Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI 10.53555/68zhqm37

INVESTIGATIONS ON CLINICAL VALIDATION OF MUTATED ZNF208 AS A NOVEL BIOMARKER OF BLAST CRISIS IN CHRONIC MYELOID LEUKEMIA

Nawaf Alanazi[1,2], Ameer Mahmood[3], Abdulkareem Al-Garni[1,2], Arbila Umrani[3], Abdulaziz Haji Siyal[3], Salman Basit[4], Sameera Shaheen[3], Masood Shammas[5], Sarah AlMukhaylid[6], Aamer Aleem[7], and Zafar Iqbal[8*]

[1] Division of Hematology/Oncology, Department of Pediatrics, King Abdulaziz Hospital, Al-Ahsa.
[2]College of Applied Medical Sciences (CoAMS), King Saud Bin Abdulaziz University for Health Sciences, Al-Ahsa.
[3]Stem Cell Unit, department of Anatomy, College of Medicine, King Saud University, Riyadh.
[4]Centre for Genetics and Inherited Diseases, Taiba University, Madina.
[5]Dana Farbar Cancer Institute, University of Harvard, Boston USA.
[6]Clinical laboratory department, Johns Hopkins Aramco HealthCare (JHAH), Alahsa.
[7]Department of Medicine, Division of Hematology/Oncology, College of Medicine and King Khalid University Hospital, King Saud University, Riyadh.
[8*]Genomic Medicine & Oncology/Hematology Group **(GeM-On-Hem)** & Quality Assurance and Accreditation Unit (QAAA), CoAMS-A, King Saud Bin Abdulaziz University for Health Sciences & King Abdullah International Medical Research Centre (KAIMRC) / SSBMT, King Abdulaziz Medical City, National Guard Health Affairs, Al-Ahsa.

***Corresponding Author(s):** Prof. Dr. Zafar Iqbal,
*GeM-On-Hem, QAAA: drzafar.medgen1@gmail.com ; iqbalz@ksau-hs.edu.sa &
Dr. Amer Mahmood: ammahmood@ksu.edu.sa

Tweetable Abstract:
Blast crisis Chronic Myeloid Leukemia (BC-CML) is fatal due to lack of early biomarkers. In this study, we discovered mutated ZNF208 gene as novel biomarker for this clinical manifestation and its clinical validation. It will help early identify patients as risk of progression**.**

Abstract:
BCR-ABL1, the hallmark of cancer, promotes genomic instability leading to further mutation acquisition in CML. This transforms the manageable Chronic Phase (CP-CML) into an accelerated phase (AP-CML) and fatal crisis phase (BC-CML). Highly specific biomarkers for early detection of BC-CML are lacking. Transcription factor (TF) mutations universally cause cancer progression, relapses, and metastasis. Recently, ZNF208 TF was reported mutated in BC-CML but its reproducibility and clinical validation were required. Current studies utilized next-generation sequencing to validate mutated ZNF208 (c.64G>A) as a novel CML progression biomarker as it was detected in 0 (0%), 90% (10/20), and 100% (12/12) CP-, AP-, and BC-CML patients (p=0.0001), suggesting its high disease specificity. We recommend prospective clinical trials to further validate this novel CML progression biomarker.

Keywords: *Chronic Myeloid Leukemia; Accelerated phase; Blast crisis; Disease Progression; Molecular Biomarker; Transcription Factors; Zing Finger Proteins; Cancer.*

1. Introduction

Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm marked by excessive production of granulocytes in the bone marrow and peripheral blood. [1]. CML is characterized by the presence of Philadelphia chromosome t(9:22), a reciprocal translocation between the Abelson murine leukemia (ABL) gene on chromosome 9 and the breakpoint cluster region (BCR gene) on chromosome 22 [2]. The fusion gene results in persistent expression of the BCR-ABL tyrosine kinase on hematopoietic stem cells (HSCs), causing leukemic transformation of stem cells (LSCs) and facilitating uncontrolled production and replication of granulocytes [3]. An inherent characteristic of CML is the presence of genomic instability induced by BCR-ABL itself. This instability results in the emergence of more mutations in both BCR-ABL and other genes as the disease progresses [4].

The development of BCR-ABL tyrosine kinase inhibitors (TKIs) has been a major advancement in the treatment of CML, making overall survival of CML patients nearing the general population, at least in the technologically advanced countries [2]. The TKIs have resulted in a marked reduction in the rate of progression of CML patients from the chronic phase (CP) to the accelerated phase (AP) or blast crisis (BC) [5]. Despite the high efficacy of TKIs, some patients experience resistance resulting in therapy failure and progression to advanced phase CML [6]. However, the exact mechanisms responsible for the progression of CML are not fully understood, and there is a paucity of reliable molecular markers associated with the progression of CML to AP and BC [3]. As the survival of CML patients who develop BC still remains poor, early recognition of patients at risk of disease progression remains an important issue in CML management [5, 6].

Transcription factors (TFs) are responsible for initiation, invasion, metastasis, drug resistance, and relapse of many types of cancers [7]. TFs are proteins that selectively attach to particular DNA sequences, facilitating the transfer of genetic information from DNA to messenger RNA (mRNA) and thus function as regulators of gene expression [8]. The activity of TFs can be modified in cancer through many direct and indirect mechanisms including chromosomal translocations, gene amplification, deletions, point mutations, altered gene expression, noncoding RNAs, DNA methylation, and through epigenetic factors [9]. Although TFs are recognized to have a significant role in the development of acute leukemias, their precise role in the progression of CML is still not known.

We recently reported a novel transcription factor ZNF208 with a missense mutation (c.64G>A) associated with the progression of CML [10]. Some markers of disease progression in CML like BCR-ABL kinase domain mutations are available in clinical practice, they are not detected in all AP- and BC-CML patients and there is a need for additional markers which may help in the diagnosis of disease progression with high specificity [3,4,6]. Keeping in view that no universal molecular biomarker(s) are currently available to detect CML patients at risk of progression, and specifically for BC-CML [3, 5, 6], this study was conducted with the objective to clinically validate mutant ZNF208 as a new biomarker for the progression of CML [10].

2. Materials and Methods

Patients

This study included CML patients who were managed at King Abdulaziz National Guard Hospital, Al-Ahsa, Saudi Arabia. The study period spanned from January 2019 to Dec 2021. The experimental group consisted of 20 patients with accelerated phase (AP-CML) and 12 patients with blast crisis (BC-CML). The study also included 41 patients diagnosed with chronic phase (CP) CML as the control group. All CML patients received imatinib mesylate (IM) as their first-line treatment. Patients who did not respond well to IM, were subsequently switched to second- and/or third-generation TKI [11].

Definitions

The definitions of clinical stages of CML were adopted according to the previous ELN guidelines [11, 12, 13]. Briefly, the presence of resistance to two or more TKIs, identification of a mutation in the kinase domain (KD) of the BCR-ABL1 gene, or the development of additional chromosome abnormalities in Philadelphia chromosome-positive (Ph+) cells (ACA), along with the clinical parameters like resistant splenomegaly and rising white cell count, were considered as the indication of progression of the disease [11]. The criteria and evaluation for hematological and cytogenetic responses were also based on the ELN guidelines [11, 12] and the studies carried out previously [14-16]. Molecular response definitions were also adopted from the ELN 2020 guidelines [11].

Overall survival refers to the duration of time from the diagnosis and initiation of IM treatment, to either the date of the patient's death or the most recent follow-up [17]. Progression-free survival (PFS) was defined and evaluated from the initiation of IM treatment till the development of AP or BC. The survival status of the patients who did not attend their last scheduled follow-up before the data collection, was confirmed by reaching out to them using their recorded contact information. The Kaplan-Meier Method was used to conduct survival analysis [18, 19].

All procedures used in this study were approved by King Abdullah International Medical Research Center (KAIMRC). Study was approved by the institutional review boards of the participating centers. All patients enrolled in the study provided written informed consent. Study was conducted according to the codes of Declaration of Helsinki [20].

Sampling and DNA extraction

Ten milliliters of peripheral blood was drawn for the study and placed in EDTA tubes (BD Vacutainer Systems, Franklin Lakes, New Jersey.) The QIAamp DNA Blood Mini Kit (QIAGEN) was utilized for DNA extraction, and the NanoDrop Spectrophotometer (NanoDrop Technologies, Inc., USA) was employed for DNA quantification. After dilution, aliquots of 70–80 ng/µl were created to detect mutations using whole exome sequencing (WES). To perform Sanger sequencing, the residual DNA was diluted to 40 ng/µl. DNA samples were kept and stored in a refrigerator at -80°C till further processing [21-22].

Next-Generation Sequencing (NGS) Experiments:

Next-generation sequencing (NGS) was employed to carry out whole exome sequencing (WES). Samples from CP-, AP-, and BC-CML patients were subjected to NGS analysis. The Illumina® DNA Prep with Enrichment, (S) Augmentation kit (Cat. # 20025523) was used to enrich the target DNA [22, 23]. The initial stage of NGS involved the process of DNA fragmentation, which was followed by tagmentation. Tagged DNA fragments were amplified and subsequently purified using magnetic beads. Next, Oligos were employed to capture specific regions of interest. The amplified libraries were enriched using PCR and their quantity was determined using a Qubit fluorometer. The library size distribution was measured using an Agilent Bioanalyzer. The Illumina NextSeq500 instrument was used to perform cluster generation and exome sequencing. The quantified DNA libraries were loaded onto the flow cell for this purpose [22, 23]. As we were looking for the common biomarker of CML progression, WES was utilized to shortlist only those gene(s) mutated in all BC-CML and in none of the CP-CML and healthy controls.

Analysis of Next-Generation Sequencing (NGS) Data:

The BCL2FASTQ software was used to convert the output files, specifically the BCL files, into FASTQ files. The BWA aligner was used to align the FASTQ data to the human genome, utilizing the BWA-MEM algorithm. The genome analysis tool kit (GATK) was used to identify variants. The genomic variations were annotated and filtered using Illumina Variant Studio [22,24].

Primary Analysis:

Filtering techniques that utilized the identification of uncommon genetic variations and the exclusion of intron and synonymous variations were employed. In addition, any variants that had a known prediction of being either benign (B) or tolerant (T), were eliminated. According to Carson (2014), certain variants were labeled as B if they had a frequency of 70% or higher for B, while other variants were categorized as T if their frequency for T was 70% or higher [25]. Any variants with a population frequency greater than 0.005 in the dbSNP and Exome Sequencing Project (ESP) database were excluded. Therefore, the process of identifying variants was restricted to only those with significant protein impacts and splice variants. In addition, the data were subjected to additional analysis to examine new genetic variants that are found in patients with advanced phase (AP and BC) CML, but not in patients with CP-CML, or healthy individuals. This suggests that these mutations may have a role in the progression of the disease [26, 27]. The data generated by NGS were submitted to NCBI, and can be accessible from the National Center for Biotechnology Information (NCBI) at https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1119181.

Strategy for screening of common biomarker for AP-CML:

Although some markers of disease progression, like BCR-ABL kinase domain, ASXL1, and GATA2 mutations are available, no universal and exclusively specific molecular biomarkers exist to early diagnose CML patients at risk of CML progression for timely therapeutic interventions to delay or minimize fatal blast crisis transformation in CML (BC-CML) [3,4,6]. In our previous study, our group found that all AP- and BC-CML patients harbored the ZNF208 mutation c.64G>A [10]. In that study, by utilizing WES-based screening for universal AP-/BC-CML biomarkers,, they had shortlisted only those genes that were mutated in all BC-CML patients and in none of CP-CML or healthy controls, as their objective was to find a "Common and very specific biomarker for BC-CML" [10]. Moreover, our previous study was needed to be reproduced as well as further clinical validation of that study was required using a larger number of CML patients. Therefore, we utilized same approach to shortlist only that gene(s) mutated in all or majority (90% or more) of AP-CML or BC-CML. Our NGS sequencing in current study found same ZNF208 in all (100%) BC-CML and all but two (18/20, 90% of AP-CML patients. Therefore, ZNF208 gene was screened for further clinical validation studies.

Sanger Sequencing to Validate Mutation(s):

Sanger sequencing was used to confirm the variants identified by WES. The University of California Santa Cruz's genome database browser was used to retrieve the genomic primers for the variants found in the indicated genes **(Table 1).** The primers were synthesized from Applied Biosystems (California, USA). The process of amplifying template DNA involved PCR. DNA sequencing reactions were performed using ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction kits (Applied Biosystems, California, USA) [28]. The ABI Prism 3730 Genetic Analyzer (Applied Biosystems, California, USA) was used to perform Sanger sequencing on both forward and reverse DNA templates [27-30].

Table 1. Common variants in Accelerated/Blast Phase-Chronic Myeloid Leukemia patients (AP, n=5, BC; n=7), primers designed to amplify the regions around the detected variants in FANCD2 and ZNF208 genes.

#	Gene Symbol	Sequence	Size (bp)	Tm
1	ZNF208-F	5` TCTGCCCATGTCCAGTTGAT 3`	234	61.5°C
2	ZNF208-R	5` GGTCTGCAGCAAAATGAAACC 3`		61.9°C

Legend: F= forward primer, R= reverse primer, bp= base pair, Tm= melting temperature, °C= degree centigrade

Analysis of the ZNF208 mutation

Genomic mapping of ZNF208 mutation c.64G>A was carried out using NCBI Variation Viewer as well as UCSC Genome Browser on Human and its interaction with other genes [31, 32]. Gno-mAD browser was utilized to calculate its frequency in general population [33] Genome Browser on Human (GRCh37/hg19) was employed to study the interaction of ZNF208 with many other important genes [32].

Analysis of Patient Clinical Data Using Statistical Methods:

The normalcy test provided evidence for percentages, absolute numbers, and categorical data. For continuous data, the same procedure was used in addition to a suitable measure of variance. We used Fisher's exact test or Chi-Square test to compare two means of categorical data. The Mann-Whitney U test or a two-sample independent test was utilized for continuous data. Furthermore, ANOVA or Kruskal-Wallis tests were performed to ascertain the variance for groups of ≥3. The survival outcome was measured using Kaplan-Meier survival analysis curves [18]. The group comparison was conducted using the log-rank test. SAS/STAT software (SAS Institute Inc., Cary, NC, USA), version 9.4 was used for data analysis. For statistical computation, the R foundation was used (Vienna, Austria) [34]. The Sokal risk score was calculated per standard methods [11, 35].

Our overall methodologies show that a standard procedure was utilized to investigate and validate mutant ZNF208 as clinical biomarker of CML disease progression [36-59].

3. Results

Patients' demographics

A total of 73 patients with CML were enrolled in this study. The mean age of the group was 34.6 years, and the male-to-female ratio was 1.6:1, with 60.3% males and 40.7% females. The mean hemoglobin, WBC count, and platelets were 10.1 x g/dl, 317.9 x 10^9, and 400.2 x 10^9, respectively (Table 2). Most of the patients had intermediate or high SOKAL score (Table 2).

The mean ages of CP-, AP-, and BC-CML patients were 33.5, 35.6, and 38.1 years, respectively. There was a male preponderance in all the phases of CML; male and female patient numbers in 3 phases of CML are given in table 3. There was a higher trend of CML progression in male patients, though the differences were not statistically significant (Table 3). CP-, AP-, and BC-CML patients showed statistically significant differences with respect to leukocyte count of 50 × 10^9/L or higher (p=0.04), splenomegaly of 5 cm or more, hepatomegaly (p=0.006), and survival (p=0.001). Figure 1 illustrates the overall survival (OS) of BC-CML patients (n=13) in months. The mean follow up time was 14.2 months (range: 2-28). The mean OS of BC-CML patients was 14 months with 9 deaths (75% confirmed deaths).

Table 2. Patients' overall demographics and laboratory parameters

Characteristics	**Number of Patients, %**
Total (n=73)	
Age in years (mean, range)	34.6 (15-50)
Gender	
Male	44 (60.3%)
Female	29 (39.7%)
Sokal Score	
<0.8 (low risk)	5 (6.8%)
0.8–1.2 (intermediate risk)	39 (53.4%)
>1.2 (high risk)	29 (39.8)
Hemoglobin (g/dL), Mean	10.1
WBC count (× 10^9/L), Mean	313.7
Platelets (× 10^9/L) (n =108), Mean	400.2

Table 3: Comparison between CP-, AP-, and BC-CML patients in this study in regard to their demographic and laboratory characteristics.

Characteristics	Patient groups, number (%)			
	CP-CML	AP-CML	BC-CML	P value
Patients' number (%)	41 (56.2)	20 (27.4)	12 (16.4)	
Mean age Range))	33.5 (range 7-69)	35.6 (range=27-43)	38.1 (29–50)	p=0.92205
Gender				
Male	25 (60.1)	13 (65)	8 (66.7)	Overall p=0.70745
Female	16 (39.9)	7 (35)	4 (33.3)	Overall p=0.70675
Sokal Score				
<0.8 (low risk)	5 (12.3)	0 (0)	0 (0)	P=0.05
0.8–1.2 (intermediate risk)	24 (58.5)	12 (60)	3 (25)	
>1.2 (high risk)	12 (29.2)	8 (40)	9 (75)	
Hemoglobin g/dL)				
<12g/dl	34 (82.9)	17 (85)	9 (75)	Overall p=0.1988
≥12g/dl	7 (17.1)	3 (15)	3 (25)	Overall p=0.43545
WBC $X10^9/L$				
<50	20 (48.8)	3 (15)	2 (16.7)	p=0.89185
Platelets (× 10^9/L)				
<450	25 (61)	13 (65)	10 (83.3)	p=0.4543
≥450	16 (39)	7 (35)	2 (16.7)	p=0.58755
Imatinib				
Yes	41 (100)	13 (65)	0 (0)	p=0.05
Chemotherapy				
Yes	0	0	8 (66.7)	P=0.0003
Splenomegaly				
<5cm	2 (4.8)	0	0	p=0.03
5-8cm	13 (31.7)	3 (15)	3 (25)	
>8cm	23 (56.1)	17 (85)	9 (75)	

Hepatomegaly				
Yes	12 (29.3)	14 (70%)	8 (66.7)	p=0.006
Survival Status				
Confirmed Deaths	0	1(5)	9 (75)	CP vs BC, p < 0.0001
Alive at last follow-up	41 (100)	19 (85)	3 (25)	CP vs BC, p < 0.0001
Patient positive for mutated ZNF208	0	18 (90)	12 (100)	CP vs BC, p < 0.0001 CP vs AP, P=0.0001

CML, chronic myeloid leukemia; CP, chronic phase; AC, accelerated phase; BC, blast crisis; WBC, white blood cell

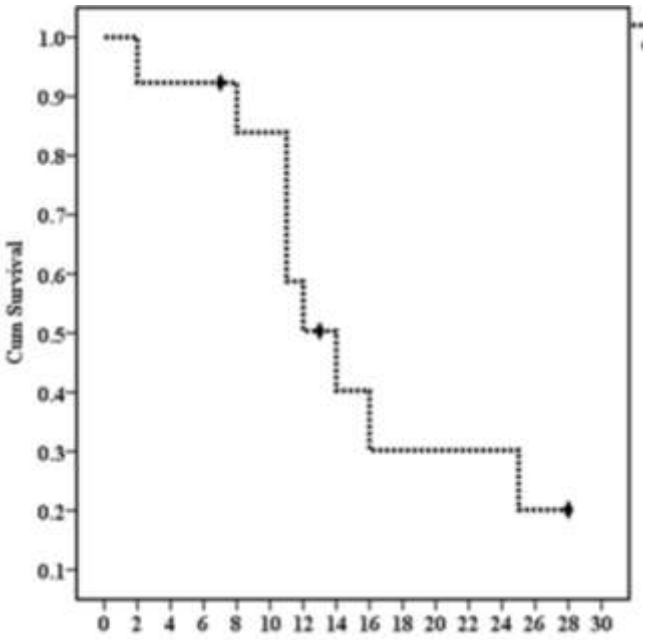

Figure 1: Overall survival of Blast Crisis-Chronic Myeloid Leukemia patients (n=12).

(Figure egends: OS: Overall survival, BC-CML: Blast crisis-chronic myeloid leukemia, n= number of patients)

Despite the differences, none of these variables was helpful in early prediction of CML progression. Therefore, it warranted utilization WES to screen gene(s) exclusively associated with CML progression.

3.2 Clinical Validation of ZNF208 by WES

WES-based screening utilized to screen gene(s) exclusively associated with CML progression found ZNF208 gene mutation (c.64G>A) corresponding to chromosome 19 locus 22,171,651 in 100% of BC-CML and 90% (18/20) AP-CML. It showed that ZNF208 is a novel biomarker of CML progression (AP- and BC-CML patients) as it was not detected in any of the controls (CP-CML patients as well as healthy controls). The results were confirmed by Sanger sequencing.

The mutation detected in ZNF208 gene (c.64G>A) is a novel point mutation in which guanine was replaced by adenine at condon 22 in position 64 downstream of ZNF208 promoter (Figure 2a & Figure 2b), corresponding to overall position of 22171651G>A on chromosome 19p12 (Figure 3).

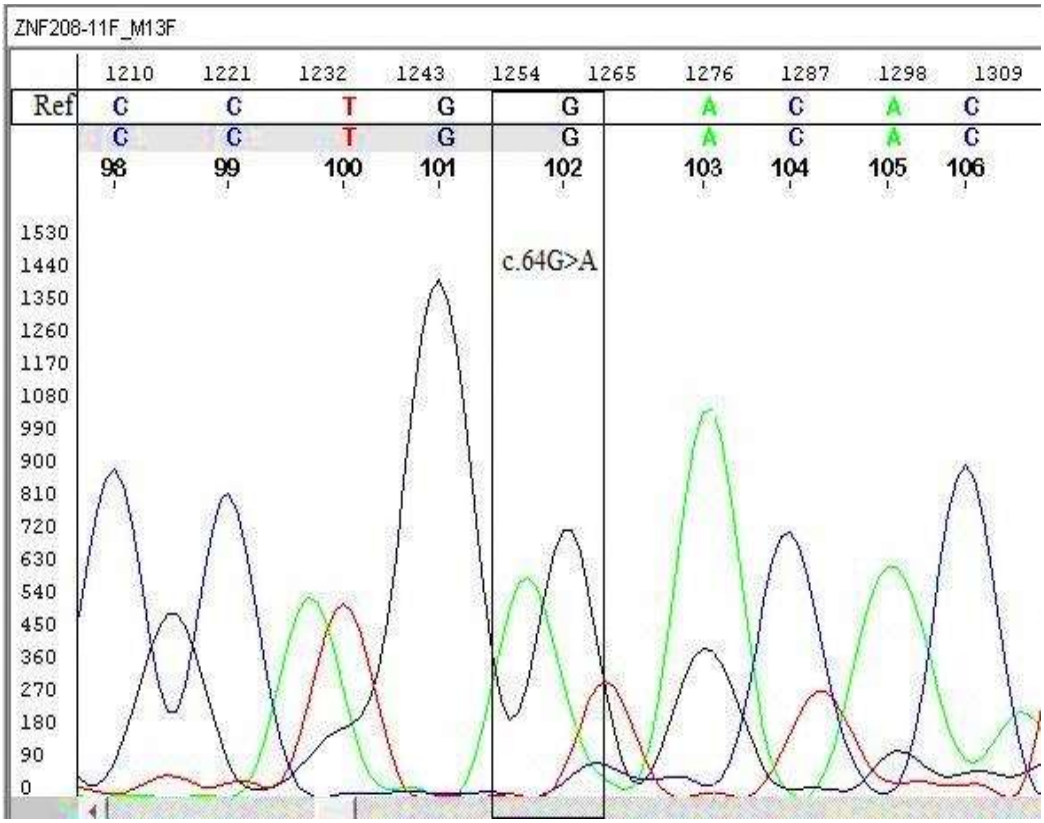

Figure 2a: *Validation of missense mutation ZNF208 (c.64G>A) by Sanger sequencing.*

Figure 2b: *Analysis of missense mutation ZNF208 (c.64G>A)*

https://www.ncbi.nlm.nih.gov/nuccore/PP485041.1?report=graph&rid=609EGY4W016[PP485041.1]&tracks=[key:sequence_track,name:Sequence,display_name:Sequence,id:STD1,category:Sequence,annots:Sequence,ShowLabel:true][key:gene_model_track,CDSProductFeats:false][key:alignm

ent_track,name:other%20alignments,annots:NG%20Alignments|Refseq%20Alignments|Gnomon%20Alignments|Unnamed,shown:false]&v=25:105&appname=ncbiblast&link_loc=fromHSP

Figure 3: UCSC Genome Browser on Human (GRCh37/hg19) shows the genomic mapping of the *ZNF208 (c.64G>A) variant (https://genome.ucsc.edu/trash/hgt/hgt_genome_20ade_f229d0.png).*

UCSC Genome Browser on Human (GRCh37/hg19) showed the genomic mapping of the ZNF208 (c.64G>A) variant (Figure 3). Gno-mAD browser showed that the frequency of this gene variant in general population to be very low (0.0004084). It shows that ZNF208 (c.64G>A) leads to asparagine instead of aspartate at the protein level (p.Asp22Asn) that is a missence mutation. Gene-gene network analysis using UCSC Genome Browser on Human (GRCh37/hg19) showed that ZNF208 interacts with many other important genes like TP53, TNF, UBC etc. (Figure 4) that have roles in many important functions including DNA repair, Cell proliferation, differentiation, cellcycle arrest, apopsosis etc. The tissue-specfic MRNA expression from GeneAtlas U133A, gcrma shows that this ZNF208 is highly expressed in blood, bone marrow and myeloid cells indicating its important role in haematopoiesis (Figure 5; http://biogps.org/#goto=genereport&id=7757).

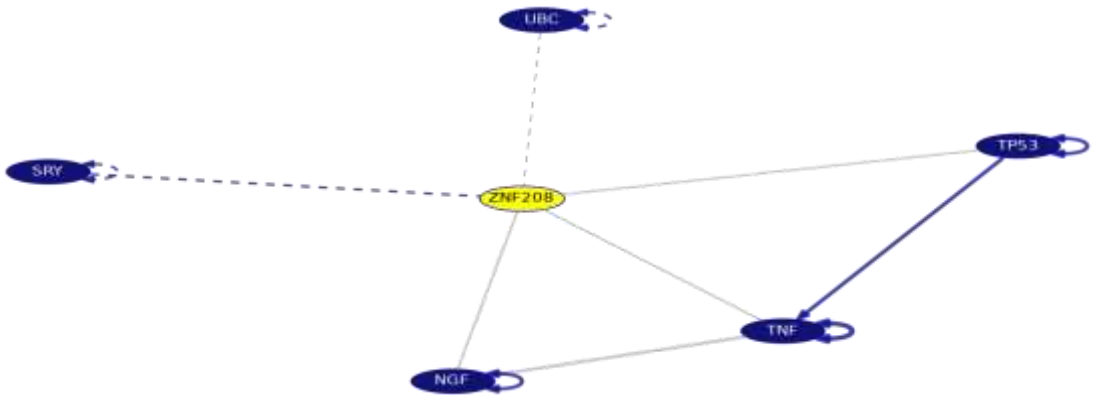

Figure 4: Gene-gene network analysis shwingd ZNF208 interactions with other important genes

(https://genome.ucsc.edu/cgi-bin/hgGeneGraph?gene=ZNF208&1=OK&supportLevel=text&geneCount=25&hgsid=2288082666_Dg8nyt475tNNrb3b6wKDLMH7VjKS)

Our further analysis of ZNF genes found another ZNF-family gene ZNF141 with multiple mutations in 100% of AP-CML patients, all leading to protein-level changes in ZNF141 (Table 4). This shows that mutations in ZNF-gene family could be an early event in AP-CML.

Table 4. Whole exome sequencing indicating mutations in *unknown gene variants* common in Accelerated Phase-Chronic Myeloid Leukemia patients

Pos	Gene_Name	HGVS.c	HGVS. p	Effect
338,197	ZNF141	c.204_205insGA	p.Lys69fs	frameshift_variant
338,200	ZNF141	c.207G>C	p.Lys69Asn	missense_variant
338,201	ZNF141	c.209_210delTC	p.Ile70fs	frameshift_variant

Overall, the results of our study confirmed that ZNF208, a member of the zinc-finger binding transcription factor family, was mutated in all BC-CML, and 90% AP-CML patients. These findings suggest that ZNF208 (c.64G>A) is a likely-to-be-pathogenic mutation and a valueable new molecular marker of CML progression with a specificity of 90% to 100%, and can help in early identification and hence timely therapeutic interventions of patients at risk of transformation to BC-CML.

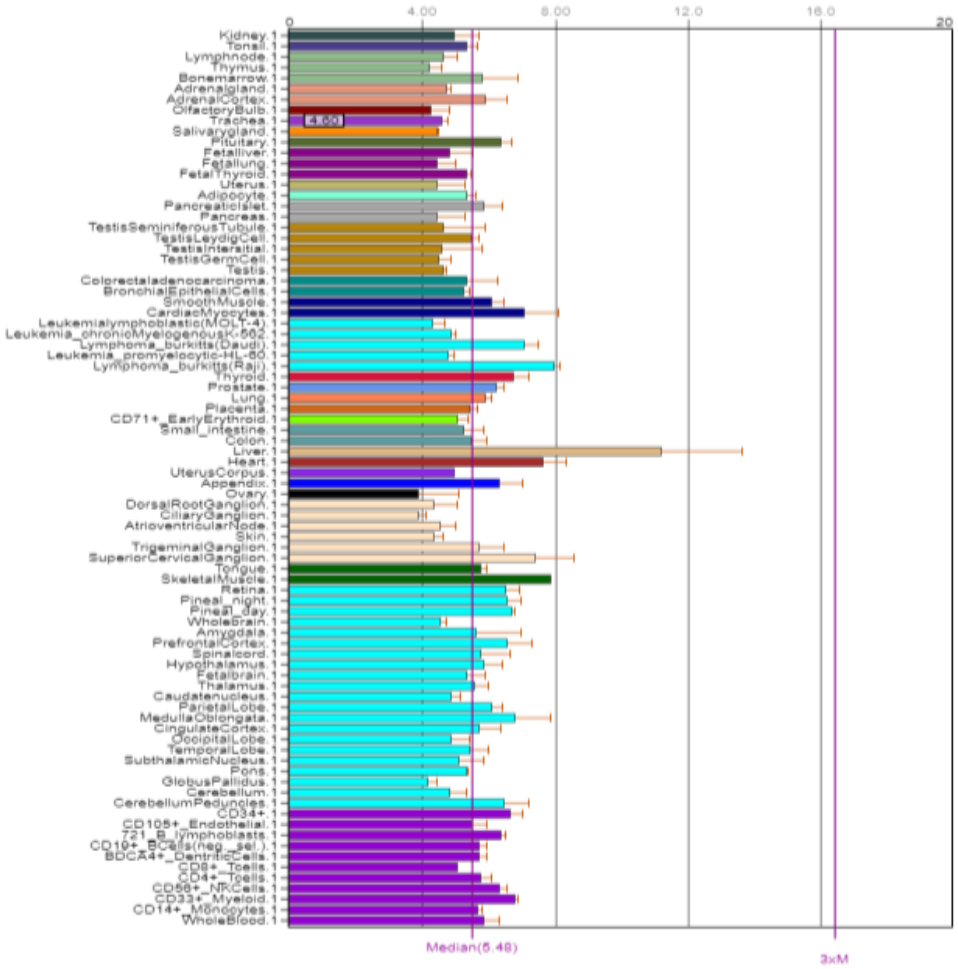

Figure 5: The tissue-specfic MRNA expression showing high expression of ZNF208 gene in blood, bone marrow and myeloid (Source: GeneAtlas U133A, gcrma, http://biogps.org/#goto=genereport&id=7757)

4. Discussion

This study included 73 subjects who were diagnosed and managed during different phases of chronic myeloid leukemia (CML). Our findings revealed that the ZNF208 gene, a member of the biologically important group of genes known as Zinc Finger genes, remained unaltered in chronic phase CML patients as well as in healthy individuals. However, it was found to be mutated in all patients with BC-, and 90% of AP-CML. The presence of mutated ZNF208 in the advanced phase CML suggests its role in the progression of CML and the development of BC. The human genome contains a large number of zinc finger (ZNF) genes, with around 500-600 members [50]. Zinc finger proteins have diverse molecular functions including a role in controlling the genes and the process of development, and they have mostly remained the same throughout evolution [51].

We conducted this study as case-control-based clinical validation of potential disease biomarkers which is a gold standard in the discovery and clinical validation of novel biomarkers [36, 37]. We applied NGS and Sanger sequencing for clinical validation of ZNF208 (c.G64A) mutation in AP- and BC-CML. Targeted resequencing NGS and Sanger sequencing along with other similar techniques are routinely being used for clinical validation of molecular biomarkers [38-48]. Moreover, the incorporation of NGS and other high-throughput techniques have refined the discovery and validation of diagnostic and prognostic biomarkers and their incorporation into precision medicine [43-51].

Research has indicated that the aberrant synthesis of many ZNF proteins has an impact on cancer development through several mechanisms [52]. ZNF309 has been detected in various types of cancers, including colorectal cancer, multiple myeloma, prostate cancer, cervical cancer, ovarian cancer, and neuroblastoma [53]. A relationship was discovered between elevated gene expression of ZNF-281 and many cancers including pancreatic, colon, breast, neuroblastoma, and ovarian malignancies. It was shown that there was an overexpression of the ZNF-281 protein, which was associated with the development of disease [53]. While excessive expression of ZNF-281 may be associated with a negative prognosis, in a contrasting manner, it suppresses the development of cancer in glioma and non-small cell lung cancer [52]. ZNF proteins have recently been identified as protectors of genomic integrity and mutations in ZNF genes can cause genomic instability through well-known mechanisms [54].

As previously mentioned, we identified a missense mutation in the ZNF208 gene that was specifically linked to patients with advanced phase CML. This missense mutation results in the substitution of aspartate with asparagine in the ZNF208 protein. The ZNF208 gene is situated on chromosome 19 in the p12 region [55]. It interacts with DNA and regulates the process of gene transcription [50, 56]. Furthermore, research has demonstrated a correlation between ZNF208 and various other medical conditions. A Genome-Wide Association Study (GWAS) identified a correlation between telomere length and the ZNF208 gene [57]. A study conducted in China reported a correlation between single nucleotide polymorphisms (SNPs) in ZNF208 and susceptibility to Hepatitis B virus (HBV) infection. The study included 300 healthy volunteers and 242 patients who tested positive for HBV. The results suggested that ZNF208 variations may have a substantial impact on the progression of HBV. The study found that ZNF208 polymorphisms significantly increased susceptibility to HBV infection [50]. This part about Hepatitis-B is nor very relevant and should be deleted!

Moreover, mutations in the ZNF208 gene are associated with an inclination towards developing esophageal cancer [58]. One study showed that the likelihood of developing laryngeal cancer increases with particular variations in the ZNF208 gene, specifically the rs8103163 A and rs7248488 A alleles [59]. Another study conducted in China indicated that ZNF208 variations had an impact on telomere length in individuals with chronic obstructive pulmonary disease [60]. It was shown recently that cirZNF208 has a function in the development of cervical cancer and has an impact on its prognosis. This demonstrates the significance of ZNF208 in the field of biology, particularly in relation to health and disease. Our study adds valuable insight in the role played by ZNF208 in the progression of CML.

Mutations in the ZNF208 gene have the potential to serve as a new biomarker for monitoring the progression of CML. Additionally, these mutations can aid in tailoring treatment for patients with

advanced phase CML. ZNF432 has recently been discovered to function as an effector of HR-based DNA repair. This finding suggests that PARylation may play a regulatory role in this mechanism [54]. PARYlation is known to coordinate the recruitment of crucial proteins involved in the DNA damage response and guide the DNA repair pathways. The expression of ZNF432 was reduced in cases of resistance to the PARP inhibitor olaparib. Moreover, altering the expression of ZNF432 in ovarian cancer cells was observed to increase their susceptibility to PARP inhibitors such as olaparib [54, 62, 63-66]. These observations suggest that ZNF208 gene mutations may have a role in treatment response in advanced phase CML, and needs further investigation.

Some novel investigations have unraveled the mechanism of ZNF-mediated repression of transcription in the genome. The proteins coded by ZNF genes have a Canonical Kruppel-associated box (KRAB) domain that has an affinity for KRAB-associated protein-1 (KAP-1) or Trim28 [67]. KRAB-ZFP identifies a specific region in the genome that is intended for suppression. The engagement with dimeric Trim28 subsequently attracts the epigenetic apparatus to achieve strong transcriptional suppression [67-69]. As the ZNF208 c.G64A mutation under our investigation also lies in the KRAB domain of the ZNF208 protein, it may have a role in KAP1-mediated repression of some other target proteins in the genome, leading to CML progression, and utilizing agonists of ZNFs and KP-1 may lead to the development of novel anti-cancer drugs [69]. Recently, ZNF208 has been reported to be involved in ALL leukemogenesis ([70]. Similarly, latest reports have shown zinc finger genes associated with different types of acute leukemias [71]. Various other studies show that by integrating multi-omics with artificial intelligence (AI), further studies can help unravel mechanism of CML blast crisis development and expedite cancer drug development for cancers like BC-CML with limited treatment options [43-49]. Further studies are needed to investigate the role of mutated ZNF208 in CML progression, its validation as a biomarker in prospective clinical trials, and explore this mutation as a potential drug target in advanced phase CML [71-75].

5. Conclusions

In conclusion, we found that ZNF208, a member of the zinc-finger binding transcription factor family, is mutated in all patients (100%) with BC-CML and in 90% of patients with AP-CML. This mutation affects the sequence of the ZNF208 protein, which suggests that it is a "likely pathogenic" mutation. Therefore, it appears to be an important and novel molecular biomarker for the progression of CML, which can help in the early identification of patients at risk of transformation to BC-CML. Larger studies are needed to further evaluate the role of ZNF208 (c.G64A) mutation as a novel biomarker and a possible drug target for advanced phase CML.

Author Contributions: Conceptualization, Z.I., N.A. and A.A..; methodology, Z.I., A.M.,A.S., N.A., S.A., M.S. and S.B. ; software, S.B..; validation, Z.I., A.M.,and A.A..; formal analysis, X. Z.I., A.M.,A.S., N.A., S.A., M.S. and S.B.; investigation, Z.I., A.M.,A.S., N.A., S.A., M.S. and S.B.; N.A., A.A., A.M, Z.I.; data curation, Z.I., A.M.,A.S., N.A., S.A., M.S. and S.B..; writing—original draft preparation, Z.I., A.M.,A.S., N.A., S.A., M.S. and S.B.—review and editing, Z.I, N.A., A.A., S.B.; visualization, Z.I., A.M.,A.S., N.A., S.A., M.S. and S.B.; supervision, Z.I., A.M., N.A., M.S. and S.B.; project administration, Z.I., A.M., N.A., M.S. and S.B; funding acquisition, Z.I., A.M. All authors have read and agreed to the published version of the manuscript."

Funding Information. This work was funded by the National Plan for Science, Technology and Innovation (MAARIFAH), King Abdul-Aziz City for Science and Technology, Kingdom of Saudi Arabia, Grant Number 14-Med-2817-02.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki. It was approved by Intuitional Review Board (IRB) of King Abdullah International Medical Research Centre (KAIMRC), National Guard Health Affairs, Saudi Arabia

through project # RA17/002/A, dated 4th Feb 2019., although no research funding was provided by KAIMRC.

Informed Consent Statement: Written informed consent has been obtained from the patients to publish this paper.

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/bioproject/PRJNA1119181 (SRA accession number PRJNA1119181).

Acknowledgments: We acknowledge Institutional Review Board (IRB) of King Abdullah International Medical Research Centre (KAIMRC), National Guard Health Affairs, Saudi Arabia for ethical approval of this project (project # RA17/002/A, dated 4th Feb 2019)., although no research funding was provided. This study was partially supported by the College of Medicine Research Centre, Deanship of Scientific Research, King Saud University, Riyadh, Saudi Arabia. All authors have read and have consented to the acknowledgement.

Conflicts of Interest: The authors acknowledge no financial or other conflicts of interest.

References

1. hmed HG, Alshammari MS, Alharbi HH, et al. Epidemiology of hematological malignancies in Hail region, Northern Saudi Arabia. Medical Science. 2020;24(102):699-705
2. Jabbour E, Kantarjian H. The evolution of treatment strategies for chronic myeloid leukemia. Review of Clinical Medicine. 2021;18(4):337-349. doi:10.1007/s11845-021-02634-8
3. Chen Q, et al. Impact of BCR-ABL1 Transcript Type on Treatment Outcomes in Chronic Myeloid Leukemia: A Systematic Review and Meta-Analysis. Leukemia. 2020;34(2):385-393. doi:10.1038/s41375-019-0467-6
4. Zeng F, Peng Y, Qin Y, et al. Regulation of DNA Damage Response and Its Impact on Imatinib Resistance in Chronic Myeloid Leukemia. Oncotarget. 2020;11(22):2109-2122. doi:10.18632/oncotarget.27572.
5. Ruggeri A, Labopin M, Bacigalupo A, Afanasyev B, Cornelissen JJ, Elmaagacli A, Itälä-Remes M, Blaise D, Meijer E, Koc Y, Milpied N, Schouten HC, Kroeger N, Mohty M, Nagler A. Post-transplant cyclophosphamide for graft-versus-host disease prophylaxis in HLA matched sibling or matched unrelated donor transplant for patients with acute leukemia, on behalf of ALWP-EBMT. J Hematol Oncol. 2018 Mar 15;11(1):40. doi: 10.1186/s13045-018-0586-4. PMID: 29544522; PMCID: PMC5855999.
6. Chimge NO, Chen MH, Nguyen C, et al. Metabolic Adaptations of Quiescent Leukemic Stem Cells: The Role of Fatty Acid Oxidation and Mitochondrial Function. Blood. 2019;133(8):806-816. doi:10.1182/blood-2018-09-875947
7. Goldman JM, Apperley JF, Jones L, et al. Bone marrow transplantation for patients with chronic myeloid leukemia. N Engl J Med. 1986;314(4):202-207. doi:10.1056/NEJM198601233140403
8. Wang G, Wang F, Huang Q, Li Y, Liu Y, Wang Y. Understanding Transcription Factor Regulation by Integrating Gene Expression and DNase I Hypersensitive Sites. *Biomed Res Int.* 2015;2015:757530. doi:10.1155/2015/757530
9. Bushweller JH. Targeting transcription factors in cancer - from undruggable to reality. *Nat Rev Cancer.* 2019;19(11):611-624. doi:10.1038/s41568-019-0196-7
10. Mologni L, Piazza R, Khandelwal P, Pirola A, Gambacorti-Passerini C. Somatic mutations identified at diagnosis by exome sequencing can predict response to imatinib in chronic phase chronic myeloid leukemia (CML) patients. Am J Hematol. 2017;92

11. Hochhaus A, Baccarani M, Silver RT, et al. European LeukemiaNet 2020 recommendations for treating chronic myeloid leukemia. *Leukemia.* 2020;34(4):966-984. doi:10.1038/s41375-020-0776-2
12. Baccarani M, Deininger MW, Rosti G, et al. European LeukemiaNet recommendations for the management of chronic myeloid leukemia: 2013. Blood. 2013;122(6):872-884. doi:10.1182/blood-2013-05-501569
13. Baccarani M, Deininger MW, Rosti G, et al. European LeukemiaNet recommendations for the management of chronic myeloid leukemia: 2013. *Blood*. 2013;122(6):872-884. doi:10.1182/blood-2013-05-501569
14. Cagnetta A, Garuti A, Marani C, et al. Evaluating treatment response of chronic myeloid leukemia: emerging science and technology. *Curr Cancer Drug Targets*. 2013;13(7):779-790. doi:10.2174/15680096113139990084
15. Cross NC, White HE, Müller MC, Saglio G, Hochhaus A. Standardized definitions of molecular response in chronic myeloid leukemia. *Leukemia*. 2012;26(10):2172-2175. doi:10.1038/leu.2012.104
16. Mahon FX, Etienne G. Deep molecular response in chronic myeloid leukemia: the new goal of therapy?. *Clin Cancer Res*. 2014;20(2):310-322. doi:10.1158/1078-0432.CCR-13-1988
17. Driscoll JJ, Rixe O. Overall survival: still the gold standard: why overall survival remains the definitive end point in cancer clinical trials. *Cancer J*. 2009;15(5):401-405. doi:10.1097/PPO.0b013e3181bdc2e0
18. Goel MK, Khanna P, Kishore J. Understanding survival analysis: Kaplan-Meier estimate. *Int J Ayurveda Res*. 2010;1(4):274-278. doi:10.4103/0974-7788.76794
19. Abdulla MAJ, Chandra P, Akiki SE, Aldapt MB, Sardar S, Chapra A, Nashwan AJ, Sorio C, Tomasello L, Boni C, Yassin MA. Clinicopathological Variables and Outcome in Chronic Myeloid Leukemia Associated With BCR-ABL1 Transcript Type and Body Weight: An Outcome of European LeukemiaNet Project. Cancer Control. 2021 Jan-Dec;28:10732748211038429. doi: 10.1177/10732748211038429. PMID: 34789006; PMCID: PMC8619745.
20. Goodyear MD, Krleza-Jeric K, Lemmens T. The Declaration of Helsinki. *BMJ*. 2007;335(7621):624-625. doi:10.1136/bmj.39339.610000.BE
21. Ip SC, Lin SW, Lai KM. An evaluation of the performance of five extraction methods: Chelex® 100, QIAamp® DNA Blood Mini Kit, QIAamp® DNA Investigator Kit, QIAsymphony® DNA Investigator® Kit and DNA IQ™. *Sci Justice*. 2015;55(3):200-208. doi:10.1016/j.scijus.2015.01.005
22. AlAsiri S, Basit S, Wood-Trageser MA, et al. Exome sequencing reveals MCM8 mutation underlies ovarian failure and chromosomal instability. *J Clin Invest*. 2015;125(1):258-262. doi:10.1172/JCI78473
23. Gnirke A, Melnikov A, Maguire J, et al. Solution hybrid selection with ultra-long oligonucleotides for massively parallel targeted sequencing. *Nat Biotechnol*. 2009;27(2):182-189. doi:10.1038/nbt.1523
24. Retterer K, Juusola J, Cho MT, et al. Clinical application of whole-exome sequencing across clinical indications. *Genet Med*. 2016;18(7):696-704. doi:10.1038/gim.2015.148
25. Carson AR, Smith EN, Matsui H, et al. Effective filtering strategies to improve data quality from population-based whole exome sequencing studies. *BMC Bioinformatics*. 2014;15:125. Published 2014 May 2. doi:10.1186/1471-2105-15-125
26. Branford S, Wang P, Yeung DT, et al. Integrative genomic analysis reveals cancer-associated mutations at diagnosis of CML in patients with high-risk disease. Blood. 2018;132(9):948-961. doi:10.1182/blood-2018-02-832253
27. Xu J, Wu M, Sun Y, Zhao H, Wang Y, Gao J. Identifying Dysregulated lncRNA-Associated ceRNA Network Biomarkers in CML Based on Dynamical Network Biomarkers. *Biomed Res Int*. 2020;2020:5189549. Published 2020 Feb 18. doi:10.1155/2020/5189549

28. Tsiatis AC, Norris-Kirby A, Rich RG, et al. Comparison of Sanger sequencing, pyrosequencing, and melting curve analysis for the detection of KRAS mutations: diagnostic and clinical implications. J Mol Diagn. 2010;12(4):425-432. doi:10.2353/jmoldx.2010.090188
29. Beck TF, Mullikin JC; NISC Comparative Sequencing Program, Biesecker LG. Systematic Evaluation of Sanger Validation of Next-Generation Sequencing Variants. Clin Chem. 2016;62(4):647-654. doi:10.1373/clinchem.2015.249623
30. Diaz-Horta O, Duman D, Foster J 2nd, et al. Whole-exome sequencing efficiently detects rare mutations in autosomal recessive nonsyndromic hearing loss. PLoS One. 2012;7(11):e50628. doi:10.1371/journal.pone.0050628
31. NCBI Variation Viewer. (https://www.ncbi.nlm.nih.gov/variation/view/?assm=GCF_000001405.25) ; accessed on 3rd Jun 2020.
32. UCSC Genome Browser on Human (GRCh37/hg19). 32. https://genome.ucsc.edu. Accessed on 3rd Jun 2020.
33. R Core Team (2018). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: https://www.R-project.org/.
34. Sokal JE, Cox EB, Baccarani M, et al. Prognostic discrimination in "good-risk" chronic granulocytic leukemia. *Blood*. 1984;63(4):789-799.
35. Kuntegowdanahalli LC, Kanakasetty GB, Thanky AH, et al. Prognostic and predictive implications of Sokal, Euro and EUTOS scores in chronic myeloid leukaemia in the imatinib era-experience from a tertiary oncology centre in Southern India. *Ecancermedicalscience*. 2016;10:679. Published 2016 Oct 6. doi:10.3332/ecancer.2016.679
36. Amaravadi RK, et al. Best practices for the development and fit-for-purpose validation of biomarker methods. AAPS Open. 2019;5(1):1-15. doi:10.1186/s41120-019-0024-2
37. Oh et al. 2020. Development of an aptamer-based procalcitonin (PCT) detection platform for sepsis diagnosis. Molecular & Cellular Toxicology, 2020
38. Has-miR-146a polymorphism (rs2910164) and cancer risk: a meta-analysis. Springer, 2019
39. Wyllie AL, et al. Saliva or nasopharyngeal swab specimens for detection of SARS-CoV-2. N Engl J Med. 2020;383(13):1283-1286.
40. Morris TJ, Butcher LM, Feber A, et al. ChAMP: 450k Chip Analysis Methylation Pipeline. Bioinformatics. 2014;30(3):428-430. doi:10.1093/bioinformatics/btt684.
41. Musunuru et al. Genetic Testing for Inherited Cardiovascular Diseases: Scientific Statement from the American Heart Association. Circ Genom Precis Med. 2020;13
42. Ahmed Z, et al. Multi-omics strategies for personalized and predictive medicine: past, current, and future translational opportunities. Emerging Topics in Life Sciences. 2021
43. Barh D, et al. Omics Approaches in Breast Cancer: Towards Next-Generation Diagnosis, Prognosis, and Therapy. Springer, 2014
44. Abbasi EY, Deng Z, Ali Q, et al. Integration of Machine Learning and Multi-Omics Data for Enhanced Prediction of Leukemia. Bioinformatics. 2020;36(15):4328-4336. doi:10.1093/bioinformatics/btaa213
45. Wells JN, Chang NC, McCormick J, et al. Transposable elements and their role in the evolution of zinc finger genes. Genome Res. 2018;28(1):57-66. doi:10.1101/gr.226256.117.
46. Miao YR, Liu W, Zhong Z, You Y, Tang Y, Li W, Zhu X, Guo AY. Case Report: Multi-Omics Analysis and CAR-T Treatment of a Chronic Myeloid Leukemia Blast Crisis Case 5 Years After the Discontinuation of TKI. Front Oncol. 2021 Sep 21;11:739871. doi: 10.3389/fonc.2021.739871. PMID: 34621680; PMCID: PMC8490701.
47. Debmalya Barh et al. Omics Approaches in Breast Cancer: Towards Next-Generation Diagnosis, Prognosis, and Therapy. Springer, 2014

48. Hehlmann R, Lauseker M, Saußele S, et al. Assessment of imatinib as first-line treatment of chronic myeloid leukemia: 10-year survival results of the randomized CML study IV and impact of non-CML determinants. *Leukemia*. 2017;31(11):2398-2406. doi:10.1038/leu.2017.253
49. O'Brien SG, Guilhot F, Larson RA, et al. Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia. *N Engl J Med*. 2003;348(11):994-1004. doi:10.1056/NEJMoa022457.
50. Ahmad R, Tripathi AK, Tripathi P, Singh S, Singh R, Singh RK. Malondialdehyde and protein carbonyl as biomarkers for oxidative stress and disease progression in patients with chronic myeloid leukemia. *In Vivo*. 2008;22(4):525-528.
51. O'Sullivan J, Kothari C, Caron MC, et al. The role of ZNF432 in DNA damage response and repair mechanisms. Nucleic Acids Res. 2017;45(18):10540-10554. doi:10.1093/nar/gkx800
52. Becker KG, Nagle JW, Canning RD, Biddison WE, Ozato K, Drew PD. Rapid isolation and characterization of 118 novel C2H2-type zinc finger cDNAs expressed in human brain. *Hum Mol Genet*. 1995;4(4):685-691. doi:10.1093/hmg/4.4.685
53. Jen J, Wang YC. Zinc finger proteins in cancer progression. *J Biomed Sci*. 2016;23(1):53. Published 2016 Jul 13. doi:10.1186/s12929-016-0269-9.
54. Yang L, Zhang L, Wu Q, Boyd DD. Unbiased screening for transcriptional targets of ZKSCAN3 identifies integrin beta 4 and vascular endothelial growth factor as downstream targets. *J Biol Chem*. 2008;283(50):35295-35304. doi:10.1074/jbc.M806965200
55. O'Sullivan J, Kothari C, Caron MC, et al. Role of ZNF432 in Regulating DNA Repair Pathways and Its Impact on Sensitivity to PARP Inhibitors. DNA Repair. 2020;92:102897. doi:10.1016/j.dnarep.2020.102897
56. Codd V, Nelson CP, Albrecht E, et al. Identification of seven loci affecting mean telomere length and their association with disease. *Nat Genet*. 2013;45(4):422-427e4272. doi:10.1038/ng.2528
57. Munro D, Ghersi D, Singh M. Two critical positions in zinc finger domains are heavily mutated in three human cancer types. *PLoS Comput Biol*. 2018;14(6):e1006290. Published 2018 Jun 28. doi:10.1371/journal.pcbi.1006290
58. Walsh KM, Whitehead TP, de Smith AJ, et al. Common genetic variants associated with telomere length confer risk for neuroblastoma and other childhood cancers. *Carcinogenesis*. 2016;37(6):576-582. doi:10.1093/carcin/bgw037
59. Wang H, Yu J, Guo Y, et al. Genetic variants in the ZNF208 gene are associated with esophageal cancer in a Chinese Han population. *Oncotarget*. 2016;7(52):86829-86835. doi:10.18632/oncotarget.13468
60. Sun W, Hang D, Han S, et al. circRNA expression profiles and their potential roles in cervical cancer. Gene. 2019;716:144-151. doi:10.1016/j.gene.2019.02.034
61. Ding Y, Zhou X, Wu C, et al. Telomere length, ZNF208 genetic variants and risk of chronic obstructive pulmonary disease in the Hainan Li population. *J Gene Med*. 2018;20(12):e3061. doi:10.1002/jgm.3061
62. Sun W, Hang D, Han S, et al. Role of circRNAs in the Regulation of Cervical Cancer Progression and Their Potential as Diagnostic Biomarkers. Oncotarget. 2020;11(11):954-964. doi:10.18632/oncotarget.27358
63. Liu Z, Kruhlak MJ, Thiele CJ. CASZ1 transcription factor and its role in DNA damage response. Biochem Biophys Res Commun. 2018;503(2):1071-1078. doi:10.1016/j.bbrc.2018.08.021
64. Kang H, Park S, Jo A, et al. Phase separation of PARIS and its implications in cellular stress responses. EMBO Rep. 2019;20(7). doi:10.15252/embr.201847744
65. Rosspopoff O, Trono D. KRAB zinc-finger proteins and their roles in gene regulation. Trends Genet. 2017;33(6):420-431. doi:10.1016/j.tig.2017.03.007
66. Rosspopoff O, Trono D. The KRAB Domain and Its Role in Gene Regulation: Insights from Recent Studies. Trends Genet. 2020;36(8):637-647. doi:10.1016/j.tig.2020.05.006

67. Chang YJ, Lin S, Kang ZF, et al. TRIM28 regulation of gene expression through acetylation and its implications in cancer. Int J Mol Sci. 2018;19(12):3896. doi:10.3390/ijms19123896
68. Bolouri H, Farrar JE, Triche T Jr, Ries RE, Lim EL, Alonzo TA, Ma Y, Moore R, Mungall AJ, Marra MA, Zhang J, Ma X, Liu Y, Liu Y, Auvil JMG, Davidsen TM, Gesuwan P, Hermida LC, Salhia B, Capone S, Ramsingh G, Zwaan CM, Noort S, Piccolo SR, Kolb EA, Gamis AS, Smith MA, Gerhard DS, Meshinchi S. The molecular landscape of pediatric acute myeloid leukemia reveals recurrent structural alterations and age-specific mutational interactions. Nat Med. 2018 Jan;24(1):103-112. doi: 10.1038/nm.4439. Epub 2017 Dec 11. Erratum in: Nat Med. 2018 Apr 10;24(4):526. Erratum in: Nat Med. 2019 Mar;25(3):530. PMID: 29227476; PMCID: PMC5907936.
69. Yan L, Tan S, Wang H, Yuan H, Liu X, Chen Y, de Thé H, Zhu J, Zhou J. Regulation of gene expression during neutrophil development by the Brd4-Smrt complex. Leukemia. 2019;33(9):2173-2183. doi:10.1038/s41375-019-0467-6
70. Shi ZY, Wang X, Chen WM, Li LD, Hao Y, Li JY, Sun K, Zhao XS, Jiang H, Jiang Q, Huang XJ, Qin YZ. Role of ZNF384 in the prognosis and treatment of B-cell acute lymphoblastic leukemia. Hematol Oncol. 2019;37(3):234-241. doi:10.1002/hon.2477
71. Robbins DJ, Pavletich TS, Patil AT, Pahopos D, Lasarev M, Polaki US, Gahvari ZJ, Bresnick EH, Matson DR. GATA2 mutations in myelodysplastic syndromes and their impact on disease progression. Blood. 2018;131(7):792-802. doi:10.1182/blood-2017-08-796424
72. Yang X, Yang L, Luo A, Liu S, Zhang X, Liu X, Liu X, Luo A, Cai M, Yan Y, Wu X,
73. Huang K, Xu L, Jiang H. Genetic variations in IKZF3 and susceptibility to acute lymphoblastic leukemia in children. Cancer. 2019;125(2):298-306. doi:10.1002/cncr.31934.
74. Pavlovic ZJ, Hsin-Yu Pai A, Hsiao TT, Yen CF, Alhasan H, Ozmen A, New EP, Guo X, Imudia AN, Guzeloglu-Kayisli O, Lockwood CJ, Kayisli UA. Role of GATA2 and GATA6 in endometrial dysfunction and reproductive health. Fertil Steril. 2019;112(4):785-792. doi:10.1016/j.fertnstert.2019.06.019