)	12 (66.7)	8 (66.7)	0.0065
Hydroxyurea				
Yes	82 (66.7)	9 (50)	10 (83.3)	0.9967
Interferon				
Yes	41 (33.3)	0 (0)	0 (0)	0.0038
Chemotherapy				
Yes	0	0	9 (75%)	0.0000
Splenomegaly				
No	40 (32.5)	0 (0)	0 (0)	0.0014
Yes	83 (67.5)	18 (100)	12 (100)	
<5cm	4 (3.2)	0 (0)	0 (0)	0.5051
5-8cm	9 (7.3)	3 (16.7)	3 (25)	
>8cm	70 (56.9)	15 (83.3)	9 (75)	
Hepatomegaly				
Yes	35 (28.5)	12 (66.7)	8 (66.7)	0.0014
Anemia				
Yes	97 (78.9)	15 (83.3)	9 (75)	0.9807
Pregnant				
Yes	4 (3.2)	0 (0)	0 (0)	0.2090
Survival Status				
Confirmed deaths	10 (8.1)	0 (0)	9 (75)	<0.001
Alive at last follow-up	113 (91.9)	18 (100)	3(25)	

**12 out of 18 AP-CML patients progressed to BC-CML during the study course.*

Whole Exome Sequencing

As no specific biomarkers exist for CML disease progression or for early detection of the patient group at risk of disease progression, and as most of the BC-CML patients specifically showed resistance to all drugs, indicating the non-availability of effective medications for BC-CML, advanced-phase CML patient samples were subjected to WES to find out druggable mutations. WES detected numerous variants in the above-mentioned study subjects. We included genes mutated only in advanced phases of CML patients, i.e., in AP-CML and BC-CML, but not in CP-CML patients and healthy control DNA sequences taken from genomic databases. The total number of variants shared by our advanced-phase CML was 4175 (Figure 1).

The mutated genes with the highest variant frequency in both AP-CML and BC-CML patients, in addition to the *ABL* gene, were *RPTOR* (7.3%), followed by *BRCA1/BRCA2* (7.0%), *BCR* (6.0%), *STAB1* (4.6%), *NF1* (4.4%), *ACIN1* (4.4%), *EGFR* (3.9%), *NDRG2* (3.7%), *ERG* (3.3%) and *MYH11* (3.1%).

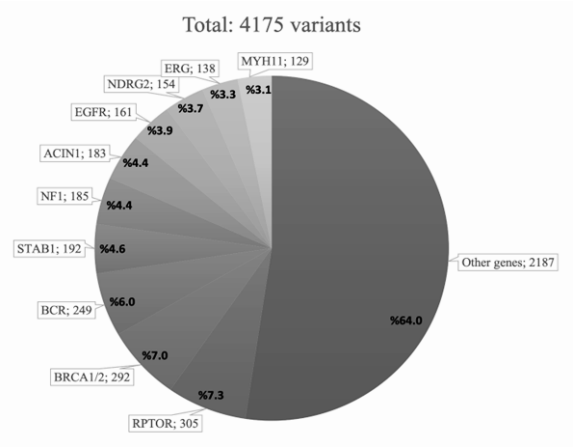

Figure 1. *Mutations shared by advanced phase CML patients (AP-CML + BC-CML).*

The number of variants and their frequencies in each gene were compared between AP-CML and BC-CML (Figure 2). CP-CML patients had only 114 mutations. AP-CML samples had 1644 variants (14.4 times higher than CP-CML), whereas BC-CML samples had 2531 variants (22.2 times higher than CP-CML), with a 54% gain in mutations from AP-CML to BC-CML (*P*=0.0000) (Figure 2). The gain of mutation from AP-CML to BC-CML in the genes with the highest variant frequency was also significantly high, with the highest increase percentage of 79.3% observed in *EGFR* (a membrane receptor gene), followed by *BRCA1/BRCA2* (72.9%), *ACIN1* (69.1%), *NF1* (60.6%), *STAB1* (59.5%), *MYH11* (58.0%), *RPTOR* (56.3%), *ERG* (46.4%), *NDRG2* (36.9%) and *BCR* (26.4%). Moreover, the low-frequency genes significantly increased from 866 variants in AP-CML to 1321 variants in BC-CML (*P*=0.0000) (Figure 2).

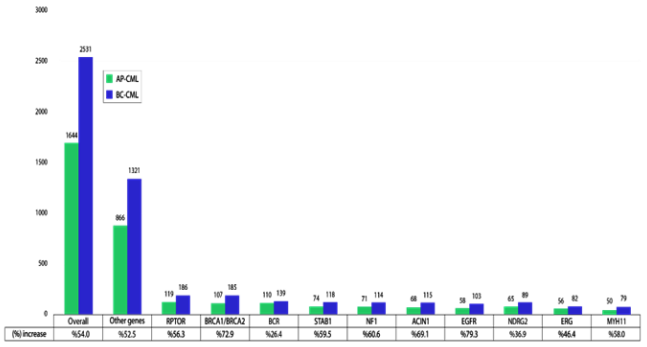

Figure 2. *A comparison of the variants' percentage increase from AP-CML to BC-CML in the highest variant frequency genes found in our study subjects.*

Druggability of Pan-Leukemic Mutations of BC-CML and Drug Repurposing

This study centers on genes amenable to pharmacological intervention, irrespective of the frequency of their variants. A gene is deemed druggable if any pharmacological agent associated with this gene has been identified for treating AML in the pandrugs2 database and relevant literature, whether as a targeted therapy or as part of chemotherapy. Among the 64 mutated genes identified in our BC-CML patients, 9 were druggable genes that have received FDA approval for use in AML or ALL patients. The variant frequencies of these druggable genes were notably low within our BC-CML patient samples. The gene exhibiting the highest variant frequencies among those amenable to pharmacological intervention, alongside mutations in the *ABL* gene, was *NPM1* (1.98%), succeeded by *DNMT3A* (1.86%), *PML* (1.82%), *AKT1* (1.62%), *CBL* (1.30%), *JAK2* (0.71%), *TET2* (0.59%), *IDH1* (0.32%), and *BCL2* (0.20%) (Figure 3). Despite the low variant frequencies of these genes among our study subjects, the observed variants exhibited a notable percentage increase from AP-CML to BC-CML, underscoring their critical involvement in disease progression (Figure 4).

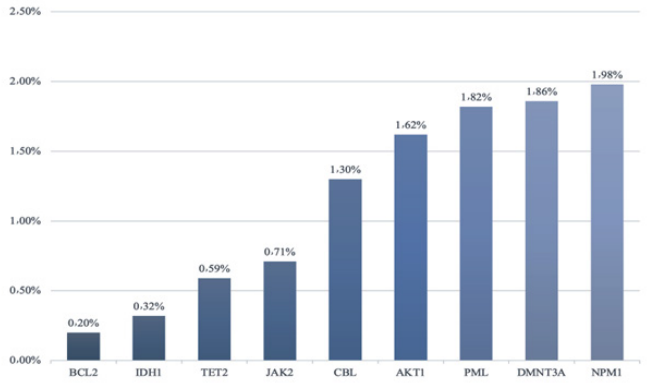

Figure 3. *Variant frequencies of the druggable genes found in our BC-CML study subjects, which have FDA-approved drugs to treat AML or ALL.*

Most of these genes were associated with FDA-approved targeted therapies for treating AML patients broadly, except for *IDH1*, which had an FDA-approved drug specifically for patients with IDH1-positive AML (Table 2). The medications sanctioned by the FDA include venetoclax, bortezomib, doxorubicin, mitoxantrone, tretinoin, quizartinib, decitabine, azacitidine, arsenic trioxide, and ivosidenib. *BCL2* emerged as the most important gene associated with FDA-approved pharmacological interventions for treating ALL patients, specifically venetoclax, doxorubicin, and vincristine (Table 2). Pharmaceuticals sanctioned by the FDA for various forms of leukemia are classified based on their genetic interaction mechanisms, including drugs that directly target specific gene mutations found in leukemia cells (Table 3).

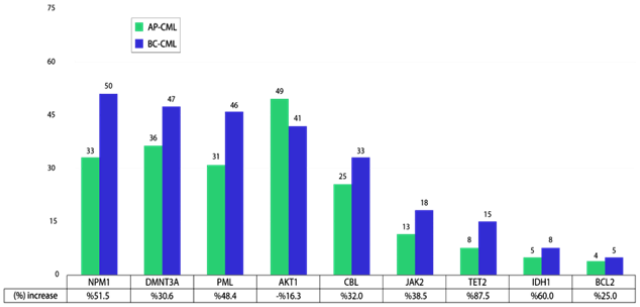

Figure 4. *Variants in the druggable genes that have FDA-approved drugs to treat AML or ALL, along with their percentage increase from AP-CML to BC-CML patients in our study subjects.*

Table 2.

List of the most significant druggable mutations in BC-CML with available FDA-approved drugs.

Gene	Drug	FDA approved	Clinical trial	Act
BCL2	VENETOCLAX*	AML (combined with azacitidine, decitabine, or low-dose cytarabine) & CLL with 17p deletion	CML	DT
	DOXORUBICIN	AML & ALL	BCL2-AML	BM
	MITOXANTRONE	AML	BCL2-AML	BM
	TRETINOIN (ATRA)	APL	BCL2-AML	BM
	VINCRISTINE	ALL & lymphoid BC-CML	BCL2-ALL	BM
	BORTEZOMIB*	MM and MCL	-	DT
CBL	QUIZARTINIB	FLT- AML	CBL-AML	BM
GATA2	BORTEZOMIB*	MM and MCL	CML	BM
DNMT3A	DECITABINE	CMML (combined with cedazuridine) & AML	DNMT3A-AML	DT
	AZACITIDINE	AML	DNMT3A-AML	BM
FGFR3	QUIZARTINIB	FLT- AML	CBL-AML	BM
IDH1	IVOSIDENIB	IDH1-AML	CML	DT
	AZACITIDINE*	AML	IDH1-ALL (combined with VENETOCLAX)	BM
JAK2	PACRITINIB	Myelofibrosis	-	DT
NPM1	VENETOCLAX*	AML (combined with azacitidine, decitabine or low-dose cytarabine) & CLL with 17p deletion	NPM1-AML	BM

** Effective against more than one type of mutation. DT, direct target; BM, biomarker.*

Table 2 (continued).

List of the most significant druggable mutations in BC-CML with available FDA-approved drugs.

Gene	Drug	FDA approved	Clinical trial	Act
PML	ARSENIC TRIOXIDE + TRETINOIN (ATRA)	PML-RARA-APL	-	DT
TET2	DECITABINE	CMML (combined with cedazuridine) & AML	TET2-CML	BM
	AZACITIDINE*	AML	TET2-CML	BM
AKT1	ARSENIC TRIOXIDE	APL	AKT1-APL	DT

** Effective against more than one type of mutation.*
DT, direct target; BM, biomarker

Table 3.

FDA-approved drugs, anti-leukemic targeted therapies that can directly target BC-CML mutations.

Drug	Disease for which FDA-approval granted	Gene targets (PanDrugs2)
VENETOCLAX	AML [22]	*BCL2, BCR, EZH2, IDH1,* and *NPM1*
TRETINOIN	APL [23]	*BCL2, PDK4, PML,* and *RARA*
BORTEZOMIB	MM and MCL [24]	*BCL2, FGFR3,* and *GATA2*
ZANUBRUTINIB	CLL and SLL [25]	*EGFR and JAK2*
IVOSIDENIB	*IDH1*+ AML [26]	*IDH1*
TAZEMETOSTAT	*EZH2*+-Follicular lymphoma [27]	*EZH2*
RUXOLITINIB	Myelofibrosis [28]	*JAK2, MPL*
PACRITINIB	Myelofibrosis [29]	*JAK2*
ARSENIC TRIOXIDE	APL [30]	*AKT1, PML*
TRETINOIN (ATRA)	APL [23]	*BCL2, PDK4, PML,* and *RARA*

In summary, notable genetic alterations were identified in patients who had progressed to the advanced stages of CML. The laboratory findings and clinical characteristics linked to the progression of CML included anemia, leukocytosis, splenomegaly, and hepatomegaly. Patients with BC-CML exhibited more variants within their mutated genes, demonstrated suboptimal treatment responses, and experienced diminished overall survival rates. The genes exhibiting the highest variant frequencies in the advanced stages of CML were found to overlap with those associated with AML/ALL lineages (pan-leukemic genes). Many genes are amenable to pharmacological intervention using drugs already approved by the FDA and EMA for treating different hematological malignancies harboring target mutations. Both FDA- and EMA-approved and investigational drugs currently undergoing clinical trials, targeting these specific gene mutations, represent promising avenues for personalized therapeutic strategies in the context of BC-CML, particularly in the TKI and post-TKI landscape.

Discussion

An extensive investigation was conducted on the clinical characteristics and pan-leukemic gene mutations in advanced-phase CML patients to gain a profound understanding of the biology, genetics, and potential precision medicine of severe and fatal clinical manifestations (blast crisis) of this treatable cancer.[4-8] Our study revealed a noteworthy association between BC-CML and hepatomegaly (P=0.0014), while splenomegaly exhibited significance in AP-CML (P=0.0014). The mean hemoglobin level, WBC count, platelet count, hepatomegaly, splenomegaly, and survival status of patients in the advanced phase of CML exhibited significant differences from those in the CP of CML. Nevertheless, the limitations of these clinical parameters in the early identification of patients susceptible to CML progression and in the pursuit of novel therapeutic agents for challenging BC-CML cases underscore the necessity for comprehensive genetic analysis. Such analysis is crucial for uncovering molecular biomarkers indicative of early progression and identifying druggable gene mutations beyond BCR-ABL.[31] The WES employed in our study was utilized to identify druggable gene mutations, representing one of the most effective methodologies for this objective.

Currently, five TKIs, namely imatinib, dasatinib, nilotinib, bosutinib, and ponatinib, are commercially available and have received approval for the treatment of CML by both the FDA and the EMA, while another TKI called asciminib is on the verge of approval very soon.[31,32] These approvals come with specific recommendations that consider various factors, including the stage of CML at diagnosis, dosage, BCR-ABL mutations, and reimbursement considerations. However, it is noteworthy that approximately 20%-30% of patients encounter resistance to these inhibitors, which can lead to treatment failure and disease relapse, ultimately resulting in BC-CML. Furthermore, considering the availability of all first-, second-, third-, and fourth-generation TKIs, the median overall survival and failure-free survival for BC-CML are recorded at 23.8 months and 5 months, respectively.[5,32,33] Furthermore, certain TKIs have demonstrated the potential to induce liver complications, including hepatotoxicity and hepatomegaly, a phenomenon also observed in our cohort of patients receiving TKI treatment.[33] This underscores the imperative to identify supplementary drug targets and ensure the availability of alternative pharmacological options for treating BC-CML. This may be accomplished by repurposing existing drugs currently utilized in the clinical management of AML and ALL, given that BC-CML presents as either M-BC-CML or L-BC-CML.[31]

Consequently, we have curated a selection of genes previously identified in AML and ALL to evaluate their potential for drug targeting, given that numerous gene mutations in these conditions are currently recognized as targets for FDA and EMA-approved therapies, in addition to various experimental drugs.

Around 80% of BC-CML patients are classified as M-BC, whereas 20% fall under L-BC, with B cells demonstrating a

greater prevalence than T cells.[34] Nevertheless, individuals diagnosed with L-BC exhibit more favorable outcomes than those with M-BC. The assessment of NGS panels to identify frequently mutated lymphoid or myeloid genes may be contemplated; however, it continues to be regarded as an experimental methodology in CML. Nonetheless, integrated therapeutic approaches have been implemented for myeloid instead of lymphoid BC-CML, drawing upon treatment strategies for AML and ALL, respectively. Recent small, single-arm studies have reported on M-BC, indicating that the combination of a second-generation TKI or ponatinib with decitabine or azacytidine yields complete cytogenetic response rates ranging from 33% to 43%, alongside a median overall survival of 13.8 to 27.4 months.[35] Strati et al.[36] assessed the efficacy of imatinib (400-800 mg daily) and dasatinib (50-140 mg daily) in conjunction with hyperfractionated cyclophosphamide, vincristine, adriamycin, and dexamethasone (hyperCVAD) in a cohort of 42 patients. The rates of complete cytogenetic response (CCyR) and complete hematologic response (CHR) were found to be 90% and 58%, respectively. In a more measured approach, Rea et al.[37] assessed the combination of IM 800 mg with vincristine and dexamethasone in a cohort of 13 patients with lymphoid blast phase. Among the 12 patients assessed, 11(91.7%) attained a complete hematologic response, while 4(33.3%) reached complete cytogenetic response.

The results from our WES revealed that the genes exhibiting the highest variant frequencies in BC-CML samples were *RPTOR* (7.3%), followed closely by *BRCA1/BRCA2* (7.0%), *BCR* (6.0%), *STAB1* (4.6%), *NF1* (4.4%), *ACIN1* (4.4%), *EGFR* (3.9%), *NDRG2* (3.7%), *ERG* (3.3%), and *MYH11* (3.1%). The gene with the highest frequency of variants that drugs can target was *NPM1* (1.98%), followed closely by *DNMT3A* (1.86%), *PML* (1.82%), *AKT1* (1.62%), *CBL* (1.30%), *JAK2* (0.71%), *TET2* (0.59%), *IDH1* (0.32%), and *BCL2* (0.20%). The genes associated with AML lineage have been linked to nine FDA-approved drugs, whereas those related to ALL lineage are associated with only one FDA-approved drug. This is logical, as both AML and CML originate from the myeloid lineage, sharing a greater number of gene mutations than does ALL. Furthermore, ivosidenib received FDA approval for treating AML patients harboring IDH1 mutations, establishing its status as a targeted therapeutic agent. While all other FDA-approved medications identified in this study are indicated for treating AML/ALL irrespective of a specific gene mutation, they are classified as targeted therapies due to their mechanisms of action, which involve the targeting of genes. This suggests that IVO and other FDA-approved medications for AML and ALL should be regarded as targeted therapies for CML patients exhibiting these gene mutations.

The FDA has sanctioned a range of pharmacological agents for the treatment of gene mutations associated with AML and ALL (venetoclax [VEN], bortezomib, ivosidenib [IVO], azacitidine [AZA], decitabine [DEC], doxorubicin [DOX], mitoxantrone [MIT], vincristine [VIN], quizartinib [QUI], arsenic trioxide [ATO], all-trans retinoic acid [ATRA], and pacritinib [PAC]), which are also relevant to our BC-CML patients. Arsenic trioxide (ATO) and all-trans retinoic acid (ATRA) have obtained FDA approval for their application in treating acute promyelocytic leukemia.[23,30] A study conducted in China has indicated that increased levels of ATO successfully sustain ERK phosphorylation, which in turn activates CDKN1A expression and results in apoptosis. Conversely, therapies utilizing reduced doses of ATO lead to an elevation in AKT1 expression, suppressing CDKN1A promoter activity and diminishing apoptosis. Consequently, ATO exhibits its therapeutic efficacy primarily at elevated doses.[38] Furthermore, patients with CML who demonstrate gain-of-function mutations in *AKT1* may show a positive response to standard doses of ATO, given that their *AKT1* expression profiles resemble those seen with low-dose ATO treatments. In the interim, ATRA has demonstrated promise in inhibiting *BCL2* and *MCL1*, indicating its potential as a targeted therapeutic approach for CML patients with *BCL2* mutations.[39] Moreover, the FDA has sanctioned a combined treatment approach of ATRA and ATO specifically for patients with acute promyelocytic leukemia who possess the PML-RARA fusion. This fusion protein serves as a direct target for the combination of drugs that have demonstrated significant rates of complete remission, improved overall survival, enduring and profound molecular responses, and a minimal relapse risk.[31] As a result, patients with CML exhibiting PML alterations could derive substantial advantages from either combination therapy or monotherapy utilizing ATRA or ATO. VEN has received FDA approval as a targeted therapy that specifically inhibits *BCL2* in individuals diagnosed with AML and those exhibiting chromosome 17p deletions in CLL.[40,41] Furthermore, the integration of dasatinib and VEN has demonstrated both safety and efficacy in managing CP-CML, attaining response rates comparable to those seen with dasatinib monotherapy.[42] This underscores the possible significance of VEN in managing CML patients presenting *BCL2* variants, particularly those who may show resistance to TKIs. Patients with AML exhibiting *NPM1* mutations have demonstrated notable sensitivity to VEN.[43] Furthermore, VEN has shown significant effectiveness when used alongside HMAs or chemotherapy in treating *IDH1*-mutated AML, and it has successfully managed relapse in ALL associated with an *IDH1* mutation.[44]

The results indicate that VEN may provide advantages for CML patients possessing *IDH1*, *NPM1*, or *BCL2* variants. Another drug targeting *IDH1* that has received FDA approval is IVO, which is indicated for patients with relapsed or refractory AML.[8] The FDA has approved AZA to be utilized as a maintenance therapy for adult patients with AML who are unable to pursue additional intensive curative treatments and have achieved complete remission, regardless of whether they have experienced incomplete blood count recovery following intensive induction chemotherapy.[45] AZA has proven effective when utilized alongside targeted therapies like VEN, which focus on variants in *BCL2* and *IDH1*.[46] A separate study indicated that elderly AML patients or those deemed unfit for intensive chemotherapy, when treated with VEN in conjunction with HMAs like AZA, have not only demonstrated improved overall survival but have also set a new

standard of care, exceeding the results achieved with HMAs alone.[47] Moreover, the FDA has sanctioned the combination of AZA and IVO for the treatment of newly diagnosed *IDH1*-mutated AML patients, encompassing those who are elderly or present with comorbidities.[26] Furthermore, the incorporation of AZA into chemotherapy protocols has resulted in prolonged survival for AML patients possessing the distinct *DNMT3A* variant, R882. This observation indicates its potential role as a biomarker for AZA efficacy in chemotherapy, independent of additional targeted treatments.[48] AZA's extensive applicability and established efficacy highlights its significant therapeutic influence in the management of leukemia, as well as its potential as a targeted treatment for CML patients harboring mutations in *DNMT3A*, *IDH1*, or *BCL2*, whether utilized as a monotherapy or in conjunction with other pharmacological agents. DEC is an HMA and DNMT inhibitor that has been granted FDA approval for use in conjunction with VEN to address newly diagnosed AML patients who are either elderly or unsuitable for intensive therapy.[43] Moreover, HMAs have demonstrated efficacy in individuals possessing *TET2* variants.[49] DOX, belonging to the anthracycline class of chemotherapeutic agents, has received FDA approval to treat various cancers, including ALL and AML.[50] The concentration of the drug exhibited an inverse relationship with the expression of a *BCL2* variant throughout treatment. Notably, DOX exhibits a selective ability to induce apoptosis in relapsed AML cells, which can be partially ascribed to its targeted inhibition of the *BCL2* variant.[51] Mitoxantrone (MIT) has received FDA approval for the treatment of adult AML.[52] Investigations have demonstrated that MIT induces programmed cell death in AML cells, alongside a significant reduction of *BCL2*.[53] The findings suggest that MIT may specifically focus on mutated *BCL2*, indicating its potential application in treating CML patients exhibiting *BCL2* variants. VIN has received FDA approval to treat a range of malignancies, encompassing ALL, B-cell ALL, and lymphoid BC-CML.[54] Moreover, an experimental investigation has revealed that VIN significantly diminishes BCL2 protein levels in human ALL cells. The effect is further amplified when VIN is combined with Huaier aqueous extract.[55]

In addition, quizartinib (QUI) is an FDA-approved FLT3 inhibitor designated for patients with FLT3-positive AML.[56] Loss-of-function variants in CBL correlate with a phenotype characterized by either hyperactivation or heightened sensitivity of FLT3. Research indicates that QUI significantly suppresses cell proliferation in wild-type FLT3 cells; a phenomenon linked to the diminished activity of cCBL E3 ligase resulting from the loss-of-function variant in CBL.[57] The results indicate that CBL mutations may function as a biomarker for the effectiveness of FLT3 inhibitors, presenting a potentially advantageous therapeutic strategy for CML patients harboring CBL variants. Pacritinib (PAC) is a tyrosine kinase inhibitor that demonstrates comparable effectiveness against both *FLT3* and *JAK2*, and it has received FDA approval for the management of myelofibrosis.[29] Studies indicate that PAC effectively inhibits the phosphorylation of *IRAK1* in AML cells, resulting in diminished viability and survival in AML cell lines with diverse mutations. Moreover, *IRAK1* has been identified as being overexpressed in chronic myelomonocytic leukemia (CMML). The administration of PAC demonstrates independent efficacy in primary CMML cells, and it has enhanced effects when combined with AZA. Furthermore, PAC extensively inhibits kinase-signaling pathways involved in tumor progression, including the driver mutations associated with *JAK2*. Exhibiting clinical tolerability and efficacy in conditions such as chronic myeloproliferative diseases, PAC distinguishes itself through its limited myelosuppressive and immunosuppressive characteristics in contrast to other *JAK2* inhibitors that similarly influence *JAK1* signaling.[58] These attributes highlight PAC's potential for incorporation into current treatment protocols for CML.

Nevertheless, the *RPTOR* gene was a highly mutated gene in our AP- and BC-CML patients, underscoring its critical role as a drug target for precision medicine in BC-CML. RPTOR constitutes a component of the mTOR pathway, which is subject to modulation by mTOR inhibitors.[31] The mTOR inhibitors that have received FDA approval include the rapamycin derivatives temsirolimus, everolimus, and ridaforolimus; however, their use is not indicated for blood cancer.[59] Reports indicate that temsirolimus and everolimus exert an inhibitory effect on mTORC2 in AML cells by activating the AKT signaling pathway. Furthermore, it has been noted that additional clinical studies involving patients with hematologic malignancies who were administered these medications yielded analogous outcomes.[60] The findings indicate that mTOR inhibitors may serve as a targeted therapeutic approach for leukemia patients exhibiting mutations in *AKT1*, *AKT2*, and *RPTOR*. Moreover, an experimental investigation has demonstrated that everolimus proved effective in addressing TKI-resistant CML cells, with a synergistic effect noted when combined with imatinib.[31] A further investigation revealed that ponatinib-resistant CML cells might acquire resistance via mechanisms that are not reliant on BCR-ABL, predominantly by activating the mTOR pathway. mTOR inhibitors have been recognized as potent agents in combating these resistant cells, facilitating the process of autophagy. Furthermore, it has been observed that the suppression of autophagy could amplify the cytotoxic effects of mTOR inhibitors in these cells. The combination of hydroxychloroquine, an autophagy inhibitor, markedly diminished cell viability in vitro and enhanced survival in vivo when compared to treatment with the mTOR inhibitor alone.[61] The findings highlight the significance of mTOR inhibitors in managing TKI-resistant CML, particularly for patients with *RPTOR* variants, which were notably mutated among our study subjects. This observation advocates for further investigation into combination therapies that could potentially augment the effectiveness of current treatment modalities.

The *BCR* (B-cell antigen receptor) gene has also been found to be a highly mutated gene in our advanced-phase CML patients in this study. The signaling of BCR plays a crucial role in the progression of CLL. Alterations in the BCR gene can influence the signaling pathway, thereby promoting the proliferation and survival of CLL cells. An essential element in this pathway is Bruton's Tyrosine Kinase (BTK), whose inhibition interferes with BCR signaling, diminishing

the growth and viability of CLL cells. Consequently, BTK has become a pivotal focus for innovative treatments in CLL and various B-cell-associated conditions. At present, multiple generations of BTK inhibitors are available. Ibrutinib, as the inaugural BTK inhibitor, has profoundly transformed the therapeutic landscape for CLL, attaining unprecedented response and survival rates. Second-generation BTK inhibitors, including acalabrutinib and zanubrutinib, provide enhanced specificity for BTK, thereby reducing side effects. Furthermore, the novel BTK inhibitor, pirtobrutinib, has been formulated to enhance efficacy and tackle the resistance observed with earlier BTK inhibitors, and it has recently received FDA approval for CLL.[62] It is prudent to consider the application of these inhibitors for treating BC-CML with BCR variants, especially when used in conjunction with TKIs, as the presence of variants in BCR may contribute to TKI resistance in patients with BC-CML.

Gilteritinib and midostaurin are FDA-approved FLT3 inhibitors utilized in the treatment of AML. These agents are employed in a range of therapeutic protocols, demonstrating efficacy in both newly diagnosed FLT3-mutated AML and in instances of relapse or refractory cases.[63] Quizartinib (QUI) represents an additional FLT3 inhibitor that has received FDA approval for patients with FLT3-positive AML.[56] Moreover, loss-of-function variants in CBL correlate with a phenotype characterized by either hyperactivation or heightened sensitivity of FLT3. Research indicates that QUI significantly hampers cell proliferation in wild-type FLT3 cells, a phenomenon linked to diminished cCBL E3 ligase activity resulting from the loss-of-function variant in CBL.[57] A study has illustrated the importance of NPM1 in AML patients as a noteworthy positive prognostic indicator in assessing the response to midostaurin. [64] The findings indicate that *CBL* and *NPM1* mutations may function as a biomarker for the effectiveness of FLT3 inhibitors, presenting a potentially advantageous therapeutic strategy for CML patients harboring CBL or NPM1 variants.

Moreover, Xiang et al.[65] discovered that pyrvinium, an FDA-approved medication for pinworm infections, demonstrates efficacy in treating BC-CML patients. The mechanism of action varies according to the specific type of cancer being addressed. Pyrvinium demonstrates an inhibitory effect on mitochondrial respiration in the context of CML, and its combination with dasatinib enhances apoptosis, thereby improving therapeutic outcomes for patients exhibiting resistance to TKI treatment. Nevertheless, it has yet to receive approval for patients with CML.

Status of Drug Repurposing in Evidence-Based Cancer Treatment

Despite the existence of numerous treatment protocols, these interventions often lead to the emergence of drug resistance in malignant cells, thereby diminishing the effectiveness of anticancer agents. This underscores the pressing necessity for innovative therapies to address the challenge of drug resistance. Nonetheless, developing a new drug approved for clinical use is a protracted endeavor, typically spanning 10 to 15 years and incurring costs ranging from 1 to 2 billion dollars. This intricate process commences with the design phase and progresses through various stages until safety and efficacy are established.[66] Furthermore, clinical evaluations are essential to ascertain appropriate dosing and effectiveness, and the shift to clinical application necessitates adherence to various regulatory and commercial stipulations. In addressing this challenge, drug repurposing is employed, wherein existing medications are utilized for alternative therapeutic applications that extend beyond their original indications. In contrast to the conventional de novo drug development approach, drug repurposing presents numerous benefits, such as improved efficiency, decreased time and financial expenditures, and a lower likelihood of failure. Furthermore, it leverages established understanding of drug mechanisms, thus enhancing the efficacy of clinical translation.[67]

In recent years, notable advancements in computational resources have enabled a methodical approach to drug repurposing. These resources employ diverse information sources, including electronic health records, genome-wide association analyses, gene expression profiles, pathway mappings, compound structures, target binding assays, and phenotypic profiling data. Furthermore, there has been a recent emphasis on computational repurposing methodologies, especially those centered around machine learning algorithms. Moreover, databases are meticulously crafted to facilitate in-silico drug repurposing, such as the Drug Repurposing Hub, repoDB, pandrugs, PathSurveyor, and RepurposeDB.[68,69] Consequently, our findings present a pragmatic approach to integrating genomics and multi-omics technologies alongside existing computational resources, facilitating the discovery of novel biomarkers, drug targets, and therapeutic options for various clinical manifestations with limited treatment modalities, such as BC-CML.[70-72]

Future Perspective

Our integrative genomics and AI-guided drug repurposing approach for BC-CML highlights a paradigm shift beyond traditional cancer therapeutics. By uncovering actionable, druggable mutations already targeted by approved therapies,[70,71] our strategy provides a highly pragmatic blueprint for precision medicine, especially for relapsed, refractory, and untreatable malignancies. As the success of drug repurposing during the COVID-19 pandemic has shown,[66,67] harnessing existing pharmacological arsenals through advanced multi-omics and AI technologies[72] can dramatically accelerate treatment innovation, offering hope for many patient populations historically considered beyond therapeutic reach. Our current study builds upon our earlier propositions advocating AI-assisted drug repositioning as a pragmatic route to precision oncology post-COVID-19.[73]

Building upon our findings identifying actionable mutations in myeloid/lymphoid lineage genes of BC-CML, practical oncology clinics can now immediately apply targeted repurposing strategies to manage otherwise untreatable cases. FDA-approved agents such as venetoclax, ivosidenib, and decitabine, traditionally used for AML and other hematologic malignancies, can be stratified based on mutational profiling at diagnosis or relapse. Integrating AI-guided drug-matching platforms into routine workflows will enable oncologists to

select patient-specific therapies rather than relying solely on TKIs, which often fail in the blast phase.[74] By 2026 and beyond, precision repurposing will likely be incorporated into standard clinical practice guidelines for advanced-phase CML, supported by the evolving paradigm of molecular reclassification in clinical trial designs[75] and predictive oncology models.[76] Advances in biomarker-driven therapies and personalized molecular-targeted strategies will further catalyze this transformation.[77] Our approach complements emerging international frameworks for repositioning strategies in aggressive leukemias, potentially reshaping future NCCN and ESMO guidelines.[78]

Conclusions

In our study, significant numbers of druggable mutations in genes associated with the AML/ALL lineage are identified in BC-CML patients when employing highly sensitive NGS techniques. The NGA technique, boasting coverage of 100X or greater, facilitates the identification of druggable gene mutations within AML-/ALL-lineage genes across nearly all BC-CML patients. Many drugs target these AML-/ALL-lineage gene mutations, and many are currently undergoing active trials or have already secured FDA approval. Consequently, our research offers valuable guidance for repurposing existing pharmaceuticals and exploring innovative therapeutic alternatives, thereby facilitating a more personalized approach to treatment for patients with BC-CML. Extensive, large-scale, and prospective investigations concerning druggable pan-cancer genes are essential to yield comprehensive insights into the molecular oncogenesis of BC-CML. Such studies are crucial for identifying novel biomarkers, drug targets, and therapeutic alternatives for this severe clinical presentation in CML.

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Ethical Considerations

The study was conducted in accordance with the Declaration of Helsinki. The Institutional Review Board (IRB) of King Abdullah International Medical Research Centre (KAIMRC), National Guard Health Affairs, through project # RA17 /002, approved it on 4 February 2019, although KAIMRC did not provide research funding. Written informed consent was obtained from each patient.

Competing Interests

The authors declare that they have no competing interests.

Data Availability Statement

Access to data made by next-generation sequencing can be obtained from NCBI, to which it was submitted, at https://www.ncbi.nlm.nih.gov/sra/PRJNA734750.

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