Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Evaluation of *TP53* Expression and Mutations among Hematological Malignancies Patients in Saudi Arabia

Heba Alkhatabi
Department of Medical Laboratory Technology, Faculty of Applied Medical Sciences, King Abdulaziz University, Jeddah, Saudi Arabia

Elrashed B. Yasin (eyasin@kau.edu.sa)
Department of Medical Laboratory Technology, Faculty of Applied Medical Sciences, King Abdulaziz University, Rabigh, Saudi Arabia.

Raed Alserihi
Department of Medical Laboratory Technology, Faculty of Applied Medical Sciences, King Abdulaziz University, Jeddah, Saudi Arabia

Raed Felimban
Department of Medical Laboratory Technology, Faculty of Applied Medical Sciences, King Abdulaziz University, Jeddah, Saudi Arabia

Aisha Elaimi
Department of Medical Laboratory Technology, Faculty of Applied Medical Sciences, King Abdulaziz University, Jeddah, Saudi Arabia

Lina Alharbi
Center of excellence in genomic medicine research (CEGMR), King AbdulAziz University

Manal Shaabad
Center of excellence in genomic medicine research (CEGMR), King AbdulAziz University

Hameeda Ahmed
Center of excellence in genomic medicine research (CEGMR), King AbdulAziz University

Abdulrahman M Alameer
Sabya General Hospital, Jazan

Abdullah Ebraheem Mathkoor
Jazan Armed forces hospital

Mohammed Alqahtani
Department of Medical Laboratory Technology, Faculty of Applied Medical Sciences, King Abdulaziz University, Jeddah, Saudi Arabia

Research Article

Keywords: TP53 mutation, Hematological malignancies, TP53 deletion, Myelodysplastic syndromes, FISH

Posted Date: September 27th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-928882/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Abstract

Tumor protein 53 (*TP53*) is the most frequently mutated gene in human cancer which located on the short arm of chromosome 17. In hematological malignancies, the prevalence of *TP53* mutations is low compared to other tumors but associated with a complex karyotype, poor prognosis, and poor response to chemotherapy. However, no data on the prevalence and prognostic value of *TP53* mutations and the significance of *TP53* deletion among hematological malignancies in Saudi patients. The objective of this study was to evaluate the frequency and prognostic significance of *TP53* mutations in different hematological malignancies in Jeddah, Saudi Arabia. 20 samples from different hematological malignancies were tested using a next-generation sequencing (NGS) and targeted panel for *TP53* deletion using fluorescence in situ hybridization (FISH) and *TP53* mutations. Based on the application of the FISH technique, 6 out of 20 patients showed a deletion in *TP53* majority of them were Acute lymphoplastic leukemia (ALL), and the deletion was prominent in a child compared to an adult. Furthermore, the result of next-generation sequencing on 10 selected samples showed that 1 out of 10 samples had a heterozygous mutation at codon 175 (exon 5) of the *TP53* gene, replacing histidine with arginine (H175R). Also, there were 90 other mutations detected on 13 genes that were sequenced besides *TP53*. None of the other gene mutations showed any clinical impact or association with *TP53* except for two mutations in two different genes; *ASXL1* (K1368T) and *SETBP1* (V231L). Despite the small size of this study, the identified mutations are considered variants with unknown significance and need further validation and investigation on a larger cohort to determine the pathogenicity of these abnormalities. This study recommends further analysis of genomic mutations, utilizing high throughput technologies, associated with hematological malignancies in Saudi populations.

Introduction

TP53 is the most frequently mutated gene in most human cancers, with a frequency of 50% [1, 2]. Alterations consist of mutations and deletions and are generally related to advanced stages of the disease, inadequate therapy-response, and poor prognosis [3–7]. The transcription component *TP53* has a central regulatory characteristic in numerous signaling pathways, including cell cycle arrest, apoptosis, and DNA repair [8, 9]. Owing to its essential function in maintaining genome stability, the p53 protein has been described as 'the guardian of the genome.' *TP53* deletions are frequently observed to be related to *TP53* mutations of the second allele, assisting the 'two-hit' hypothesis, which indicates that alteration of each copy of a tumor suppressor gene is required to result in and/or force most cancers development [2, 10–13]. Activation of p53 takes place in response to DNA damage or different strain conditions (for example, metabolic changes, hypoxia, or oncogene activation), leading to activation or repression of its target genes, precisely inflicting G1 cell cycle arrest and apoptosis induction, a procedure this is disrupted with the aid of using *TP53* mutation/deletion in cancer[14–16]. Deletions in *TP53* regularly result from large deletions of the short arm of chromosome 17, wherein *TP53* is located, which may be detected through interphase FISH (fluorescence in situ hybridization), figuring out the copy-number state of a gene. Thus, the *TP53* feature is commonly preserved within a *TP53* deletion without accompanying *TP53* mutation

within the other allele. Mutations in *TP53* generally bring about a lack of character of the p53 protein that could encompass complete or partial absence of characteristic, depending on the site of the mutation [17]. Whereas tumor suppressors are usually inactivated through frameshift or nonsense mutations, the most common mutation form of *TP53* in tumors is represented through missense mutations withinside the coding region [10, 18]. Although the cancer-related *TP53* mutations are determined in lots of different places throughout the *TP53* sequence, they generally cluster within the DNA-binding domain, disrupting the ability of p53 to bind to its target DNA sequences, therefore preventing transcriptional transcription activation of the respective genes [19]. About 30% of the missense mutations are located in six 'hotspot' residues (p.R175, p.G245, p.R248, p.R249, p.R273, and p.R282) withinside the DNA-binding domain of p53, with R273 and R248 being the maximum often mutated ones [20, 21]. Interestingly, even though *TP53* mutations usually abolish the tumor suppressor activity of the protein (loss-of-function mutations), gain-of-function mutations have additionally been defined that cause acquisition of additional oncogenic functions that sell cell growth and provide survival advantages to the cell [21].

Mutations and deletions in *TP53* are determined in all hematological malignancies at a different frequency. Whereas *TP53* mutations had been determined to arise pretty frequently in ALL (16%) [22] and AML (12%) [23, 24], the frequencies are decreased in CLL (7%) [7, 25–27] and MDS (6%)[28–30]. Like most cancer types, *TP53* mutations in hematological malignancies had been determined to expose a negative effect on survival. Moreover, *TP53* mutations had been proven to be enriched in therapy-related diseases such as t-AML and t-MDS. They were also determined excessively in relapse cases, which were related to poor outcomes [31, 32]. Therefore, the proposed role of *TP53* mutations in therapy-associated patients and relapsed disease appears to be because of the selective gain of the individual cells due to their resistance to therapy [10, 33].

We aimed to study the prevalence of *TP53* concerning the type and frequency of *TP53* mutations and accompanying deletions in hematological entities. For this purpose, we carried out a comprehensive analysis of the *TP53* mutation/deletion patterns in different hematological malignancies, such as AML, MDS, and ALL. In addition, we analyzed (i) the frequencies of *TP53* mutations and deletions, (ii) the types of mutation, (iii) the mutation load, (iv) the correlations to cytogenetic aberrations, and (v) the age dependency.

Patients And Methods

Patients

This study evaluated 20 cases of hematological malignancies (AML: n = 9, ALL: n = 9, non-Hodgkin's lymphoma: n = 1, MDS: n = 1; bone marrow samples) reported in King Abdul-Aziz University Hospital, throughout 2015–2017. Informed consent was obtained from each patient before obtaining bone marrow sample under an approved protocol code (01-CEGMR-Bioeth-2019). The samples were selected based on the availability of DNA, clinical data, and cytogenetic results. The study followed the rules of the Helsinki

Declaration. Ethical approval was obtained from the ethical committee of the Center of Excellence in Genomic Medicine Research (CEGMR) (Bioethical approval code: 01-CEGMR-Bioeth-2019).

Cytogenetic and FISH.

Chromosomal analysis was carried out for all cases as formerly described according to standard methods [34–36]. In addition, karyotyping was performed following the ISCN guidelines (2016) [37]. In all patients, interphase FISH using probes for *TP53* (from vysis) spanning a 167 kb region in 17p13, including the complete sequence of *TP53*, was performed to determine the copy-number state of *TP53*. The following steps were employed for the FISH technique: preparation of the interphase/metaphase spreads, denaturation of the target DNA (Interphase/metaphase spreads) and DNA probes, hybridization over one day, post-hybridization washes, and counterstaining for image analysis using a fluorescence microscope.

Tp53mutation Detection By Ngs

The sequencing analysis was performed on 10 out of the 20 samples, selected according to the variability in disease diagnosis (AML, ALL, MDS, and LNH). Therefore, it included different cytogenetic abnormalities and FISH results.

ClearSeq AML HS panel (G9963A, Agilent Technologies), designed to target 48 selected exons in 20 genes commonly mutated in myeloid leukemia, was applied to investigate the mutational hotspot regions of *TP53* (ENST00000269305, exons 5–8) in all patients (Table 1). Genomic DNA was extracted from patient bone marrow samples using QIAGEN kits (according to the manufacturer's instructions). The quantitative method assessed the quality of extracted DNA using a 2000c spectrophotometer (NanoDrop Technologies, Wilmington, DE). Purity was measured by calculating the absorbance ratio (A_{260}/A_{280}). Pure DNA should have an A_{260}/A_{280} ratio of 1.7–1.9.

Table 1
ClearSeq AML HS panel

Gene List (targeted exons)							
GENE	**EXON**	**GENE**	**EXON**	**GENE**	**EXON**	**GENE**	**EXON**
ASXL1	12	EZH2	8, 17,18	MPL	10	SF3B1	13–15,17
CSF3R	14, 17	FLT3	14, 20	NPM1	11	SRSF2	1
CBL	8, 9	IDH1	4	NRAS	2, 3	TET2	3, 9, 10, 11
CEBPA	1	IDH2	4	RUNX1	3, 4, 8	TP53	5–8
DNMT3A	4, 8, 13, 15, 16, 18–23	JAK2	12, 14	SETBP1	3	U2AF1	2, 6

Table 2
Patient information and cytogenetic result

Impacted Gene	***TP53***
Type of Mutation	SNP (Heterozygous)
Chromosome	17
Ref. Allele	T
Alt. Allele	C
Function Class	MISSENSE
AA	H175R
Codon	cAt/cGt
Quality	Pass
Allele Frequency	0.447
Number of Variant Alleles	10232
Filtered Read Depth (per sample)	22870
Effect	UNKNOWN
Exon ID	NM_001126118.ex.5

Case #	Age	Sex	Diagnosis	Cytogenetic Result
1	2	M	ALL	46, XY
2	2	M	ALL	AL,57 ~ 43, XY, +X, dup (1) (q21q31), + 4, +5, + 6, +7, -8, + 9, +10, + 14, +17, + 18, -19, -20, + 21, +22[cp50]
3	5	F	ALL	Leukemia,46, XX, der (19) t (1;19) (q25; p13.3) [17]/46, XX, idem, +der (21) t (1;21) (p13; p11.2) [14]/46, XX [19]
4	5	M	ALL	46, XX[20]
5	7	F	ALL	46, XX[20]
6	9	M	AML	46,XY[20]
7	12	M	ALL	46, XY, t (8; 21)(q22; q22) [29]/46, XY [21]
8	31	M	ALL	46,XY[20]
9	36	F	AML	46, XX[20]
10	43	M	AML	46,XY[20]
11	44	F	ALL	ALL,45, XX, +X, -9, t (9;22) (q34; q11.2), -13[cp34]/45, XX, t (9;22) (q34; q11.2) [cp8]/46, XX[cp8]
12	45	F	ALL	46, XX[20]
13	59	F	Lymphoma, NHL	46, XX[20]
14	63	F	AML	46, XX[20]
15	65	F	MDS	65 – 58, XX, + 1, +2, der(2) t(2;5) (q12; q37), + 5, +6, + 8, +9, + 10, +11, der(17) t (12;17) (p10; p10), + 13, del (13) (q21), + 21, +21 [cp50]
16	72	M	AML	45, XY,der (7;12), (q11.2; p12) [30]
17	13 Y	F	AML	46,XX[20]
18	35 Y	F	AML	46,XY[20]
19	42 Y	M	AML	46,XX[20]
20	69 Y	M	AML	46,XY[20]

Data Analysis:

Data acquisition and analysis were performed using Agilent's SureCall V2. (Agilent Technologies) that incorporates BWA, SAM tools (Agilent Technologies) for alignment, variant calling, and annotation.

Results

Clinical characteristics of patients

The subjects included 10 males and 10 females with a median age of 43 years, ranging from 5–69 years (Table.2). Most cases consisted of AML (45% of patients), ALL (45%), Non-Hodgkin's lymphoma (5%), and MDS (5%).

Cytogenetic and FISH Results

The cytogenetic results for the patients showed that most cases were found to have a normal karyotype (70%), four with a complex karyotype (20%), and two with a single chromosomal abnormality (10%). The data of the patients are summarized in (Table.2). Figure 1 represents an example of a different karyotype. As for FISH results based on the analysis of 200 interphase cells on each sample, seven patients showed normal signaling for TP53. Another seven patients showed a deletion in the *TP53* gene (on average of 11 to 45% of the examined cells on each sample). However, one patient harbored three different cell lines, including 15% normal cells, 45% of the cells showed deletion in TP53, and 40% of the cells showed three signals for TP53. Additionally, two patients showed extra signals for TP53 (three signals) in an average of 20 and 30% without deletion of TP53. Figure 2 represents examples of the FISH result. The TP53 deletion was mainly detected in patients with ALL 55% (5/9) and only presented in 1 AML patient. Both NHL and MDS have shown abnormality in TP53 gene deletion and amplification, respectively. There was no gender association with the deletion. However, TP53 deletions were detected in 62% (5 /8) child samples compared to 16% (2/12) adult patient (Table 3).

Next-generation sequencing analysis

Sequencing analysis revealed a heterozygote point mutation (H175R) in the *TP53* gene (MDS patient), showing an alteration in amino acid sequence from T to C, which resulted in a change in amino acid residue from histidine to arginine at codon 175 (Table.4). The frequency assessment of the *TP53* mutations in our study demonstrated that most of the investigated samples, either with TP53 deletion or not as detected by the FISH technique did not show any mutation except for one case representing 10% of all cases.

Correlation of *TP53* Mutation with Cytogenetic & FISH Results

In assessing the relationship of *TP53* mutation to cytogenetic and FISH results, the mutation was observed in MDS patients with a complex karyotype. Interestingly, the FISH result for this patient showed a TP53 gene that revealed a signal gain in 60 cells of the *TP53* gene.

Correlation of TP53 Mutation with Other Genes Mutations

Using targeted NGS with ClearSeq AML HS panel (Agilent Technologies), we identified a total of 91 mutations in 14 of the 20 genes analyzed in our cohort. The analysis showed that patient with *TP53* mutation also had mutations in *NPM1*, *TET2*, *SRSF2*, *ASXL1*, *SETBP1* (Table.5). Furthermore, among these mutations, the identified mutation in both *ASXL1* (K1368T) and *SETBP1* (V231L) was exclusively associated with *TP53* mutation and did not show in the other patients (see Table.6, Figure 3).

Discussion

TP53 is a major tumor suppressor which plays an important role in tumorigenesis, proliferation, and cell survival in most human cancers [38]. The previous research confirmed that greater than 80% of human cancers have mutations in TP53. The current model of the IARC database (R20, July 2019) includes over 29900 somatic mutations and 9200 variations reported in SNP databases ("Database Development", 2019). Nowadays, it is undisputed that the inactivation of the TP53 gene due to a mutation is a critical step in tumor transformation and progression[1]. The activity of TP53 lies in its ability to activate and suppress a broad set of target genes whose products regulate, among other things: the cell cycle arrest and apoptosis when the DNA is damaged [39].

TP53 gene might not have a specific role in developing all tumors. However, mutations of this gene have been related to a complicated karyotype, poor prognosis, and poor response to chemotherapy [40-42]. There is a lack of the published data in Saudi Arabia that describe the frequency of the TP53 mutations and their relationship with cytogenetic and clinical phenotype in hematological neoplasms. Therefore, we endeavored in this study to evaluate the TP53 deletion using the FISH technique and TP53 mutations screening TP53 using NGS technology and their relationship with cytogenetics and clinical phenotype in leukemia patients.

Previous studies have shown that FISH is a powerful cytogenetic technique used to evaluate the TP53 alterations in patients with hematological malignancies [43, 44]. In our study, 20 patients samples were examined, and the TP53 deletion was detected in 35% of the cases. Similarly, there were about 35% of cases with normal signaling of TP53 and two cases with extra signals in TP53.

TP53 deletion was identified in about 62.5% of all investigated child samples, whereas the deletion was detected at a lower rate (16%) of adult cases. Furthermore, the highest average of TP53 deletion has been noticed in patients with ALL (55%), which is in concordance with and even higher than what was reported by other studies (56%) [45] .

TP53 changes were mainly seen in a hypodiploid subtype of ALL, mainly due to germline changes, which changed the disease manifestation to Li-Fraumeni syndrome. Therefore, it becomes important to know if the identified variant is a secondary event contributing to risk stratification and treatment response [46].

According to the analysis of 10 samples by NGS, only one (MDS patient) was harboring a TP53 mutation in exon 5. The detected mutation was a heterozygote point mutation (T to C) that changed amino acid residue from histidine to arginine at codon 175 of the TP53 gene. The mutation was found in an MDS patient who was the only case in the study. Based on our knowledge and from the search on different databases (ClinVar - NCBI", 2020; "IARC TP53 Search", 2020; "Search results on cosmic for H175R", 2020), this particular mutation (H175R) we observed in our study was not reported previously in MDS or any other hematological malignancies. However, this mutation was found in lung adenocarcinoma from Korean patients[47]. According to cytogenetic and FISH results, the mutation was associated with a complex karyotype and TP53 gene amplification detected by FISH. This finding aligns with what was published before that TP53 mutation is associated with a complex karyotype and poor prognosis in MDS[48, 49].

The reason why TP53 mutations are associated with the complex karyotype remains unclear and raises the question of whether these mutations promote and induce increasing cellular instability or whether these mutations are secondary mutations that occur only after chromosomal instability. Previous studies showed that TP53 mutations in hematological malignancies are highly prevalent in a complex karyotype and deletion of chromosome 17p. At the same time, in the other cytogenetic subgroups, they are deficient, suggesting that chromosomes instability may precede mutations in TP53 [22, 50, 51]. However, further studies and examination on larger cohorts are needed to assess these possibilities.

Targeted NGS in our research enabled us to discover mutations in other genes rather than TP53. The analysis revealed that TP53 mutation was associated with other genes mutations such as TET2, SRSF2, ASXL1, U2AF1, NPM1, and SETBP1. Similar co-occurrence results for these mutations with TP53 mutation in MDS were published[52-54]. Interestingly, among these mutations, we found exclusive mutations on ASXL1 (K1368T) and SETBP1 (V231L) that were associated mainly with TP53 mutation [55]. Reported that ASXL mutations are frequently seen in MDS in association with SETBP1 mutations, inhibiting myeloid differentiation and inducing leukemic transformation [56]. Furthermore, they reported that SETBP1 is a driver for ASXL1 mutation, and ASXL1 is a poor prognostic biomarker associated with short survival. Another study focused on TP53 and ASXL1 prognosis in AML and MDS reported that they are two independents factors associated with poor prognosis and short survival; nevertheless, none of the studies had reported the pathogenic significance of the particularly identified mutations on these genes, their importance on disease pathogenicity cannot be ignored and further functional validation should be done [57].

Based on our knowledge, mutation of ASXL1 (K1368T) was also not previously reported, and its pathogenicity was not assessed or examined before. On the other hand, a SETBP1 (V231L) mutation was found in Schinzel-Giedion Midface Retraction Syndrome with a mild effect, as reported by Illumina Clinical Services Laboratory ("VCV000159885.1 - ClinVar - NCBI", 2020). Therefore, the exclusiveness of the identified mutations in this project will be considered variants with unknown significance. As for the correlation of TP53 mutations with tumor type and cytogenetic abnormalities, in AML, all patients were

found with wild-type TP53 (six patients had a normal karyotype and one with a single chromosomal abnormality). In addition, one patient has TP53 deletion by FISH. This finding is consistent with other published work, which indicated that TP53 mutations are infrequent in AML without a complex karyotype, highlighting its importance as a therapeutic target through activation of the intact gene[50, 58, 59].

In the Lymphoma patient, there was no TP53 mutation. Instead, the patient had a normal karyotype with a TP53 deletion based on FISH. This finding is consistent with Ahmad et al., study, which revealed that TP53 mutations in Saudi non-Hodgkin's lymphoma are infrequent, as, from 45 patients, only one patient showed a mutation in the TP53 gene [60].

Further examination and screening on a larger cohort are highly recommended to confirm research findings. Also, the used panel covers only 4 exons from TP53, representing the exons that include the most reported hotspot mutations in the gene. That limits the study finding as there might be a chance of detecting other variants of the TP53 gene on the uncovered regions. Therefore, whole gene sequencing for TP53 is important to confirm the absence of any changes on the gene. That will support the recommendation of utilizing the activation of the Wilde type gene in controlling tumor progression. Moreover, the FISH technique remains a powerful tool for clinical diagnosis, and further screening on the clinical impact of FISH analysis for TP53 on AML and ALL manifestation is recommended.

Declarations

Ethics statements

The study followed the rules of the Helsinki Declaration. Ethical approval was obtained from the ethical committee of the Center of Excellence in Genomic Medicine Research (CEGMR) (Bioethical approval code: 01-CEGMR-Bioeth-2019).

Consent for publication

All authors agreed to publish this manuscript.

Author Contribution:

HAK, RF, EY and AE participated in analysis of data, helped in designing images, tables, critical review and drafted the manuscript.

HA, AA, AM, HA, MS, EY and LA carried out the experiment including: Cytogentic analysis, FISH experiment and Analysis, DNA extraction, sequencing studies. AA and AM performed data collection. HAK participated in designing the study, provided required reagents for the experiment and helped in drafting the manuscript. MA helped in providing the reagents, kits and other logistics in order to perform the study. All authors read and approved the final manuscript.

Competing interests:

The authors declare that they have no competing interests.

References

1. Rivlin, N. *et al.* Mutations in the p53 Tumor Suppressor Gene: Important Milestones at the Various Steps of Tumorigenesis. *Genes & Cancer*, **2** (4), 466–474 (2011).
2. Preudhomme, C. & Fenaux, P. THE CLINICAL SIGNIFICANCE OF MUTATIONS OF THE P52 TUMOUR SUPPRESSOR GENE IN HAEMATOLOGICAL MALIGNANCIES. *British journal of haematology*, **98** (3), 502–511 (1997).
3. Soussi, T. *et al.* Multifactorial analysis of p53 alteration in human cancer: a review. *International journal of cancer*, **57** (1), 1–9 (1994).
4. Wickremasinghe, R., Prentice, A. & Steele, A. p53 and Notch signaling in chronic lymphocytic leukemia: clues to identifying novel therapeutic strategies., **25** (9), 1400–1407 (2011).
5. Lane, D. P. p53, guardian of the genome. Nature 1992. **358**(6381): p. 15–16.
6. Fenaux, P. *et al.* Mutations of the P53 gene in acute myeloid leukaemia. *British journal of haematology*, **80** (2), 178–183 (1992).
7. Zenz, T. *et al.* TP53 mutation and survival in chronic lymphocytic leukemia. *Journal of Clinical Oncology*, **28** (29), 4473–4479 (2010).
8. Rotter, V. *et al. Does wild-type p53 play a role in normal cell differentiation?* in *Seminars in cancer biology*. 1994.
9. Wynford-Thomas, D. Cellular senescence and cancer. *The Journal of pathology*, **187** (1), 100–111 (1999).
10. Agirre, X. *et al. TP53 is frequently altered by methylation, mutation, and/or deletion in acute lymphoblastic leukaemia.* Molecular Carcinogenesis: Published in cooperation with the University of Texas MD Anderson Cancer Center, 2003. **38**(4): p. 201–208.
11. Knudson, A. G. Mutation and cancer: statistical study of retinoblastoma. Proceedings of the National Academy of Sciences 1971. **68**(4): p. 820–823.
12. Venot, C. *et al.* The requirement for the p53 proline-rich functional domain for mediation of apoptosis is correlated with specific PIG3 gene transactivation and with transcriptional repression. *The EMBO journal*, **17** (16), 4668–4679 (1998).
13. Harms, K. L. & Chen, X. The C terminus of p53 family proteins is a cell fate determinant. *Molecular and cellular biology*, **25** (5), 2014–2030 (2005).
14. Shaw, P. *et al.* Regulation of specific DNA binding by p53: evidence for a role for O-glycosylation and charged residues at the carboxy-terminus., **12** (4), 921–930 (1996).
15. Sakaguchi, K. *et al.* DNA damage activates p53 through a phosphorylation–acetylation cascade. *Genes & development*, **12** (18), 2831–2841 (1998).
16. Kruse, J. P. & Gu, W. Modes of p53 regulation., **137** (4), 609–622 (2009).

17. Rossi, D. & Gaidano, G. Molecular genetics of high-risk chronic lymphocytic leukemia. *Expert review of hematology*, **5** (6), 593–602 (2012).
18. Hainaut, P. *et al.* Database of p53 gene somatic mutations in human tumors and cell lines: updated compilation and future prospects. *Nucleic acids research*, **25** (1), 151–157 (1997).
19. Bullock, A. N. & Fersht, A. R. Rescuing the function of mutant p53. *Nature Reviews Cancer*, **1** (1), 68–76 (2001).
20. Brosh, R. & Rotter, V. When mutants gain new powers: news from the mutant p53 field. *Nature reviews cancer*, **9** (10), 701–713 (2009).
21. Li, J. *et al.* Mutants TP 53 p. R273H and p. R273C but not p. R273G Enhance Cancer Cell Malignancy. *Human mutation*, **35** (5), 575–584 (2014).
22. Stengel, A. *et al.* TP53 mutations occur in 15.7% of ALL and are associated with MYC-rearrangement, low hypodiploidy, and a poor prognosis. *Blood, The Journal of the American Society of Hematology*, **124** (2), 251–258 (2014).
23. Grossmann, V. *et al.* A novel hierarchical prognostic model of AML solely based on molecular mutations. *Blood, The Journal of the American Society of Hematology*, **120** (15), 2963–2972 (2012).
24. Rücker, F. G. *et al.* TP53 alterations in acute myeloid leukemia with complex karyotype correlate with specific copy number alterations, monosomal karyotype, and dismal outcome. Blood. *The Journal of the American Society of Hematology*, **119** (9), 2114–2121 (2012).
25. Döhner, H. *et al.* Genomic aberrations and survival in chronic lymphocytic leukemia. *New England Journal of Medicine*, **343** (26), 1910–1916 (2000).
26. Rossi, D. *et al.* The prognostic value of TP53 mutations in chronic lymphocytic leukemia is independent of Del17p13: implications for overall survival and chemorefractoriness. *Clinical cancer research*, **15** (3), 995–1004 (2009).
27. Jeromin, S. *et al.* SF3B1 mutations correlated to cytogenetics and mutations in NOTCH1, FBXW7, MYD88, XPO1 and TP53 in 1160 untreated CLL patients., **28** (1), 108–117 (2014).
28. Cazzola, M., Della Porta, M. G. & Malcovati, L. The genetic basis of myelodysplasia and its clinical relevance. *Blood, The Journal of the American Society of Hematology*, **122** (25), 4021–4034 (2013).
29. Papaemmanuil, E. *et al.* Clinical and biological implications of driver mutations in myelodysplastic syndromes. *Blood, The Journal of the American Society of Hematology*, **122** (22), 3616–3627 (2013).
30. Haferlach, T. *et al.* Landscape of genetic lesions in 944 patients with myelodysplastic syndromes., **28** (2), 241–247 (2014).
31. Hof, J. *et al.* Mutations and deletions of the TP53 gene predict nonresponse to treatment and poor outcome in first relapse of childhood acute lymphoblastic leukemia. *Journal of clinical oncology*, **29** (23), 3185–3193 (2011).
32. Wada, M. *et al. Analysis of p53 mutations in a large series of lymphoid hematologic malignancies of childhood.* 1993.

33. Wong, T. N. *et al.* Role of TP53 mutations in the origin and evolution of therapy-related acute myeloid leukaemia. *Nature*, **518** (7540), 552–555 (2015).
34. Schoch, C. *et al.* Comparison of chromosome banding analysis, interphase-and hypermetaphase-FISH, qualitative and quantitative PCR for diagnosis and for follow-up in chronic myeloid leukemia: a study on 350 cases., **16** (1), 53–59 (2002).
35. Dicker, F. *et al.* Immunostimulatory oligonucleotide-induced metaphase cytogenetics detect chromosomal aberrations in 80% of CLL patients: a study of 132 CLL cases with correlation to FISH, IgVH status, and CD38 expression., **108** (9), 3152–3160 (2006).
36. Haferlach, C. & Bacher, U. *Cytogenetic methods in chronic lymphocytic leukemia, in Cancer Cytogenetics*p. 119–130(Springer, 2011).
37. Hastings, R. J. *et al.* Guidelines for cytogenetic investigations in tumours. *European Journal of Human Genetics*, **24** (1), 6–13 (2016).
38. Suzuki, K. & Matsubara, H. Recent advances in p53 research and cancer treatment. Journal of Biomedicine and Biotechnology 2011. **2011**.
39. Vogelstein, B., Lane, D. & Levine, A. J. Surfing the p53 network. Nature 2000. **408**(6810): p. 307–310.
40. Peller, S. & Rotter, V. TP53 in hematological cancer: low incidence of mutations with significant clinical relevance. *Human mutation*, **21** (3), 277–284 (2003).
41. Cazzola, A. *et al.* TP53 deficiency permits chromosome abnormalities and karyotype heterogeneity in acute myeloid leukemia., **33** (11), 2619–2627 (2019).
42. Alwash, Y. *et al. Development of TP53 Mutations Over the Course of Therapy for Acute Myeloid Leukemia* (American journal of hematology, 2021).
43. Bishop, R. Applications of fluorescence in situ hybridization (FISH) in detecting genetic aberrations of medical significance. *Bioscience Horizons*, **3** (1), 85–95 (2010).
44. Moueden, A. *et al.* Aberrant Expression of the p53 Tumor Suppressor Gene in Pediatric Acute Lymphoblastic Leukemia. *J Blood Lymph*, **8** (216), 2 (2018).
45. Annooz, A. F. *et al.* Detection p53 gene deletion in hematological malignancies using fluorescence in situ hybridization: a pilot study. *Pak J Biol Sci*, **17** (7), 891–897 (2014).
46. Qian, M. *et al.* TP53 germline variations influence the predisposition and prognosis of B-cell acute lymphoblastic leukemia in children. *Journal of Clinical Oncology*, **36** (6), 591 (2018).
47. Chun, Y. J. *et al.* Molecular characterization of lung adenocarcinoma from Korean patients using next generation sequencing. *PloS one*, **14** (11), 0224379 (2019).
48. Bejar, R. *et al.* Clinical effect of point mutations in myelodysplastic syndromes. *New England Journal of Medicine*, **364** (26), 2496–2506 (2011).
49. Misawa, S. & Horiike, S. TP53 mutations in myelodysplastic syndrome. *Leukemia & lymphoma*, **23** (5–6), 417–422 (1996).
50. Haferlach, C. *et al.* Mutations of the TP53 gene in acute myeloid leukemia are strongly associated with a complex aberrant karyotype., **22** (8), 1539–1541 (2008).

51. Kulasekararaj, A. G. *et al.* TP 53 mutations in myelodysplastic syndrome are strongly correlated with aberrations of chromosome 5, and correlate with adverse prognosis. *British journal of haematology*, **160** (5), 660–672 (2013).
52. Ratajczak, M. Z. *et al.* Cancer from the perspective of stem cells and misappropriated tissue regeneration mechanisms., **32** (12), 2519–2526 (2018).
53. La Starza, R. *et al.* NPM1 deletion is associated with gross chromosomal rearrangements in leukemia. *PloS one*, **5** (9), 12855 (2010).
54. Tefferi, A. *et al. Mutations and karyotype in myelodysplastic syndromes: TP53 clusters with monosomal karyotype, RUNX1 with trisomy 21, and SF3B1 with inv (3)(q21q26. 2) and del (11q).* Blood cancer journal, 2017. **7**(12): p. 1–4.
55. Reddel, H. K. *et al.* A summary of the new GINA strategy: a roadmap to asthma control. *Eur. Respir. J*, **46** (3), 622–639 (2015).
56. Inoue, D. *et al.* SETBP1 mutations drive leukemic transformation in ASXL1-mutated MDS., **29** (4), 847–857 (2015).
57. Devillier, R. *et al. Role of ASXL1 and TP53 mutations in the molecular classification and prognosis of acute myeloid leukemias with myelodysplasia-related changes*.Oncotarget, 2015. **6**(10).
58. Barbosa, K. *et al.* The role of TP53 in acute myeloid leukemia: challenges and opportunities. *Genes, Chromosomes and Cancer*, **58** (12), 875–888 (2019).
59. Cassier, P. A. *et al.* Targeting apoptosis in acute myeloid leukaemia. *British journal of cancer*, **117** (8), 1089–1098 (2017).
60. Ahmad, M. F. *et al.* Studies of the p53 gene mutation in Saudi non-Hodgkin's lymphoma. *Cancer letters*, **104** (2), 225–231 (1996).

Tables

Table 1: ClearSeq AML HS panel

Gene List (targeted exons)							
GENE	EXON	GENE	EXON	GENE	EXON	GENE	EXON
ASXL1	12	*EZH2*	8, 17,18	*MPL*	10	*SF3B1*	13–15,17
CSF3R	14, 17	*FLT3*	14, 20	*NPM1*	11	*SRSF2*	1
CBL	8, 9	*IDH1*	4	*NRAS*	2, 3	*TET2*	3, 9, 10, 11
CEBPA	1	*IDH2*	4	*RUNX1*	3, 4, 8	*TP53*	5–8
DNMT3A	4, 8, 13, 15, 16, 18-23	*JAK2*	12, 14	*SETBP1*	3	*U2AF1*	2, 6

Table 2: Patient information and cytogenetic result

Case #	Age	Sex	Diagnosis	Cytogenetic Result
1	2	M	ALL	46, XY
2	2	M	ALL	AL,57~43, XY, +X, dup (1) (q21q31), +4, +5, +6, +7, -8, +9, +10, +14, +17, +18, -19, -20, +21, +22[cp50]
3	5	F	ALL	Leukemia,46, XX, der (19) t (1;19) (q25; p13.3) [17]/46, XX, idem, +der (21) t (1;21) (p13; p11.2) [14]/46, XX [19]
4	5	M	ALL	46, XX[20]
5	7	F	ALL	46, XX[20]
6	9	M	AML	46,XY[20]
7	12	M	ALL	46, XY, t (8; 21)(q22; q22) [29]/46, XY [21]
8	31	M	ALL	46,XY[20]
9	36	F	AML	46, XX[20]
10	43	M	AML	46,XY[20]
11	44	F	ALL	ALL,45, XX, +X, -9, t (9;22) (q34; q11.2), -13[cp34]/45, XX, t (9;22) (q34; q11.2) [cp8]/46, XX[cp8]
12	45	F	ALL	46, XX[20]
13	59	F	Lymphoma, NHL	46, XX[20]
14	63	F	AML	46, XX[20]
15	65	F	MDS	65-58, XX, +1, +2, der(2) t(2;5) (q12; q37), +5, +6, +8, +9, +10, +11, der(17) t (12;17) (p10; p10), +13, del (13) (q21), +21, +21 [cp50]
16	72	M	AML	45, XY,der (7;12), (q11.2; p12) [30]
17	13 Y	F	AML	46,XX[20]
18	35 Y	F	AML	46,XY[20]
19	42 Y	M	AML	46,XX[20]
20	69 Y	M	AML	46,XY[20]

Table.3: FISH result in relation with the clinical diagnosis and cytogenetic finding

Diagnosis	Cytogenetic	FISH
NHL (1), ALL(5), AML(8)	Normal karyotype (14)	NHL TP53 deletion (1/1)(11%) ALL TP53 deletion (2/5) (20-22%) AML TP53 deletion (1/8) (11%) AML TP53 trisomy (1/8)(20%)
ALL (1), AML (1)	Single abnormality (2)	ALL TP53 deletion (1/1) (22%) AML TP53 deletion (0/1)
MDS(1), ALL(3)	Complex karyotype (4)	MDS (1/1)TP53 trisomy (30%) ALL (2/3) TP53 deletion (45%)

Table.4: NGS results for MDS sample (shows the mutation details)

Impacted Gene	*TP53*
Type of Mutation	SNP (Heterozygous)
Chromosome	17
Ref. Allele	T
Alt. Allele	C
Function Class	MISSENSE
AA	H175R
Codon	cAt/cGt
Quality	Pass
Allele Frequency	0.447
Number of Variant Alleles	10232
Filtered Read Depth (per sample)	22870
Effect	UNKNOWN
Exon ID	NM_001126118.ex.5

Table.5: Distribution Pattern of Coexisting Mutations in Patients With and Without TP53 Mutations

Gene	Total No. of Mutation (n = 10)	TP53-mutated (n = 1)	TP53-wt (n = 9)
ASXL1	10	1	9
CEBPA	2	0	2
DNMT3A	2	0	2
FLT3	2	0	2
IDH2	3	0	3
JAK2	1	0	1
NPM1	8	1	7
RUNX1	4	0	4
SETBP1	10	1	9
SRSF2	10	1	9
TET2	8	1	7
U2AF1	1	0	1
NRAS	1	0	1

Table.6: ASXL1 & SETBP1 mutations in all cases. The mutations marked with red color represent the exclusive association with TP53 mutation.

Sample No.	Age	Sex	Diagnosis	Gene	Type of Mutation
1	5 Y	F	ALL	ASXL1	Missense (L815P)
				SETBP1	Silent (S1275)
2	65 Y	F	MDS	ASXL1	Missense (L815P)
					Missense (K1368T)
				SETBP1	**Missense (V231L)**
					Silent (S1275)
3	59 Y	F	Lymphoma, NHL	ASXL1	Missense (L815P)
					Silent (S1253)
				SETBP1	Missense (V1101I)
					Silent (S1275)
4	63 Y	F	AML	ASXL1	Missense (L815P)
				SETBP1	Silent (S1275)
5	36 Y	F	AML	ASXL1	Missense (L815P)
					Silent (S1253)
				SETBP1	Silent (H1206)
					Silent (S1275)
					Silent (L1278)
6	43 Y	M	AML	ASXL1	Missense (L815P)
					Silent (S1253)
				SETBP1	Silent (S1275)
7	13 Y	F	AML	ASXL1	Missense (L815P)
					Silent (S1253)
				SETBP1	Silent (H1206)
					Silent (S1275)
8	35 Y	F	AML	ASXL1	Missense (L815P)
					Silent (S1253)
				SETBP1	Missense (V1101I)
					Silent (S1275)

9	42 Y	M	AML	ASXL1	Missense (L815P) Silent (S1253)
				SETBP1	Silent (S1275)
10	69 Y	M	AML	ASXL1	Missense (L815P)
				SETBP1	Silent (S1275)

Figures

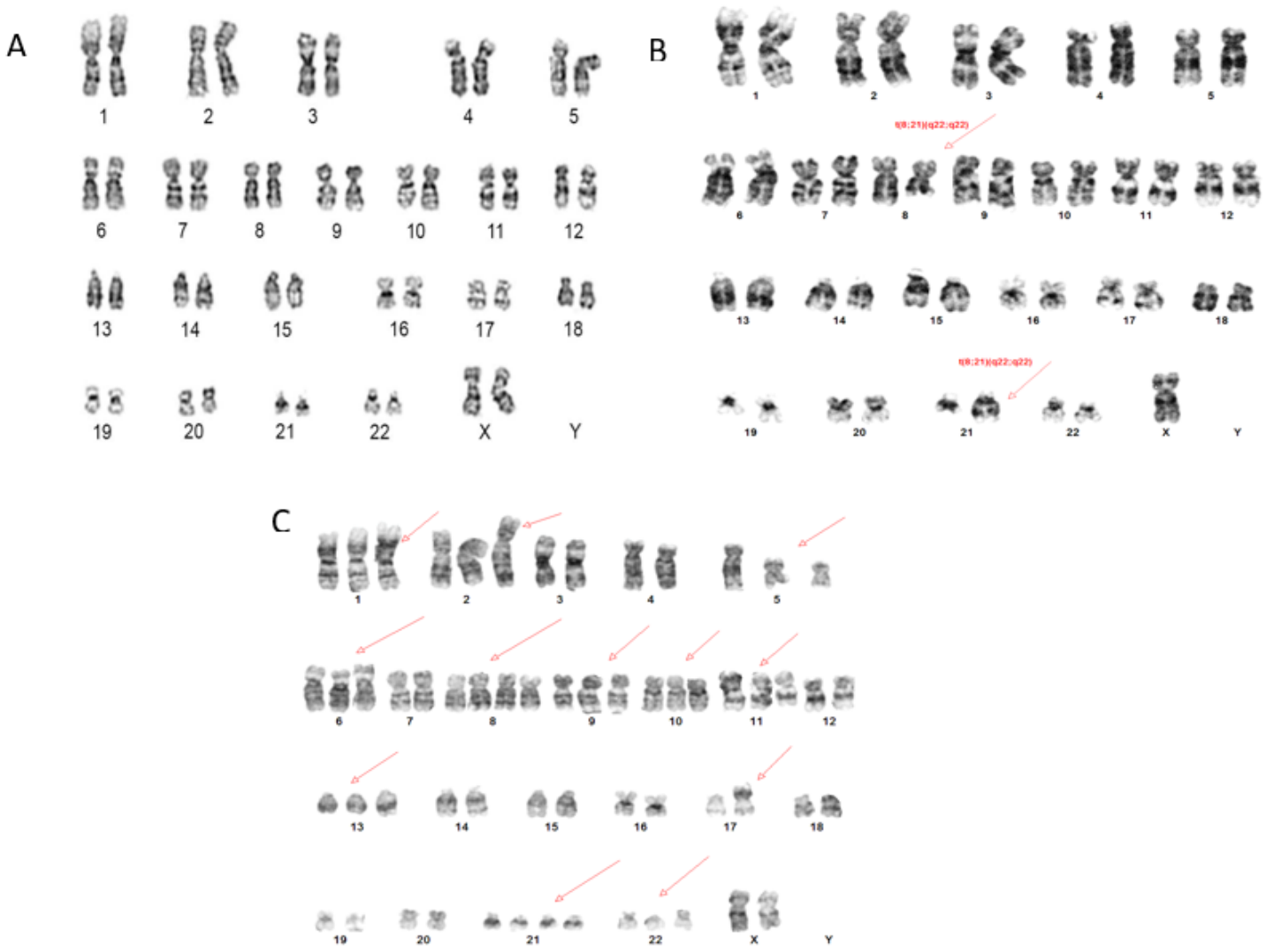

Figure 1

Demonstrated cytogenetic result. A) represented AML female with normal karyotype. B) ALL patient with single chromosome abnormality, 46,XY,t (8; 21)(q22; q22). (C) MDS patient with complex karyotype, 65-58, XX, +1, +2, der(2) t(2;5) (q12; q37), +5, +6, +8, +9, +10, +11, der(17) t (12;17) (p10; p10), +13, del (13) (q21), +21, +21 [cp50])

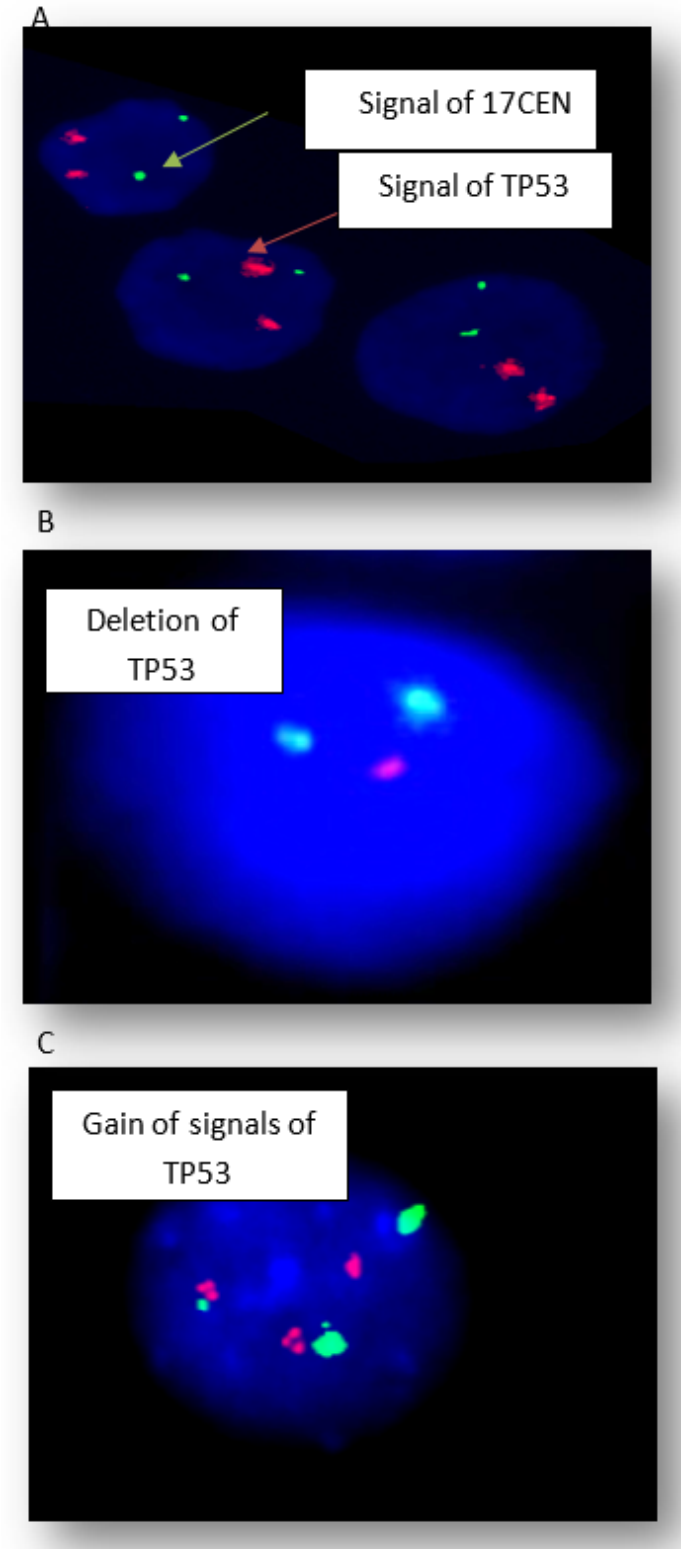

Figure 2

Demonstrated FISH result. A) represented a normal result (2 green and 2 red signals). B) TP53 deletion (2 green and 1 red signals). The last figure (C) represent cases with trisomy singles (3 green and 3 red).

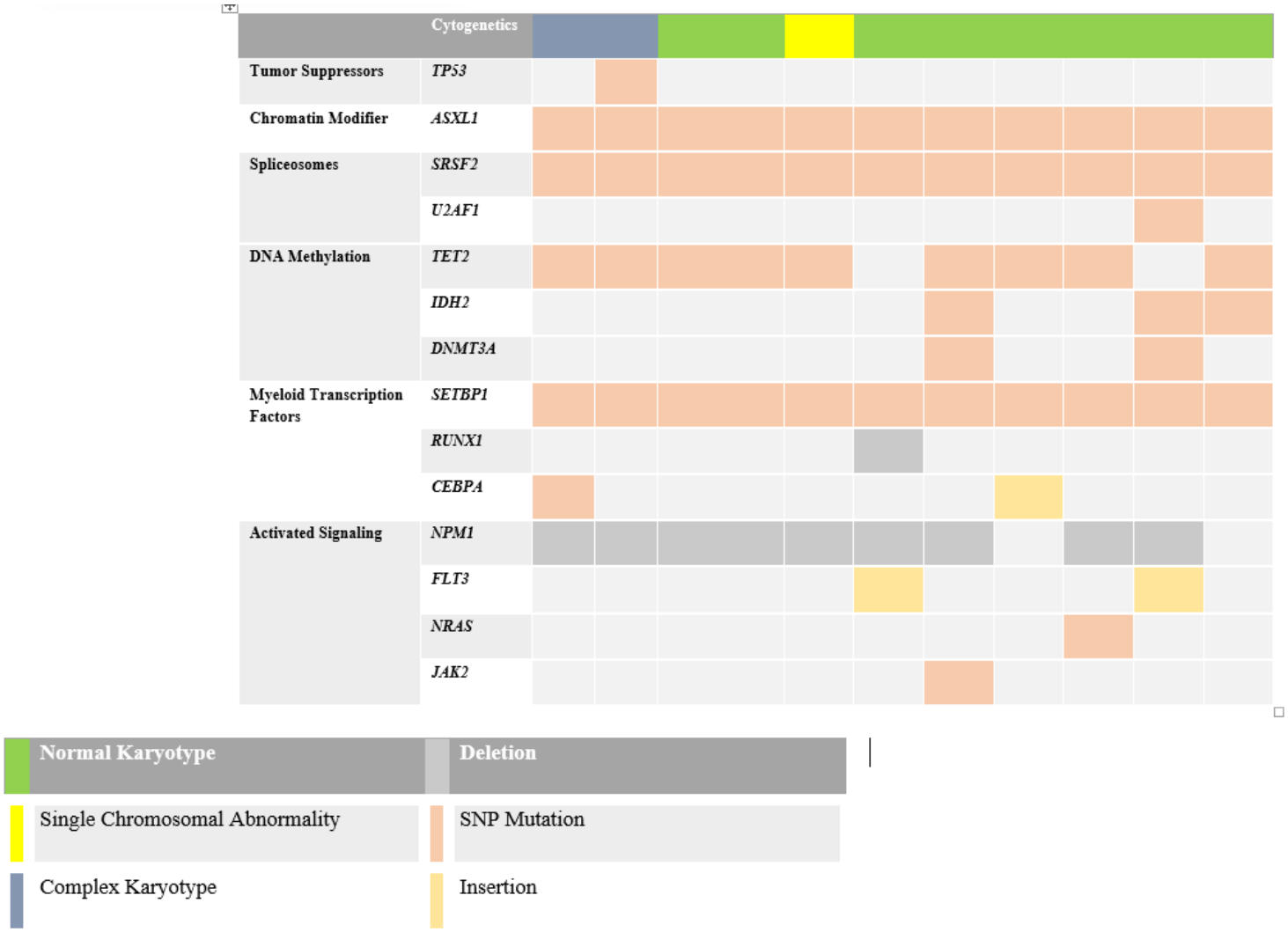

Figure 3

Mutation status according to patient characteristics & cytogenetics. The far-left column lists the 14 genes that were tested in the panel. Each column represents a single patient, and each colored bar indicates the presence of a mutation in the indicated gene. In addition, each color represents the type of mutation and cytogenetic status, as shown above. This illustrates the spectrum of coexistent mutations in all patients.