folia medica

Folia Medica 63(5):670-5
DOI: 10.3897/folmed.63.e63366

Original Article

# Clinical Characteristics, Disease Evolution, and Survival in Patients with Chronic Myeloid Leukemia, BCR-ABL1 (+), and T315I Mutation

Veselina Goranova-Marinova[1,4], Alexander Linev[2,5], Hristo J. Ivanov[2,5], Ivan Zhelyazkov[2,5], Vily Stoyanova[2,5], Zhanet Grudeva-Popova[3,4]

[1] *Section of Hematology, First Department of Internal Medicine, Faculty of Medicine, Medical University of Plovdiv, Plovdiv, Bulgaria*
[2] *Section of Medical Genetics, Department of Pediatrics, Faculty of Medicine, Medical University of Plovdiv, Plovdiv, Bulgaria*
[3] *Section of Medical Oncology, Department of Clinical Oncology, Faculty of Medicine, Medical University of Plovdiv, Plovdiv, Bulgaria*
[4] *Clinic of Clinical Hematology, St George University Hospital, Plovdiv, Bulgaria*
[5] *Department of Medical Genetics, St George University Hospital, Plovdiv, Bulgaria*

**Corresponding author:** Veselina Goranova-Marinova, Section of Hematology, First Department of Internal Medicine, Faculty of Medicine, Medical University of Plovdiv, 15A Vassil Aprilov Blvd., Plovdiv 4002, Bulgaria; E-mail: vesselina_goranova@yahoo.com; Tel.: +359 888 885 741

**Received:** 19 Jan 2021 ♦ **Accepted:** 17 Mar 2021 ♦ **Published:** 31 Oct 2021

**Citation:** Goranova-Marinova V, Linev A, Ivanov HJ, Zhelyazkov I, Stoyanova V, Grudeva-Popova Zh. Clinical characteristics, disease evolution and survival in patients with chronic myeloid leukemia, BCR-ABL1 (+) and T315I mutation. Folia Med (Plovdiv) 2021;63(5):670-5. doi: 10.3897/folmed.63.e63366.

## Abstract

**Introduction:** The T315I mutation in patients with chronic myeloid leukemia (CML) has been associated with therapeutic resistance and an unfavourable prognosis.

**Aim:** To study the frequency of T315I mutation in patients with CML, BCR-ABL (+), their clinical characteristics, disease evolution, and median survival.

**Patients and methods:** We studied 75 patients with CML and BCR-ABL1 (+). T315I mutation was detected by digital droplet PCR and BCR-ABL1 was analyzed by RT-PCR. A comparative analysis was performed by sex, age, disease phase, risk group, treatment, molecular response (MR), and median survival in T315I (+) and T315I (−) patients.

**Results:** T315I mutation was detected in 11 patients (14.7%). No significant difference was found in the phase, risk group, and first-line therapy. A significantly higher proportion of T315I (+) did not achieve MR >3.5 log: 8 (72.7%) vs. 22 (34.4%) ($p$=0.023). The lowest mean BCR-ABL1 levels were significantly higher in the CML T315I (+) group compared to the CML T315I (−) group: 12.1±6.0 vs. 3.77±1.28 ($p$=0.009). The median survival of T315I (+) patients was significantly shorter: 73 months vs. 175 months ($p$<0.0001, CI 95%).

**Conclusions:** Our data confirm the world experience on the frequency of T315I mutation, including the unfavourable evolution, resistance to TKI treatment and short survival. ddPCR is a highly sensitive method for early detection of genetic mutations which gives the chance for effective treatment.

## Keywords

CML, clinical evolution, mutation T315I

## INTRODUCTION

The discovery of the Philadelphia chromosome in patients with chronic myeloid leukemia (CML) is a fundamental achievement in medical science. As a result of the successful targeted inhibition of its fusion protein, the mutant tyrosine kinase BCR-ABL1, today patients with CML have an expected survival comparable to that of people without CML in the same age group. However, as a result of additional mutations in the kinase domain of the BCR-ABL1 gene, resistance to the effect of tyrosine kinase inhibitors (TKI) occurs.[1,2] The T315I mutation in the BCR-ABL1 gene affects the common ABL kinase binding site and results in high-grade cross-resistance to TKIs. Currently, five TKIs are used in clinical practice, with different drugs covering a different spectrum of genetic mutations. The T315I mutation is one of the most difficult to treat.[3] It is associated with resistance to treatment, failure or early loss of molecular response (MR), progression to the acceleration phase and blast crisis, and shortened survival.[4] Currently, RTq-PCR, Sanger sequencing, and next-generation sequencing are the golden standard for monitoring of MR and mutation analysis in patients with CML.[5] In recent years, emulsion digital PCR (ddPCR) has become increasingly important as a highly informative, fast and cost-effective methodology.[6]

## AIM

To investigate the frequency of T315I mutation detected by ddPCR in patients with CMR BCR-ABL1 (+), to analyze their clinical features, disease evolution, and median survival, and to compare those in CML patients without the T315I mutation.

## PATIENTS AND METHODS

We studied 75 patients with CML, BCR-ABL1 (+), who did not achieve an optimal therapeutic response within the defined timeframes, or with a loss of the achieved molecular response. Patients were diagnosed, treated and monitored between March 2000 and December 2018 in the Clinic of Clinical Hematology and Clinic of Medical Oncology, St George University Hospital, Medical University of Plovdiv. The detection and quantitative measurement of the T315I mutation was performed by ddPCR using the QX200 Droplet Digital PCR system in the Department of Medical Genetics, Medical University of Plovdiv under the PERIMED project. Digital droplet PCR is based on the division of a standard PCR reaction into tens of thousands of nano-droplets that contain one (or more) or do not contain the target sequence. Amplification is performed in each droplet and the number of the sequences is calculated from the proportion of positive and negative droplets using the Poisson distribution.[7] Molecular genetic tests for detection and quantitative measurement of fusion transcripts BCR-ABL1 were performed by RT-PCR in the Specialized Hospital for Active Treatment of Hematological Diseases in Sofia and presented on an international scale, according to the criteria of European Leukemia Net. **Table 1** presents the characteristics of the studied contingent of patients at the time of diagnosis. The male to female ratio was 1.2/1.0, the mean age was 54.64±14.4 years.

**Table 1.** Characteristics of patients at diagnosis

| Parameter | N (%) |
|---|---|
| Sex | |
| Male | 34 (45.3) |
| Female | 41 (54.7) |
| Phase of the disease | |
| Chronic | 58 (77.3%) |
| Accelerated | 12 (16.0%) |
| Blast crisis | 5 (6.7%) |
| Risk - Score Sokal | |
| Low risk | 28 (37.3%) |
| Intermediate risk | 26 (34.7%) |
| High risk | 21 (28.0%) |
| Risk - Score Hasford | |
| Low risk | 25 (33.3%) |
| Intermediate risk | 29 (38.7%) |
| High risk | 21 (28.0%) |
| Mutation T315I | 11 (14.7%) |
| MR≥3.5 log | 45 (60.0%) |
| Median of MR -1 | 26 (17.26-34.75) mos |
| Median Survival | 175 (156.69-200.43) mos |

In patients with T315I mutation (CML T315I +), a comparative analysis was performed by sex, age, disease phase, risk group, treatment, type, and duration of molecular response and median survival calculated from the time of diagnosis and compared to patients with CML without T315I mutation (CML T315I −). Statistical data processing was performed with descriptive analysis, correlation analysis and the Independent Sample t-test for comparison of mean values, at a level of significance $p<0.05$. Survival analysis was performed by the Kaplan-Meier method with a log-rank test.[8]

## RESULTS

The T315I mutation was detected in 11 (14.7%) of CML patients. The mean time from the initiation of therapy to analysis was 74 months (range 7–225). There was no statistically significant difference in demographics, baseline disease characteristics (phase and prognostic risk score), and comorbidity profile between the two groups of patients **(Table 2)**.



Folia Medica

**Table 2.** Comparative analysis between CML T315 (–) and CML T315 (+) patients

| Parameters | CML T315I (–) | CML T315I (+) | P |
|---|---|---|---|
| Sex | | | |
| Male | 30 (46.9%) | 5 (45.5%) | NS |
| Female | 34 (53.1%) | 6 (54.5%) | |
| Mean age | 55.29±14.1 | 49.27±13.5 | NS |
| Phase of the disease | | | |
| Chronic | 45 (70.3%) | 7 (63.6%) | NS |
| Accelerated | 14 (21.9%) | 4 (36.4%) | |
| Blast crisis | 5 (7.8%) | 0 | |
| Risk - Score Sokal | | | |
| Low risk | 24 (37.5%) | 6 (54.5%) | NS |
| Intermediate risk | 26 (40.6%) | 4 (36.4%) | |
| High risk | 14 (21.9%) | 1 (9.1%) | |
| Risk - Score Hasford | | | |
| Low risk | 23 (35.9%) | 4 (36.4%) | NS |
| Intermediate risk | 23 (35.9%) | 6 (54.5%) | |
| High risk | 18 (28.1%) | 1 (9.1) | |
| Comorbidities | | | |
| Cardiovascular | 34 (53.1%) | 5 (45.5%) | NS |
| Hepatic | 22 (34.5%) | 2 (18.2%) | |
| Renal | 11 (17.2%) | - | |
| Endocrino-logical | 10 (15.6%) | 2 (18.2%) | |
| Others | 14 (21.8%) | 3 (27.2%) | |

## Lines of treatment

Therapeutic choice for first-, second- and ≥ third-line therapy did not differ significantly in the patient groups CML T315I (–) and CML T315I (+), presented in **Table 3**. Imatinib was most often chosen for a first-line therapy in both groups while nilotinib was the most common therapeutic choice for second line. In patients with CML T315I (+), the mean number of treatment lines was 3.2 compared to 1.1 in the CML T315I (–), (NS).

The duration of first-line treatment was significantly shorter in patients with CML T315I (+) mutation compared to the CML T315I (–) group: 13 months (95% CI 3.424–19.710) versus 33 months (95% CI 16.184–76.657) ($p$=0.001) **(Fig. 1)**.

## Depth and duration of the molecular response

Significantly higher proportion of patients with CML T315I (+) did not achieve an optimal molecular response >3.5 log throughout the follow-up period compared to patients with CML T315I (–): 8 (72.7%) vs. 22 (34.4%) ($p$=0.023). The lowest mean BCR-ABL1 levels were significantly higher in the CML T315I (+) group compared to the CML T315I (–) group: 12.1±6.0 vs. 3.77±1.28 ($p$=0.009). The duration of the MR was significantly shorter in the CML T315I (+) group – 13 months (95% CI 10–15) vs. 113 months (95% CI 54–171) in CML T315I (–) ($p$<0.001) **(Fig. 2)**.

The median survival (MS) of patients with CML T315I (+) was significantly shorter than the MS of patients CML T315I (–): 73 months vs. 175 months ($p$<0.0001) **(Fig. 3)**.

**Table 3.** Lines of therapy in patients CML T315 I (+) and CML T315 I (–)

| Lines of therapy | CML T315I (–) | CML T315I (+) | P |
|---|---|---|---|
| First line | | | |
| Imatinib | 46 (71.9%) | 6 (54.5%) | |
| Nilotinib | 11 (17.2%) | 3 (27.3%) | 0.08 |
| Dasatinib | 7 (10.9%) | 1 (9.1%) | |
| Bosutinib | - | 1 (9.1%) | |
| Second line | | | |
| Nilotinib | 18 (73.9%) | 4(57.2%) | |
| Dasatinib | 4 (17.4%) | 1 (14.3%) | 0.224 |
| Bosutinib | 1 (4.3) | 1 (4.3%) | |
| Ponatinib | - | - | |
| Third and fourth lines | | | |
| Dasatinib | 8 (44.4%) | 2 (11.1%) | 0.243 |
| Bosutinib | 1 (5.55%) | 4 (22.2%) | |
| Ponatinib | 1 (5.55%) | 2 (11.1%) | |

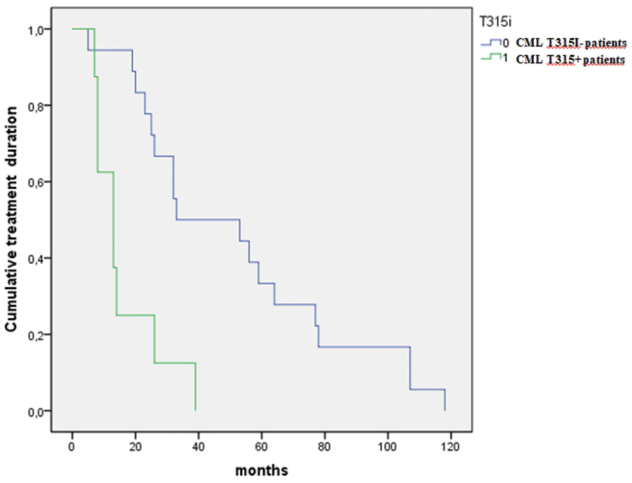


**Figure 1.** Duration of first-line treatment in patients with CML depending on the presence of the T315I mutation.

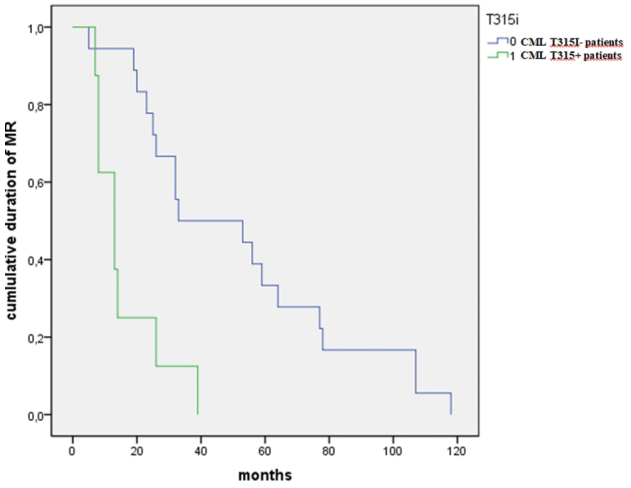


**Figure 2.** Duration of therapeutic response in first-line treatment of patients with CML depending on the presence of T315I mutation.

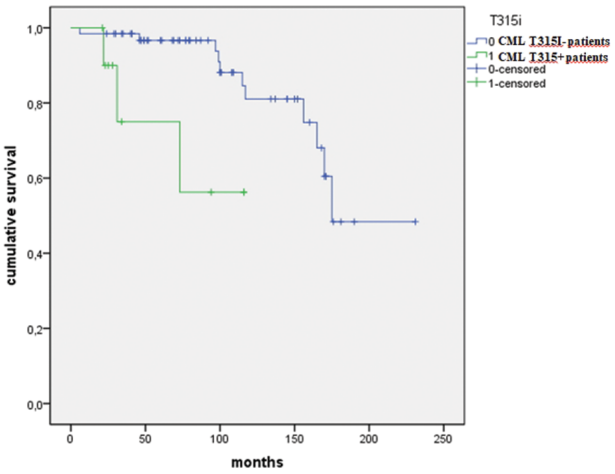


**Figure 3.** Median survival in patients with CML depending on the presence of the T315I mutation.

## DISCUSSION

In the literature, the frequency of detection of T315I mutation varies in a wide range of 2%-20% depending on the characteristics of the studied contingent and the method of analysis used. In the study of Shi DY et al. on 1093 patients, the T315I mutation was the most common mutation found in imatinib-, nilotinib-, and dasatinib-resistant patients (12.3%, 27.3%, and 34.1%, respectively).[9] Large epidemiological studies on the frequency of T315I mutation confirm that it is the most common mutation in patients with CML. Different authors focus on different aspects of the manifestation of this mutation, but unequivocally prove the T315I mutation is associated with a poor prognosis.[10,11] Nicolini et al. found short overall survival in patients with T315I mutation depending on the phase of the disease: 22.4 months, 28.4 months, 4.0 months for chronic phase, acceleration and blast crisis and progression-free survival of 11.5, 22.2, and 1.8, respectively (calculated from the time of mutation detection).[12] In the study by Shy et al., the detection of a T315I mutation was analyzed in relation to the type of drug administered and was more commonly demonstrated in second-generation TKIs. In addition, according to the same authors, it is more common in men, younger age, lack of concomitant diseases and advanced stage of disease.[9] The analysis of the results of our study did not find a statistical difference between patients with T315I (+) and T315I (–) according to the baseline characteristics of the disease at diagnosis – phase and risk group, demographics, sex and age, and the comorbidity profile of patients. Many authors, as well as our analysis, observed adverse clinical evolution, resistance to treatment and short overall survival, reasonably in the course of disease progression.[13-15] On the other hand, a large number of researchers hypothesize that if the mutation was detected earlier with the highly sensitive Sanger sequencing, the next generation sequencing or ddPCR, which are able to detect very low expression levels[16,17], this would be a prerequisite for early change of treatment strategy to the only effective TKI for these patients – ponatinib and better therapeutic results will be expected[18]. In our cohort of patients harbouring T315I mutation, the switch to ponatinib occurred only recently and the results are not ready to be reported. The analysis of the T315I mutation samples in our study was performed by ddPCR. The method is much cheaper and faster in the search for a particular mutation, but its most significant advantage is its much higher sensitivity (0.01%), which is significantly higher than that of Sanger sequencing (20%) and the next generation sequencing (1-3%).[5,6]

## CONCLUSIONS

Our results confirm the data from the accumulated world experience on the frequency of T315I mutation, as well as the unfavourable evolution, resistance to TKI treatment, and short survival in patients with CML T315I (+). ddPCR is a highly sensitive and informative method for early detection of genetic mutations, even at low levels of expression. Timely detection of the T315I mutation, immediately after failure of first-line TKI therapy in CML patients, provides a chance to choose effective treatment, improve their prognosis, and long-term survival.

## Author contributions

V.G-M. developed the concept, designed the study, took part in collecting data and samples, and wrote the manuscript. H.I., A.L., I.Zh., and V.S. took part in designing the study and performed the genetic tests for T315I mutation. Zh.G-P. and all co-authors critically revised the manuscript.

## Acknowledgements

The study was funded under Project No. BG05M2OP001-1.002-0005-C 01, Center for Competence "Personalized Innovative Medicine (PERIMED)", work package – 1,2,3; Funded by the Operational Program "Science and Education for Intelligent Growth" 2014-2020, co-financed by the European Union through the European Regional Development Fund.

## REFERENCES

1. Apperley JF. Part I: mechanisms of resistance to imatinib in chronic myeloid leukaemia. Lancet Oncol 2007; 8(11):1018–29.
2. Shah NP, Nicoll JM, Nagar B, et al. Multiple BCRABL kinase domain mutations confer polyclonal resistance to the tyrosine kinase inhibitor imatinib (STI571) in chronic phase and blast crisis chronic myeloid leukemia. Cancer Cell 2002; 2(2):117–25.
3. Khorashad JS, de Lavallade H, Apperley JF, et al. Finding of kinase domain mutations in patients with chronic phase chronic myeloid leukemia responding to imatinib may identify those at high risk of disease progression. J Clin Oncol 2008; 26(29):4806–13.
4. Hughes T, Saglio G, Branford S, et al. Impact of baseline BCR-ABL mutations on response to nilotinib in patients with chronic myeloid leukemia in chronic phase. J Clin Oncol 2009; 27(25):4204–10.
5. Soverini S, Bernardi S, Galimberti S. Molecular testing in CML between old and new methods: are we at a turning point? J Clin Med 2020; 9(12):3865.
6. Galimberti S, Guerrini F, Grassi S, et al. Digital droplet PCR is a fast and effective tool for detecting T315I mutation in chronic myeloid leukemia. EHA Library 2020:294653.
7. Sykes PJ, Neoh SH, Brisco MJ, et al. Quantitation of targets for PCR by use of limiting dilution. Biotechniques 1992; 13:444–9.
8. Kaplan EL, Meier P. Nonparametric estimation from incomplete observation. J Amer Statist Assoc 1958; 53(282):457–81.
9. Shi DY, Qin YZ, Lai YY. Variables associated with BCR-ABL kinase domain mutation in TKI-resistant patients with chronic myeloid leukemia. Zhonghua Xue Ye Xue Za Zhi 2020; 41(6):469–76.
10. Muller MC, Cortes JE, Kim DW, et al. Dasatinib treatment of chronic-phase chronic myeloid leukemia: analysis of responses according to pre-existing BCR-ABL mutations. Blood 2009; 114(24):4944–53.
11. Soverini S, Colarossi S, Gnani A, et al. Contribution of ABL kinase domain mutations to imatinib resistance in different subsets of Philadelphia-positive patients: by the GIMEMA Working Party on Chronic Myeloid Leukemia. Clin Cancer Res 2006; 12(24):7374–9.
12. Nicolini FE, Corm S, Le QH, et al. Mutation status and clinical outcome of 89 imatinib mesylate-resistant chronic myelogenous leukemia patients: a retrospective analysis from the French intergroup of CML (Fi-LMC GROUP). Leukemia 2006; 20(6):1061–6.
13. Branford S, Rudzki Z, Walsh S, et al. Detection of BCR-ABL mutations in patients with CML treated with imatinib is virtually always accompanied by clinical resistance, and mutations in the ATP phosphate-binding loop (P-loop) are associated with a poor prognosis. Blood 2003; 102(1):276–83.
14. Etienne G, Dulucq S, Huguet F, et al. Incidence and outcome of BCR-ABL mutated chronic myeloid leukemia patients who failed to tyrosine kinase inhibitors. Cancer Med 2019; 8(11):5173–82.
15. Jabbour E, Jones D, Kantarjian HM, et al. Long-term outcome of patients with chronic myeloid leukemia treated with second-generation tyrosine kinase inhibitors after imatinib failure is predicted by the in vitro sensitivity of BCR-ABL kinase domain mutations. Blood 2009; 114:2037–43.
16. Polakova KM, Kulvait V, Benesova A, et al. Next-generation deep sequencing improves detection of BCR-ABL1 kinase domain mutations emerging under tyrosine kinase inhibitor treatment of chronic myeloid leukemia patients in chronic phase. J Cancer Res Clin Oncol 2015; 141(5):887–99.
17. Soverini S, De Benedittis C, Castagnetti F, et al. In chronic myeloid leukemia patients on second-line tyrosine kinase inhibitor therapy, deep sequencing of BCR-ABL1 at the time of warning may allow sensitive detection of emerging drug-resistant mutants. BMC Cancer 2016; 16:572.
18. Cortes JE, Kim DW, Pinilla-Ibarz J, et al. Ponatinib efficacy and safety in Philadelphia chromosome-positive leukemia: final 5-year results of the phase 2 PACE trial. Blood 2018; 132(4):393–404.

# Клинические характеристики, развитие заболевания и выживаемость пациентов с хроническим миелоидным лейкозом, мутацией BCR-ABL1 (+) и T315I

Веселина Горанова-Маринова[1,4], Александр Линев[2,5], Христо Й. Иванов[2,5], Иван Желязков[2,5], Вили Стоянова[2,5], Жанет Грудева-Попова[3,4]

[1] *Секция гематологии, Первая кафедра внутренних болезней, Факультет медицины, Медицинский университет – Пловдив, Пловдив, Болгария*

[2] *Секция медицинской генетики, Кафедра педиатрии, Факультет медицины, Медицинский университет – Пловдив, Пловдив, Болгария*

[3] *Секция медицинской онкологии, Кафедра клинической онкологии, Факультет медицины, Медицински университет – Пловдив, Пловдив, Болгария*

[4] *Клиника клинической гематологии, УМБАЛ „Св. Георги", Пловдив, Болгария*

[5] *Отделение медицинской генетики, УМБАЛ „Св. Георги", Пловдив, Болгария*

**Адрес для корреспонденции:** Веселина Горанова-Маринова, Секция гематологии, Первая кафедра внутренних болезней, Факультет медицины, Медицинский университет – Пловдив, бул. "Васил Априлов" № 15А, Пловдив 4002, Болгария; E-mail: vesselina_goranova@yahoo.com; Тел.: +359 888 885 741

**Дата получения:** 19 января 2021 ♦ **Дата приемки:** 17 марта 2021 ♦ **Дата публикации:** 31 октября 2021

**Образец цитирования:** Goranova-Marinova V, Linev A, Ivanov HJ, Zhelyazkov I, Stoyanova V, Grudeva-Popova Zh. Clinical characteristics, disease evolution and survival in patients with chronic myeloid leukemia, BCR-ABL1 (+) and T315I mutation. Folia Med (Plovdiv) 2021;63(5):670-5. doi: 10.3897/folmed.63.e63366.

## Резюме

**Введение:** Мутация T315I у пациентов с хроническим миелолейкозом (ХМЛ) связана с терапевтической резистентностью и неблагоприятным прогнозом.

**Цель:** Изучить частоту мутации T315I у пациентов с ХМЛ, BCR-ABL (+), их клинические особенности, развитие заболевания и медианную выживаемость.

**Пациенты и методы:** Обследовано 75 пациентов с ХМЛ и BCR-ABL1 (+). Мутация T315I была обнаружена с помощью цифровой ПЦР в каплях, а BCR-ABL1 анализировался с помощью ОТ-ПЦР. Сравнительный анализ проводился на основании пола, возраста, фазы заболевания, группы риска, лечения, молекулярного ответа (МО) и медианы выживаемости у пациентов с T315I (+) и T315I (-).

**Результаты:** Мутация T315I обнаружена у 11 пациентов (14.7%). Не было обнаружено значительных различий в фазах, группах риска и базовом лечении. Значительно более высокое соотношение T315I (+) не достигло MR >3.5 log: 8 (72.7%) против 22 (34.4%) ($p$=0.023). Самые низкие средние уровни BCR-ABL1 были значительно выше в группе ХМЛ T315I (+) по сравнению с группой ХМЛ T315I (-) 12.1±6.0 против 3.77±1.28 ($p$=0.009). Средняя выживаемость пациентов с T315I (+) была значительно короче: 73 месяца по сравнению с 175 месяцами ($p$<0.0001, ДИ 95%).

**Заключение:** Наши данные подтверждают мировой опыт определения частоты мутаций T315I, включая неблагоприятное развитие, устойчивость к терапии TCI и короткую выживаемость. ddPCR является высокочувствительным методом раннего обнаружения генетических мутаций, позволяющий проводить эффективное лечение.

## Ключевые слова

ХМЛ, клиническая эволюция, мутация T315I