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ΕΡΕΥΝΗΤΙΚΗ ΕΡΓΑΣΙΑ

FOXO3a rs4946936 polymorphism and its clinical implications in chronic myeloid leukemia therapy

OBJECTIVE **To analyze the association between *FOXO3a rs4946936* polymorphism and treatment response in chronic myeloid leukemia (CML) patients.** METHOD **This was an analytical study with a retrospective cohort design conducted at Dr Saiful Anwar Hospital, Malang, Indonesia, during 2024. Data were retrospectively collected from medical records of CML patients meeting inclusion criteria, including clinical data, BCR-ABL test results, and *FOXO3a rs4946936* genotype. Statistical analyses were performed using Chi-square, t-test, and Mann-Whitney tests.** RESULTS **A total of 50 CML patients were included, consisting of 25 patients who achieved treatment response and 25 who did not. Significant differences were found in disease phase, EUTOS risk, hemoglobin levels, basophil counts, FOXO3a levels, and BCR-ABL levels between the two groups. Allele and genotype frequency analysis showed that patients with the T allele had a higher risk of treatment failure compared to the C allele. We also found that patients with the *TT* genotype had a higher risk of treatment failure compared to those with CC and CT genotypes.** CONCLUSIONS ***FOXO3a rs4946936* polymorphism is significantly associated with treatment response in CML patients, thus potentially serving as a predictive biomarker in CML management and personalized therapy.**

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ΑΡΧΕΙΑ ΕΛΛΗΝΙΚΗΣ ΙΑΤΡΙΚΗΣ 2026, 43(Συμπλ 1):46–51

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Ο πολυμορφισμός *FOXO3a rs4946936* και η κλινική του σημασία στη χρόνια μυελογενή λευχαιμία

Περίληψη στο τέλος του άρθρου

Key words

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Chronic myeloid leukemia (CML) is one of the major problems in hematology characterized by uncontrolled proliferation of myeloid cells.[1] The global incidence of CML is estimated at 1–2 cases per 100,000 population per year, with still relatively high mortality rates particularly among patients who do not respond to standard therapy.[2] Various factors are known to influence prognosis and treatment response in CML, such as age, disease stage, hemoglobin levels, and genetic factors.[3] One genetic factor that has recently gained attention is gene polymorphism, which can affect disease progression and treatment response.[4] Recent data show that certain genetic variations can serve as predictors of treatment success and prognosis in CML patients, making it important to identify these factors to improve clinical outcomes.[5]

The *FOXO3a rs4946936* polymorphism is a genetic variation suspected to play an important role in the pathogenesis and treatment response of CML patients.[6] FOXO3a is a transcription factor that regulates apoptosis, cell cycle, and DNA repair processes, so changes in this gene can directly

impact leukemia cell survival.[7] Several studies have shown that the T allele of *FOXO3a rs4946936* is associated with poorer prognosis and lower treatment response in various cancers, including CML.[6] However, some reports show conflicting results or no significant association between this polymorphism and clinical outcomes, indicating the need for further research to clarify the role of *FOXO3a rs4946936* in CML.[6] This uncertainty is the main reason for conducting further studies in the Indonesian population.

This study aimed to analyze the association between *FOXO3a rs4946936* polymorphism and treatment response in CML patients. It was expected that the results might provide scientific evidence supporting the use of *FOXO3a rs4946936* polymorphism as a predictive biomarker, thereby improving therapy personalization and prognosis for CML patients in the future.

MATERIAL AND METHOD

Design

This analytical study employed a retrospective cohort design conducted at Dr Saiful Anwar Hospital, Malang, Indonesia, during 2024. The study aimed to analyze the potential of *FOXO3a rs4946936* gene polymorphism as a predictive biomarker for treatment response in CML patients. The study protocol followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) checklist and received ethical approval under number 400/162/K.3/102.7/2024.

Participants and eligibility criteria

The study involved 50 patients diagnosed with CML at Dr Saiful Anwar Hospital, Malang, Indonesia. Participants were selected using total sampling, including all patients meeting inclusion criteria from 2020 to 2024. Inclusion criteria were CML patients with positive BCR-ABL test results who had received imatinib mesylate therapy for at least six months, tested positive for *FOXO3a rs4946936* polymorphism, and were at least 18 years old at study start. Exclusion criteria included patients with leukopenia (white blood cell count <4,000/µL), chronic obstructive pulmonary disease (COPD), rheumatoid arthritis, acute lymphoblastic leukemia, and vitiligo.

Data collection

Data collection was conducted at Dr Saiful Anwar Hospital, Malang, Indonesia, from 2020 to 2024. Retrospective medical records were used to obtain quantitative BCR-ABL results. Collected data included demographics, BCR-ABL test results, *FOXO3a rs4946936* genotype, treatment response status, and other clinical data. Data collection was performed by AA, BB, and CC.

Covariates

The main variable analyzed was *FOXO3a rs4946936* gene polymorphism, classified into three genotypes: *CC, TC,* and *TT*. Treatment response status was defined as "Achieved" (achieved treatment response) and "Not achieved" (did not achieve treatment response) based on clinical and laboratory evaluation during follow-up.

Statistical analysis

Categorical data were presented as n (%). The Kolmogorov-Smirnov test was used to assess normality of numerical data. Normally distributed data were presented as mean±standard deviation (SD), while non-normal data were presented as median (interquartile range, IQR). Chi-square test compared categorical variables between treatment response groups. T-test compared means of continuous variables if normally distributed; otherwise, Mann-Whitney test was used. A p-value <0.05 was considered statistically significant. All analyses were performed using GraphPad Prism software (GraphPad Software, Inc; California, USA).

RESULTS

Baseline characteristics of patients included in the study

This study included 50 CML patients divided into two groups based on treatment response: 25 patients who achieved response (Achieved) and 25 who did not (Not achieved). Baseline characteristic analysis showed significant differences in several clinical variables between groups. Significant p-values were found for disease phase, EUTOS risk, hemoglobin levels, basophil counts, FOXO3a levels, and BCR-ABL levels, indicating real differences between patients who achieved treatment response and those who did not. Specifically, BCR-ABL and FOXO3a levels were higher in the non-responder group, while high EUTOS risk was only found in the treatment failure group. Baseline characteristics data are presented in table 1.

The association between genotype and allele frequencies of *FOXO3a rs4946936* and the risk of failure treatment among chronic myeloid leukemia patients

Analysis of allele and genotype frequencies of *FOXO3a rs4946936* in all patients showed significant differences between treatment failure and responder groups. Our results found that the C allele was associated with a decreased risk of treatment failure, whereas the T allele was associated with an increased risk. Additionally, the *CC* genotype significantly conferred a protective effect against

Table 1. Baseline characteristics of patients included in our study.

Characteristics	Treatment response		p-value
	Achieved	Not achieved	
Age (years) (mean±SD)	41.72±11.17	42.08±12.54	0.9151
Genders			
Male (n %)	12 (48.0%)	14 (56.0%)	0.5713
Female (n %)	13 (52.0%)	11 (44.0%)	0.5713
Spleen (median [IQR])	14.48 (3,022)	17.50 (4,533)	0.2163
Phase			
Acceleration (n %)	5 (10.0%)	13 (26.0%)	0.0184
Chronic (n %)	19 (38.0%)	10 (20.0%)	0.0099
Blast crisis (n %)	1 (2.0%)	2 (4.0%)	0.5515
EUTOS			
Low risk (n %)	25 (50%)	15 (30.0%)	0.0004
High risk (n %)	0	10 (20.0%)	0.0004
Hemoglobin (mean±SD)	12.71±2.90	12.37±3.13	0.0017
Leukocyte (median [IQR])	6,140 (3,230)	15,540 (117,770)	0.0621
Thrombocyte (median [IQR])	190,000 (213,000)	279,000 (113,000)	0.0733
Basophil (median [IQR])	0.3 (0.2)	0.5 (0.9)	0.0014
Blast (median [IQR])	4 (7)	8 (11)	0.2318
Foxo3a levels (median [IQR])	67.65 (84.51)	22.30 (36.01)	0.0058
Bcrabl (median [IQR])	0.599 (3.77)	21.63 (20.86)	<0.0001

Values are mean±SD, n (%), median (IQR)

SD: Standard deviation, IQR: Interquartile range

treatment failure, while the *TT* genotype increased the risk of treatment failure in CML patients. The *TC* genotype did not show a significant difference. Detailed results of this analysis are shown in figure 1.

DISCUSSION

The main findings of this study indicated that the distribution of genotypes and alleles of *FOXO3a rs4946936* was significantly associated with the risk of treatment failure in CML patients. Patients carrying the T allele and *TT* genotype had a higher risk of treatment failure compared to those with the C allele and *CC* genotype. These findings align with previous studies reporting that the T allele of *FOXO3a rs4946936* is associated with poorer prognosis in CML and other cancers such as rhabdomyosarcoma.[8,9] Other studies also report associations between *FOXO3a rs4946936* polymorphism and clinical outcomes in various cancers; for example, in breast cancer patients, the T allele was linked to lower survival and poorer treatment response.[10] Similar findings in lung cancer and lymphoma suggest that *FOXO3a* gene variations influence protein expression and cancer cell sensitivity to apoptosis, impacting patient prognosis.[11] Thus, the *FOXO3a rs4946936* polymorphism in this study further strengthens its potential as an important predictive biomarker for stratifying treatment failure risk in CML patients.

Theoretically, these findings might be explained by the significant role of the *FOXO3a* gene in the regulation of apoptosis, cell cycle, and DNA repair, all of which are essential in the pathogenesis and progression of CML.[12] *FOXO3a* functions as a transcription factor controlling the expression of genes involved in programmed cell death and response to oxidative stress.[13] The rs4946936 polymorphism located in the 3'-UTR region of FOXO3a could affect either mRNA stability or microRNA interactions, inducing an effect on the expression of FOXO3a protein and how it functions.[14] The *TT* genotype is known to be associated with lower FOXO3a expression; thus, it could result in the loss of both apoptosis and DNA repair, which could allow leukemia cells to become more resistant to therapy and facilitate faster disease progression.[6] On the contrary, the *CC* genotype appears to maintain optimal FOXO3a levels, which has a protective, clinical importance against adverse clinical outcomes in CML patients.[15] Therefore, genetic variation occurring at *FOXO3a rs4946936* is related to differences in clinical response to treatment and mortality risk. Overall, our findings may reinforce the potential for *FOXO3a rs4946936* to become a useful predictive biomarker to guide treatment decisions in CML.

This study demonstrated several important clinical implications. First, the *FOXO3a rs4946936* polymorphism is a potential predictive biomarker to identify CML patients that are at increased risk of treatment failure and offers a more accurate means for physician assessment in risk identification. Second, if physicians understand patient genotype status, they could develop more individualized treatment plans, beyond monitoring and duration of treatment, for patients carrying the risk-associated *TT* genotype. Third, this study raised possibilities in developing molecular-targeted therapies that may be directed towards pathways influenced by FOXO3a. Fourth, this study provided solutions for identifying risk, which is still an issue in clinical management of CML and ultimately provides a more effective and efficient means of clinical decision making. Fifth, this study provided a basis for future studies in pharmacogenomics; molecular oncology we can develop

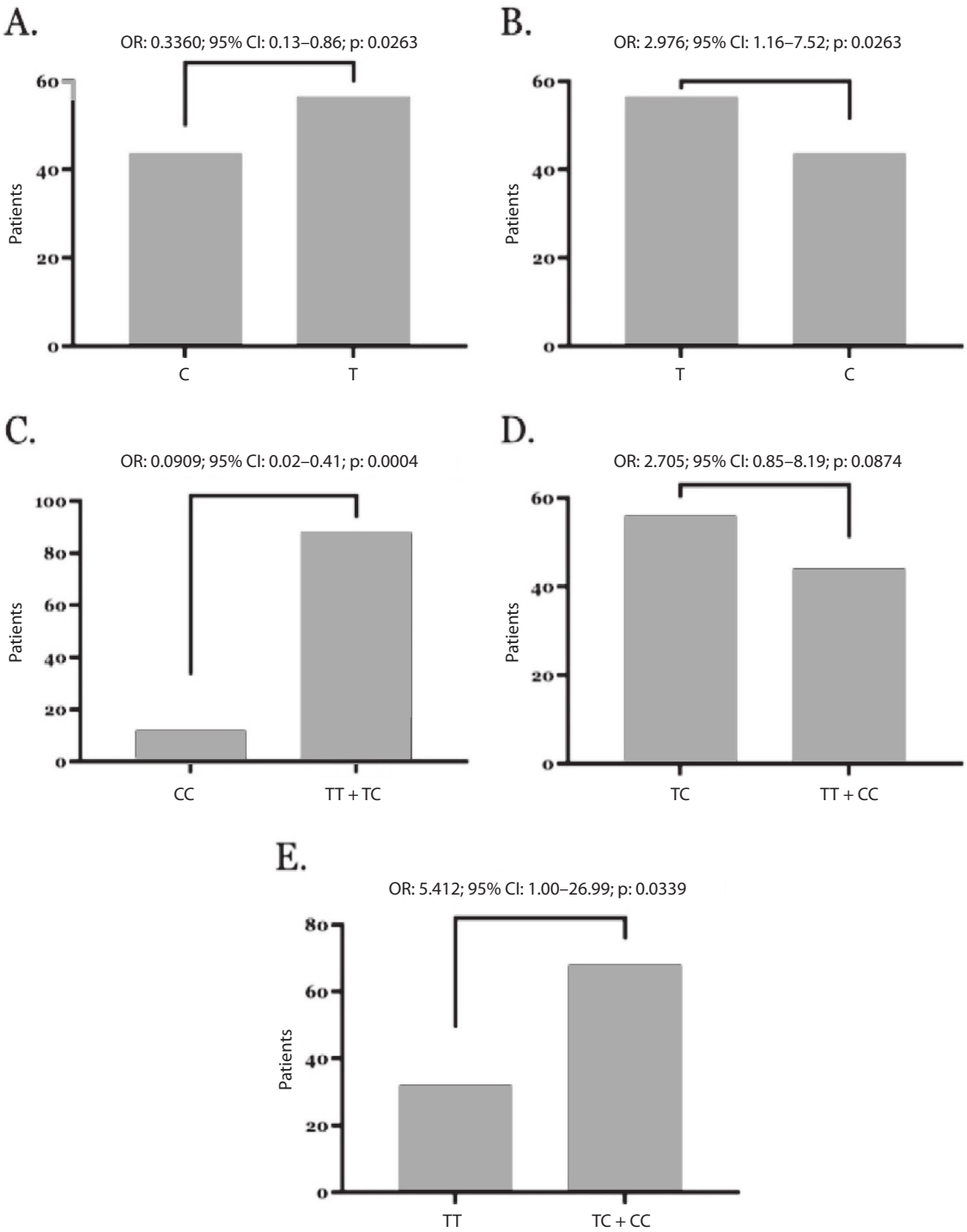

Figure 1. Analysis of allele and genotype frequencies of the *FOXO3a rs4946936* polymorphism in chronic myeloid leukemia (CML) patients who failed to achieve treatment response. **(A)** Frequency comparison of C versus T alleles (odds ratio [OR]: 0.3360; 95% confidence interval [CI]: 0.13–0.86; p: 0.0263). **(B)** Inverse comparison of T versus C alleles (OR: 2.976; 95% CI: 1.16–7.52; p: 0.0263). **(C)** Association of *CC* genotype versus combined *TT* and *TC* genotypes (OR: 0.0909; 95% CI: 0.02–0.41; p: 0.0004). **(D)** Association of *TC* genotype versus combined *TT* and *CC* genotypes (OR: 2.705; 95% CI: 0.85–8.19; p: 0.0874). **(E)** Association of *TT* genotype versus combined *TC* and *CC* genotypes (OR: 5.412; 95% CI: 1.00–26.99; p: 0.0339).

for further biomarker validation and also understand the molecular mechanisms that create the association between *FOXO3a* genotype and patient result.

This study also had several limitations that should be considered. First, the relatively small sample size limited the statistical power and generalizability of the findings to the

broader CML population. Second, the retrospective study design might introduce selection and information bias due to reliance on medical records that may be incomplete or inconsistent. Third, not all confounding factors such as comorbidities, lifestyle, or environmental influences could be fully controlled. Fourth, the study lacked advanced molecular data such as protein expression or functional analysis of *FOXO3a*, limiting deeper understanding of the biological mechanisms linking polymorphism to clinical outcomes. Fifth, prospective studies with larger samples and more comprehensive molecular analyses are needed to confirm and expand these findings and elucidate the biological mechanisms in greater detail.

In conclusion, this study demonstrated that the *FOXO3a rs4946936* gene polymorphism is significantly associated with the risk of treatment failure in CML patients, where the *TT* genotype increases the risk and the *CC* genotype provides a protective effect. It is hoped that these findings may form the basis for developing predictive biomarkers to personalize therapy and improve prognosis in CML patients in the future.

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ΠΕΡΙΛΗΨΗ

Ο πολυμορφισμός *FOXO3a rs4946936* και η κλινική του σημασία στη χρόνια μυελογενή λευχαιμία

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ΣΚΟΠΟΣ Η ανάλυση της συσχέτισης μεταξύ του πολυμορφισμού *FOXO3a rs4946936* και της ανταπόκρισης στη θεραπεία ασθενών με χρόνια μυελογενή λευχαιμία (ΧΜΛ). **ΥΛΙΚΟ-ΜΕΘΟΔΟΣ** Πρόκειται για μια αναλυτική μελέτη με αναδρομικό σχεδιασμό κοόρτης που διεξήχθη στο Νοσοκομείο Dr Saiful Anwar, στο Malang της Ινδονησίας, κατά τη διάρκεια του 2024. Τα δεδομένα συλλέχθηκαν αναδρομικά από ιατρικά αρχεία ασθενών με ΧΜΛ που πληρούσαν τα κριτήρια ένταξης, περιλαμβανομένων κλινικών δεδομένων, αποτελεσμάτων εξετάσεων BCR-ABL και γονότυπου *FOXO3a rs4946936*. Οι στατιστικές αναλύσεις πραγματοποιήθηκαν εφαρμόζοντας δοκιμασίες Chi-square, t-test και Mann-Whitney. **ΑΠΟΤΕΛΕΣΜΑΤΑ** Συμπεριλήφθηκαν συνολικά 50 ασθενείς με ΧΜΛ, οι οποίοι αποτελούνταν από 25 ασθενείς που ανταποκρίθηκαν στη θεραπεία και 25 οι οποίοι δεν ανταποκρίθηκαν. Διαπιστώθηκαν σημαντικές διαφορές στη φάση της νόσου, στον κίνδυνο EUTOS, στα επίπεδα αιμοσφαιρίνης, στον αριθμό βασεόφιλων, στα επίπεδα FOXO3a και στα επίπεδα BCR-ABL μεταξύ των δύο ομάδων. Η ανάλυση συχνότητας αλληλόμορφων και γονοτύπων έδειξε ότι οι ασθενείς με το αλληλόμορφο T είχαν υψηλότερο κίνδυνο αποτυχίας της θεραπείας σε σύγκριση με το αλληλόμορφο C. Διαπιστώθηκε επίσης ότι οι ασθενείς με τον γονότυπο *TT* είχαν υψηλότερο κίνδυνο αποτυχίας της θεραπείας σε σύγκριση με εκείνους με γονότυπους *CC* και *CT*. **ΣΥΜΠΕΡΑΣΜΑΤΑ** Ο πολυμορφισμός *FOXO3a rs4946936* σχετίζεται σημαντικά με την ανταπόκριση στη θεραπεία σε ασθενείς με ΧΜΛ, λειτουργώντας έτσι ενδεχομένως ως προγνωστικός βιοδείκτης στη διαχείριση της ΧΜΛ και στην εξατομικευμένη θεραπεία.

Λέξεις ευρετηρίου: Ανταπόκριση στη θεραπεία, Βιοδείκτες, Γονιδιακός πολυμορφισμός, FOXO3a, Χρόνια μυελογενής λευχαιμία

References

1. MOJTAHEDI H, YAZDANPANAH N, REZAEI N. Chronic myeloid leukemia stem cells: Targeting therapeutic implications. *Stem Cell Res Ther* 2021, 12:603
2. CROSS NCP, ERNST T, BRANFORD S, CAYUELA JM, DEININGER M, FABARIUS A ET AL. European LeukemiaNet laboratory recommendations for the diagnosis and management of chronic myeloid leukemia. *Leukemia* 2023, 37:2150–2167
3. IEZZA M, CORTESI S, OTTAVIANI E, MANCINI M, VENTURI C, MONALDI

C ET AL. Prognosis in chronic myeloid leukemia: Baseline factors, dynamic risk assessment and novel ınsights. *Cells* 2023, 12:1703
4. TARANNUM F, FAIZUDDIN M. Effect of gene polymorphisms on periodontal diseases. *Indian J Hum Genet* 2012, 18:9–19
5. LAUSEKER M, HOFFMANN VS, PFIRRMANN M. Prognostic scoring systems in chronic myeloid leukaemia. *Leukemia* 2025, 39:1046–1052
6. WARDHANI SO, SUSIANTI H, RAHAYU P, YUENIWATI YP, FAJAR JK. The association between *FOXO3a rs4946936* gene polymorphism and the levels of *FOXO3a* among chronic granulocytic leukemia patients treated with imatinib mesylate. *F1000Res* 2021, 10:1003
7. NESTAL DE MORAES G, BELLA L, ZONA S, BURTON MJ, LAM EWF. Insights into a critical role of the FOXO3a-FOXM1 axis in DNA damage response and genotoxic drug resistance. *Curr Drug Targets* 2016, 17:164–177
8. ZHANG X, SUN Y, NIU H, TAN P, LIU S, LIU X ET AL. *FOXO3* polymorphisms influence the risk and prognosis of rhabdomyosarcoma in children. *Front Oncol* 2024, 14:1387735
9. SANTOSO DSB, SANTOSO B, KRISTYANA S, CANDRADIKUSUMA D, NATASIA SMD, EVATTA A. Exploring a rare oncologic complication: Alveolar rhabdomyosarcoma in HIV patient. *Deka in Medicine* 2024, 1:e277
10. SALJOOGHI S, HEIDARI Z, SARAVANI M, REZAEI M, SALIMI S. Association of *FOXO1 Rs17592236* polymorphism and tumor size in papillary thyroid carcinoma. *Rep Biochem Mol Biol* 2022, 11:216–223
11. LIU Y, AO X, DING W, PONNUSAMY M, WU W, HAO X ET AL. Critical role of *FOXO3a* in carcinogenesis. *Mol Cancer* 2018, 17:104
12. RAHMIANTI ND, VENDARANI YF, MAULIDIYAH N. Right ventricular strain: Cardiovascular challenges in pulmonary diseases. *Deka in Medicine* 2024, 1:e359
13. JIN H, ZHANG L, HE J, WU M, JIA L, GUO J. Role of *FOXO3a* transcription factor in the regulation of liver oxidative injury. *Antioxidants (Basel)* 2022, 11:2478
14. WANG Y, ZHOU L, CHEN J, LI J, HE L, WU P ET AL. Association of the 3'UTR *FOXO3a* polymorphism *rs4946936* with an increased risk of childhood acute lymphoblastic leukemia in a Chinese population. *Cell Physiol Biochem* 2014, 34:325–332
15. ALVES R, GONCALVES AC, RUTELLA S, ALMEIDA AM, DE LAS RIVAS J, TROUGAKOS IP ET AL. Resistance to tyrosine kinase inhibitors in chronic myeloid leukemia – from molecular mechanisms to clinical relevance. *Cancers (Basel)* 2021, 13:4820

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