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Chronic Myeloid Leukemia in the Tyrosine Kinase Inhibitor Era: Incidence of Second Malignancies

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Abstract

The introduction of tyrosine kinase inhibitors (TKIs) as the first line treatment for chronic myeloid leukemia (CML) has substantially improved the outcome of patients with chronic phase disease. However, as this treatment could be maintained throughout life, it becomes relevant to assess the potential long-term side effects of this group of drugs, particularly the development of second malignancies (SMs). Therefore, we performed a retrospective study with the aim of evaluating the incidence and potential risk factors of SMs in CML patients treated with TKIs.

For this purpose, we reviewed the clinical files of the patients diagnosed with Philadelphia chromosome-positive (Ph+) CML between January 2005 and December 2019 at an University Hospital. We included patients diagnosed in chronic phase disease and treated only with TKIs. Clinical and therapeutic data were collected, and we compared the characteristics of patients that developed SMs with the characteristics of patients that did not develop SMs. Then, we calculated the Standardized incidence ratio (SIR) and analysed the impact of several factors in the development of second malignancies. Lastly, overall survival (OS) was assessed.

After a median follow-up of 6.4 years since the CML diagnosis, 11 out of 108 patients (10.1%) developed second malignancies, within a median time of 3.9 years since the diagnosis of CML. Nonmelanoma skin cancer and colorectal cancer were the most common SMs found. The observed risk of developing any secondary malignancy, excluding non-melanoma skin tumors, was higher than expected (SIR=2.35; 95% CI 2.06-2.67, p=0.001). Higher age at the time of diagnosis of CML and an intermediate or high risk Sokal Score were the only two factors in our study that associated with an increased risk of developing SMs. The development of SMs had no significant effect on OS.

Our results showed that CML patients treated with TKIs have a higher risk of developing second malignancies compared to the general Portuguese population. However, we found that cumulative incidence of SMs stabilizes over the follow-up time period, suggesting that this higher SM incidence may be related to other factors rather than to the TKI treatment itself. Moreover, no relevant correlations were found between the possible studied risk factors and the development of second malignancies that could explain the influence of TKIs in the higher SM incidence.

Resumo

A introdução dos inibidores de tirosina cinase (TKIs) como tratamento de primeira linha para a Leucemia Mieloide Crónica (LMC) melhorou substancialmente o prognóstico dos doentes com LMC em fase crónica. No entanto, uma vez que este tratamento pode ser mantido durante toda a vida, torna-se fundamental avaliar os potenciais efeitos adversos a longo prazo deste grupo farmacológico, nomeadamente o desenvolvimento de neoplasias secundárias (NSs). Assim, realizámos um estudo retrospetivo com o objetivo de avaliar a incidência e os potenciais fatores de risco de NS em doentes com LMC tratados com TKIs.

Para o efeito, foram revistos os processos clínicos dos doentes diagnosticados com LMC cromossoma Filadélfia positivo, entre janeiro de 2005 e dezembro de 2019, num Hospital Universitário. Foram incluídos doentes tratados apenas com TKIs e diagnosticados durante a fase crónica da doença. Começámos por realizar a recolha dos dados clínicos e terapêuticos dos doentes e descrevemos a população total de estudo e a população que desenvolveu neoplasias secundárias. De seguida, calculámos o risco de desenvolvimento de NS, comparando-o com o da população geral, e analisámos o possível impacto de vários fatores neste desenvolvimento. Por fim, calculámos a sobrevida global.

Após um follow-up mediano de 6.4 anos (95% IC: 5.7-7.2) desde o diagnóstico da LMC, 11 (10.1%) dos 108 doentes desenvolveram neoplasias secundárias, num tempo mediano de 3.9 anos. Entre as neoplasias secundárias encontradas, a neoplasia colorretal e neoplasias da pele não-melanomas foram as mais comuns. O risco de desenvolvimento de qualquer neoplasia secundária, excluindo tumores da pele não-melanomas, foi maior do que o esperado (SIR=2.35; 95% IC 2.06-2.67, p=0.001). No nosso estudo, a idade avançada ao diagnóstico da LMC e o Score de Sokal de risco intermédio ou elevado foram os únicos fatores que se associaram a um risco aumentado de desenvolvimento de NSs. O desenvolvimento de neoplasias secundárias não demonstrou ter impacto significativo na sobrevivência global.

Os resultados obtidos revelaram que os doentes com LMC tratados com TKIs apresentam um risco aumentado de desenvolver neoplasias secundárias, comparativamente com o risco da população geral portuguesa. No entanto, foi demonstrado que a incidência cumulativa de NSs estabilizava ao longo do tempo de follow-up, o que sugere que a alta incidência de NSs pode resultar de outros fatores e não do tratamento com TKIs. Além disso, não foram encontradas correlações relevantes entre os possíveis fatores de risco estudados e o desenvolvimento de neoplasias secundárias que nos permitissem esclarecer acerca da influência dos TKIs neste desenvolvimento.

Keywords

- Chronic Myeloid Leukemia
- Tyrosine Kinase Inhibitors
- Second malignancies
- BCR/ABL1
- Risk factors

Abbreviations

ABL - Abelson leukemia gene
BCR - Breakpoint Cluster Region gene
CCAs – Clonal Chromosomal Abnormalities
CCyR – Complete Cytogenetic Response
CHR – Complete Haematological Response
CML - Chronic Myeloid Leukemia
CP – Chronic Phase
CyR – Cytogenetic Response
DGS – Direção-Geral da Saúde
DMR – Deep Molecular Response
ELN - European Leukemia Net
EMA – European Medicine Agency
MMR – Major Molecular Response
MR – Molecular Response
OS – Overall Survival
Ph+ – Philadelphia Chromosome-Positive
Ph- – Philadelphia Chromosome-Negative
ROS - Reactive Oxygen Species
SIR – Standardized Incidence Rate
SMs - Second malignancies
TKIs - Tyrosine Kinase Inhibitors
α-IFN - Interferon Alpha
95% CI – 95% Confidence Interval

I. Introduction

Chronic Myeloid Leukemia (CML), with an incidence of 1 or 2 cases per 100.000 people a year, represents 15% of all leukemias [1]. CML is a myeloproliferative neoplasm hallmarked by the presence of a shortened chromosome 22 – Philadelphia Chromosome (Ph) – that arises in a haematopoietic stem cell, from a reciprocal translocation between two genes: the Abelson Leukemia gene (*ABL*) and the breakpoint cluster region gene (*BCR*), leading to the *BCR/ABL* fusion gene. The first is located on the chromosome 9q34, while the *BCR* resides on the chromosome 22q11.2. Depending on the breakpoints involved in the translocation, a variable segment of the *BCR* gene merges with a variable sequence of the *ABL* gene, resulting in different fusion transcripts (e1a2, e13a2, e14a2, e19a2). These products encode one of the various isoforms of a chimeric oncoprotein called BCR/ABL1 (p210, p190, p230) [2]. In more than 90% of CMLs, e13a2 and/or e14a2 transcripts are present, giving rise to the p210 BCR/ABL1 isoform. Regardless the isoform, this oncoprotein has a constitutive tyrosine kinase (TK) activity, leading to the activation of numerous molecular signalling pathways. The continuous TK activity dysregulates hematopoietic cells' behaviour, inhibits apoptosis and reduces cell adhesion capacity, prompting the constant activation of myeloproliferation. These mechanisms ultimately lead to malignant hematopoietic cell transformation and, consequently, to CML development [3]. According to the European Leukemia Net, CML is divided into three phases: chronic (CP), accelerated and blast[4].

The introduction of tyrosine kinase inhibitors (TKIs) as a targeted therapy for CML has dramatically changed the natural history and prognosis of patients diagnosed in CP. Thus, patients treated with TKIs are less likely to progress to accelerated and blast phases, and their overall survival (OS) is similar to the OS of the general population. Despite the excellent outcome, this increase in survival rates requires long-term observation of potential side-effects of TKIs. Recently, some studies have reported an increased incidence of second malignancies (SMs) among CML patients treated with TKIs. Nevertheless, they have failed to demonstrate whether this rise is caused by the therapy itself or due to the underlying disease (CML) [5–7]. In contrast, other studies have shown no increase in the incidence of second malignancies, compared to the incidence in the general population, raising some debate about the potential side effects of TKIs [8].

The first-generation TKI Imatinib was approved as the first-line treatment for CML in 2001 by the European Medicine Agency (EMA), and is the most widely used TKI. Currently, there are other TKIs approved for CML's treatment by the EMA: Dasatinib, Nilotinib, Bosutinib and Ponatinib [4]. By inhibiting tyrosine kinase activity, this group of drugs prevents cell proliferation, inducing tumoral regression and, eventually, remission. Current available data describe TKIs as a well-tolerated group of drugs, with a low risk of severe adverse effects [9].

However, preclinical data have evidenced an interaction between TKIs (mainly Imatinib) and the normal functioning of non-malignant cells. This should be taken into account for the study of possible adverse effects of TKIs, such as the development of second malignancies. Dendritic cells and T-lymphocytes are two cellular types whose functions are proven to be impaired by Imatinib. In the first ones, Imatinib promotes the inhibition of their differentiation, which will ultimately lead to a decrease in anti-tumoral immunity. In T-lymphocytes, this drug acts by arresting cells in checkpoint G0/G1 and thereby inhibiting their proliferation, which will strongly reduce the expansion of T cells when they need to respond to external peptides [10]. Also, it was reported that Imatinib interferes with DNA damage repair mechanisms and induces the emergence of clonal chromosomal abnormalities (CCAs) [11–13]. Ultimately, these events may play a relevant role in increasing the probability of SMs' development in patients treated with TKIs [14].

On the other hand, some studies suggest that CML itself may increase the risk of developing a secondary malignancy due to disease related factors. The fusion oncogene *BCR/ABL1* induces the production of reactive oxygen specimens (ROS). ROS promote DNA damage and genomic instability, which may increase the genetic susceptibility of these patients to acquire other malignancies besides CML. Additional chromosomal abnormalities are one of the effects of this genomic instability and they may itself contribute to enhance it, potentially playing a part in the development of second malignancies.

This study aims to examine the incidence of second malignancies among CML patients diagnosed on CP treated with TKIs and compare it to the incidence in the general Portuguese population. We also try to understand if there is any clinical or therapeutic feature that can contribute to the development of second malignancies. Lastly, we analyse and compare the overall survival rate of patients with and without SMs.

II. Materials and Methods

2.1 Research Design and Patient Selection

We performed a retrospective analysis among patients diagnosed with Chronic Phase Ph+ CML between January 2005 and December 2019 who were treated with one of the EMA's approved TKIs at Centro Hospitalar e Universitário de Coimbra. For this study we included patients only treated with TKIs. We excluded previously or concomitantly treated patients with any other drug, as well as patients submitted to an allogeneic stem cell transplantation. We also excluded patients that were in the accelerated or blast phase at the time of the diagnosis. The diagnoses of Ph+ CML and respective phases of the disease were performed according to the European Leukemia Net (ELN) [4].

2.2 Evaluation of Patients

Through the clinical files of CML patients, data were collected in order to characterize the population on demographics, co-morbidities, response to treatment, and SMs. Thus, the following variables were collected: Age, Sex, Date of CML's and SMs' diagnosis, histological type and site of SMs, stage of CML at SMs diagnosis, vital status, date and cause of death.

The following data regarding clinical features at CML diagnosis were also assessed: BCR/ABL (percentage), type of transcript, spleen distance to coastal grid (cm), complete blood counts, blasts cells, eosinophils and basophils in bone marrow (percentage), degree of bone marrow fibrosis, karyotype and clonal chromosomal abnormalities (if present). Using clinical and hematological data previously obtained, we calculated the following relative risk scores: SOKAL; EUTOS and ELTS Score.

Regarding treatment with TKI, the following data were collected: duration of treatment, first and second line TKI used, dose adjustment and motive, discontinuation of TKI and motive, additional mutations at the date of change and duration until achieving a Complete Hematological Response (CHR). Cytogenetic responses (CyR) and molecular responses (MR) were analyzed, according to ELN criteria, on the 3rd, 6th and 12th month of treatment with TKI. The best response achieved by each patient was collected before the last documented appointment/observation.

Lastly, detailed data on personal and known family history of cancer, as well as history of chemotherapy before the diagnosis of CML were assessed and collected.

2.3 Statistical Analysis

Statistical Analysis was performed using *IBM SPSS Statistics 24.* In order to better characterize our study population, we initially conducted a descriptive statistical analysis, calculating the central measures of each population variable. Standardized Incidence Ratio

(SIR) was then calculated according to the standard methodology [15] using data obtained from the National Program for Oncological Diseases by Direção-geral da Saúde (DGS) relative to the year 2010 [16]. Non-melanoma skin cancer was not included in the calculation of the SIR.

We divided our study population into two groups - patients with and without SMs - and sought to compare differences between them using non-parametric tests, namely Mann-Whitney U and Chi-square tests. Logistic regression was performed to assess the impact of various factors on the likelihood of developing SMs. Lastly, we used the Kaplan-Meier method to build survival curves, performed log rank tests to assess differences between the two groups, and used Cox Regression to analyze the effects of the development of SMs in OS. A *p* value < 0,05 was considered statistically significant.

III. Results

3.1 Study Population

Participants

For this study, we selected 162 clinical files of patients diagnosed with CML between January of 2005 and December of 2019. Of these patients, 33 were excluded (21 underwent concomitant treatment with interferon-alpha (α-IFN); 7 were on the blast phase at diagnosis of CML; 3 were on the accelerated phase at diagnosis of CML; 1 received Hydroxycarbamide as first line treatment for 2 years and 1 underwent an allogeneic stem cell transplantation). Of the remaining 129 patients we ruled out 17 patients due to lack of data and 4 patients that died within the first year of the CML's diagnosis, which resulted in a final study population of 108 patients.

Clinical characteristics

A total of 108 patients diagnosed with CML in CP treated with TKIs were enrolled in this study, including 65 males (60.2%) and 43 females (39.8%). The median age at diagnosis of CML was 55.5 years and the median follow-up time from the diagnosis of CML was 6.4 years (95% CI: 5.7; 7.2).

Cytogenetic analysis at diagnosis of CML was conducted in 107 patients, of which 98 showed a typical Ph+ karyotype (91.6%) – t(9;22)(q34;q11.2) – and 9 showed the presence of other chromosomal abnormalities, including 6 Ph variant translocations, 2 minor route CCAs and 1 CCA/Ph-. The type of transcript present at diagnosis of CML was identified in 91 patients. Typical transcripts, e14a2 and e13a2, appeared isolated in 42 (46.2%) and 35 (38.5%) patients, respectively, and together in 9 patients (9.9%). Of the remaining 5 patients (5.5%), 2 had a typical transcript in association with the atypical e1a2, and the other 3 showed distinct

atypical transcripts, namely e13a3, e8a2 and e19a2. Of the 105 patients in whom the type of fusion oncoprotein was identified, p210 BCR/ABL was present alone in 101 patients (96.2%) and paired with the p190 BCR/ABL in 3 patients (2.9%), with only 1 patient showing the p230 BCR/ABL fusion protein (1.0%). In 99 patients, the percentage of BCR/ABL1 at diagnosis was assessed, with a median of 105.0% (range: 27.5% - 486.0%).

At the time of diagnosis of CML, 13 patients out of the total of 108 had a personal history of cancer (12.0%), 3 had undergone chemotherapy in the past (2.8%) and 24 had a known family history of cancer (22.2%).

The main clinical characteristics of our study population are summarized in Table 1.

Treatment for CML

Of the 108 patients, 99 received Imatinib as first line treatment for CML (91.7%), 6 received Nilotinib (5.6%) and 3 received Dasatinib (2.8%), all in recommended standard doses. Dose adjustment was performed in 21 (18.4%) patients due to: inadequate response to treatment (*n*=14); hematological toxicity (*n*=3); gastro-intestinal adverse effects (*n*=3) or renal transplant (*n*=1). Hematological complete response was achieved in all patients with a median of 55 days (CI: 44.8; 65.2). The best response achieved with first line TKI was assessed in 106 patients with 69 achieving DMR (65.1%).

First line TKI was discontinued in 32 out of the 108 patients (29.6%) due to: treatment failure (*n*=16, 50.0%); STOP TKI (*n*=7, 21.9%); hematological toxicity (*n*=4, 12.5%); failure in therapeutic adherence (*n*=2; 6.3%); treatment intolerance (*n*=2; 6.3%) or chemotherapy initiation for SM (*n*=1; 3.1%). Research for the presence of mutations was conducted in 23 patients when the first therapeutic line was discontinued. Of these 23 patients, 10 had an altered mutational profile, all with distinct mutations.

Treatment with a second line TKI was introduced in 21 patients (19.4%), of whom 11 received Bosutinib, 5 received Dasatinib, 4 received Nilotinib and 1 received Ponatinib. The best response achieved with a second line TKI was assessed in all patients that initiated a second TKI, with 14 achieving DMR (66.7%).

The main therapeutic characteristics of our study population are summarized in Table 1.

Table 1 - Clinical and Therapeutic Characteristics of CML patients at diagnosis

		Total population, *n*=108
Sex, *n*	Male/Female	65/43
Age, years	Median (range)	55.5 (15.0-92.0)
BCR/ABL1, %	Median (range)	105.0 (27.5-486.0); *n*[1]=98
ACAs present, *n*	Yes/No	13/94; *n*[1]=107
HB level in PB, g/dL	Median (range)	12.2 (7.4-17.5)
WBC Count in PB, x10^9/L	Median (range)	72.9 (10.2-490.0)
PLT Count in PB, x10^9/L	Median (range)	331.0 (92.0-1354.0)
Eosinophils in PB, %	Median (range)	2.0 (0.0-13.6)
Basophils in PB, %	Median (range)	2.6 (0.0-11.0)
Blasts in BM, %	Median (range)	2.0 (0.0-8.1); *n*[1]=77
Eosinophils in BM, %	Median (range)	3.0 (0.0-17.0); *n*[1]=64
Basophils in BM, %	Median (range)	2.0 (0.0-9.0); *n*[1]=62
Splenomegaly, n	Yes/No	39/65 (*n*[1]=104)
Fibrosis = grade 2 or 3, *n*	Yes/No	6/43 (*n*[1]=49)
Personal history of cancer, *n*	Yes/No	13/95
Personal history of chemotherapy, *n*	Yes/No	3/105
Known family history of cancer, *n*	Yes/No	24/84
Sokal Score, *n*(%)	Low Risk	61 (58.7); *n*[1]=104
	Intermediate Risk	39 (37.5); *n*[1]=104
	High Risk	4 (3.8); *n*[1]=104
ELTS, *n*(%)	Low Risk	61 (58.7); *n*[1]=104
	Intermediate Risk	33 (31.7); *n*[1]=104
	High Risk	10 (9.6); *n*[1]=104
EUTOS, *n*(%)	Low Risk	101 (97.1); *n*[1]=104
	High Risk	3 (2.9); *n*[1]=104
First line TKI, *n*(%)	Imatinib	99 (91.7)
	Nilotinib	6 (5.6)
	Dasatinib	3 (2.8)
Total duration of treatment with TKIs, years	Median (95% CI)	6.1 (5.2-7.0)
Duration of first line TKI, years	Median (95% CI)	5.2 (4.4-6.0)
Duration of second line TKI, years	Median (95% CI)	4.9 (2.0-7.8)
Achievement of CHR, days	Median (95% CI)	55.0 (44.8-65.2)
Dose adjustment, *n*	Yes/No	21/87
DMR achieved with 1st line TKI, *n*	Yes/No	69/37 (*n*[1]=106)
Treatment discontinuation, *n*	Yes/No	32/76
Second line treatment, *n*	Yes/No	21/87

Abbreviations: CCAs = clonal chromosomal abnormalities; BM = bone marrow; CML = chronic myeloid leukemia; DMR = deep molecular response; HB = haemoglobin; PB = peripheral blood; PLT = platelets; SM = second malignancy; TKI = tyrosine kinase inhibitor; WBC = white blood cells.

[1]Due to lack of available data, this variable exhibits a different n value.

3.2 Second malignancies

After a median follow up of 6.4 years (95% CI: 5.7-7.2), 11 patients of 108 patients developed second malignancies (10.1%). The crude incidence rate of SMs was 1.42%. Standardized Incidence Ratio for second malignancies in our study population in comparison with the general Portuguese population was 2.35 (95% CI 2.06-2.67, *p*=0.001), as demonstrated in Table 2. As illustrated in Figure 1, the number of new second malignancies increased in the first 70 months (5.8 years) after CML's diagnosis. After month 73 (6.1 years), the cumulative curve is nearly flat, as almost no new second malignancy was diagnosed.

Table 2 - Standardized Incidence rate of second malignancies (excluding nonmelanoma skin cancer)

Cases	O	E	SIR (O/E)	95% CI for O/E	***p***
108	8	3.40	2.35	2.06-2.67	**0.001**
Abbreviations: E= expected; O = observed; SIR = standardized incidence ratio					

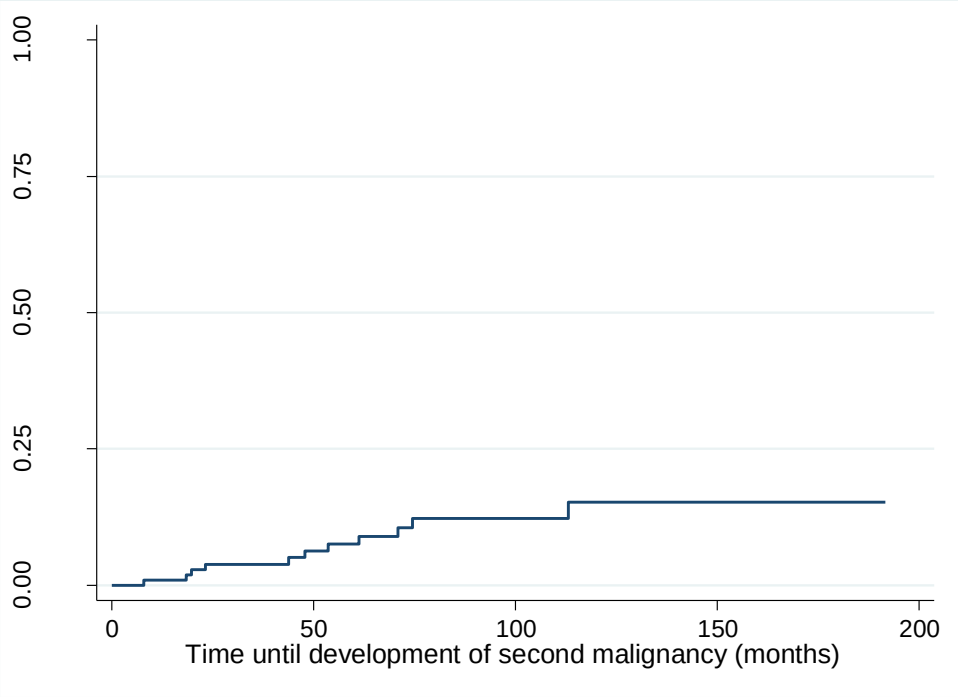


Figure 1 - Cumulative incidence of second malignancies

The incidence of second malignancies was higher in males. Of these 11 patients that developed SMs, 9 were male (13.8% of all male patients) and 2 were female (4.6% of all female patients). About 27.3% of these patients (n=3) had a personal history of cancer and 1 had been submitted to chemotherapy before the diagnosis of CML (9.1%). Two out of 11 had a known family history of cancer (18.2%). The median age at diagnosis of the first secondary malignancy was 67.0 years, while the median age of these 11 patients at diagnosis of CML was 62.0 years. The median time between diagnosis of CML and the diagnosis of a secondary malignancy was 3.9 years.

All 11 patients that developed second malignancies were treated with Imatinib as first-line therapy for CML, with only one having to perform dose adjustment after undergoing a kidney transplant procedure. Three out of these 11 patients discontinued Imatinib before the development of the secondary malignancy due to treatment failure (27.3%), changing to a second line TKI: Dasatinib (*n*=1), Nilotinib (*n*=1) and Ponatinib (*n*=1). Two other patients discontinued Imatinib after the diagnosis of SM: one due to chemotherapy initiation and the other because of the fulfilment of the criteria to STOP TKI. At the time of the diagnosis of the SM, 54.5% of the patients were in DMR (n=6).

Among these 11 patients with SMs, 3 developed skin cancer (27.3%), 3 developed colorectal cancer (27.3%) and the other 5 patients developed 5 different malignancies. One of them developed 2 other malignancies, first a colorectal cancer (adenocarcinoma) and later a basal cell carcinoma of the skin. The median time of follow-up since diagnosis of second malignancy was 2.0 years. Detailed information of the population that developed second malignancies is represented in Table 3.

Table 3- Characteristics of patients with Second Malignancies

		Patients with SMs, *n*=11
Sex, n (%)	Male	9 (81.8)
	Female	2 (18.2)
Age at diagnosis of SM, years	Median (range)	67.0 (57.0-81.0)
Age at diagnosis of CML, years	Median (range)	62.0 (53.0-80.0)
Time from CML's diagnosis to SM's diagnosis, years	Median (95% CI)	3.9 (1.2-6.6)
Type of second malignancy, n(%)	Adenocarcinoma	4 (36.4)
	Basal cell carcinoma	2 (18.2)
	Squamous cell carcinoma	1 (9.1)
	Infiltrating ductal carcinoma	1 (9.1)
	Papillary thyroid carcinoma	1 (9.1)
	Papillary renal cell carcinoma	1 (9.1)
	Unknown	1 (9.1)
Site of second malignancy, n(%)	Skin	3 (27.3)
	Colon or Rectum	3 (27.3)
	Prostate	1 (9.1)
	Thyroid	1 (9.1)
	Breast	1 (9.1)
	Kidney	1 (9.1)
	Brain	1 (9.1)
Therapeutic line for CML at diagnosis of SM, n(%)	First-line	8 (72.7)
	Second-line	3 (27.3)
DMR achieved at diagnosis of SM, n(%)	Yes	6 (54.5)
	No	5 (45.5)
Abbreviations: CCAs = clonal chromosomal abnormalities; BM = bone marrow; CML = chronic myeloid leukemia; DMR= deep molecular response; PB = peripheral blood; SM= second malignancy WBC = white blood cells.		
[1]Due to lack of available data, this variable exhibits a different n value.		

The differences in clinical and therapeutic parameters between patients with and without second malignancies, as well as the median values of these variables for each group of patients are shown in Table 4. The median age at CML diagnosis for patients who developed SMs was significantly higher than for those who did not develop other malignancies (62 vs 54 years; p=0.032). There was no statistically significant difference on sex (*p*=0.122), personal history of cancer (*p*=0.101) or chemotherapy (*p*=0.179) between each group. There were no significant differences in all blood count levels and bone marrow levels between the two groups.

Also, no statistically significant differences were obtained on duration of treatment with TKIs (76 vs 73 months; *p*=0.509), neither on time until achievement of a complete hematological response (*p*=0.622) between patients that developed SMs and patients who did not developed other neoplasms.

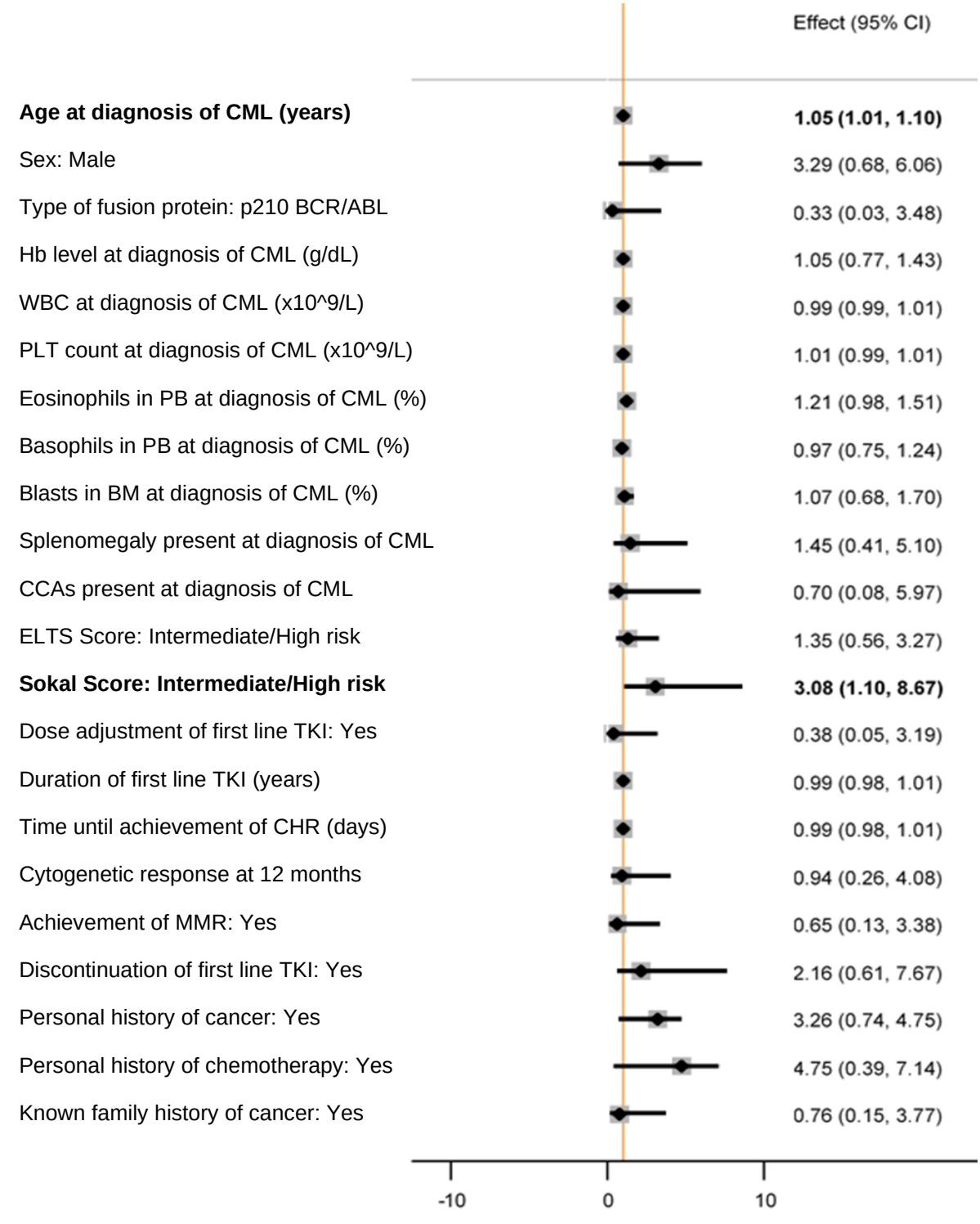
Only two of the independent variables showed statistically significant *p* values as predictors of the development of SMs: Age at diagnosis of CML (OR 1.05; *p*= 0.044; 95%CI: 1.01-1.10) and intermediate/high risk Sokal Score. Patients with an intermediate/high risk Sokal Score were associated with a 3.08-fold higher risk of developing second malignancies (*p*= 0.033; 95%CI: 1.10-8.67). Other variables were associated with a higher risk of SM's development, but not statistically significant: Personal history of cancer (OR=3.26); personal history of chemotherapy (OR=4.75); and first-line TKI discontinuation (OR=2.16). Although not statistically significant, the presence of CCAs at CML's diagnosis was associated with a lower risk (OR=0.7) of SMs' development. These results are illustrated in Figure 2.

Table 4 - Association of the Clinical and Therapeutic Parameters with the development of Second malignancies

		Patients With SM, *n*=11	Patients Without SM, *n*=97	*p*
Sex, *n*	Male/Female	9/2	56/41	0.122
Age at diagnosis of CML, years	Median (range)	62.0 (53.0-80.0)	54.0 (15.0-90.0)	**0.028**
BCR/ABL1 at Diagnosis of CML, %	Median (range)	117.0 (59.2-166.1); *n*[1]=9	99.4 (27.5-486.0); *n*[1]=90	0.301
ACAs present at Diagnosis of CML, *n*	Yes/No	1/10	12/84 (n=96)[1]	0.743
HB level in PB at Diagnosis of CML, g/dL	Median (range)	12.3 (9.0-15.8)	12.1 (7.4-17.5)	0.699
WBC Count in PB at Diagnosis of CML, x10^9/L	Median (range)	46.4 (20.4-360.5)	73.3 (10.2-490.0)	0.471
PLT Count in PB at Diagnosis of CML, x10^9/L	Median (range)	299.0 (114.0-1354.0)	335.0 (92.0-1345.0)	0.831
Eosinophils in PB at Diagnosis of CML, %	Median (range)	3.9 (0.0-10.0)	2.0 (0.0-13.6)	0.072
Basophils in PB at Diagnosis of CML, %	Median (range)	2.0 (0.0-7.0)	2.6 (0.0-11.0)	0.972
Blasts in BM at Diagnosis of CML, %	Median (range)	2.8 (1.0-4.0); *n*[1]=*8*	2.0 (0.0-8.1); *n*[1]=69	0.462
Eosinophils in BM at Diagnosis of CML, %	Median (range)	3.5 (1.0-14.0); *n*[1]=6	3.0 (0.0-17.0); *n*[1]=58	0.88
Basophils in BM at Diagnosis of CML, %	Median (range)	2.0 (0.0-4.0); *n*[1]=5	2.0 (0.0-9.0); *n*[1]=57	0.646
Splenomegaly at Diagnosis of CML, *n*	Yes/No	5/6	34/59 (*n*=93)[1]	0.564
Fibrosis = grade 2 or 3 at Diagnosis of CML, *n*	Yes/No	0/6 (*n*=6)[1]	6/37 (*n*=43)[1]	0.329
Personal history of cancer, *n*	Yes/No	3/8	10/87	0.101
Personal history of chemotherapy, *n*	Yes/No	1/10	2/95	0.179
Total duration of TKI treatment, years	Median (95%CI)	6.3 (3.9-8.6)	6.1 (5.2-7.0)	0.509
Duration of first line TKI, years	Median (95%CI)	5.2 (4.4-6.0)	4.6 (0.0-9.6)	0,443
Time until achievement of CHR, days	Median (95%CI)	42.0 (26.9-57.1)	56.0 (47.0-65.0)	0,622
BCR/ABL at 3 months of TKI therapy, %	Median (range)	1.9 (0.9-6.9); *n*[1]=7	2.9 (0.01-181.0); *n*[1]=87	0,235
BCR/ABL at 6 months of TKI therapy, %	Median (range)	0.08 (0.0-3.9); *n*[1]=9	0.3 (0.0-137.0); *n*[1]=91	0,399
BCR/ABL at 12 months of TKI therapy, %	Median (range)	0.01 (0.0-8.1); *n*[1]=7	0.06 (0.0-96.0); *n*[1]=93	0,313

Abbreviations: CCAs = clonal chromosomal abnormalities; BM = bone marrow; CHR= complete haematological response; CML = chronic myeloid leukaemia; HB = haemoglobin; PB = peripheral blood; PLT = platelets; SM = second malignancy; TKI = tyrosine kinase inhibitor; WBC = white blood cells.

[1]Due to lack of available data, this variable exhibits a different n value.



Abbreviations: CCAs = clonal chromosomal abnormalities; CHR = complete haematological response; BM bone marrow; CML = chronic myeloid leukaemia; Hb = haemoglobin; MMR = major molecular response; PB = peripheral blood; PLT = platelets; TKI = tyrosine kinase inhibitors; WBC = white blood cells.

Figure 2 - Predictors of the development of second malignancies

3.4 Overall survival

At the time of the collection of the data, 88% of the patients were alive (n=95), and 13 were deceased (12%). One patient died due to the progression of CML and only 2 of these patients died of SM. It was not possible to determine the cause of death for the remaining 10 patients.

The medians of OS could not be reached neither in patients with and without SM nor in the total population (Figure 4 and 5). In the total cohort we obtained the following results: OS at 5 years of 93.1% (95%CI 86.0-96.7%); OS at 10 years of 86.8% (95%CI 75.7-93.1%). The difference in the OS of CML patients treated with TKIs with and without SM was not statistically significant (log rank test, p=0,384). The development of SMs had no significant effect on OS (HR 1.94; p=0.392; 95%CI 0.42-8.87).

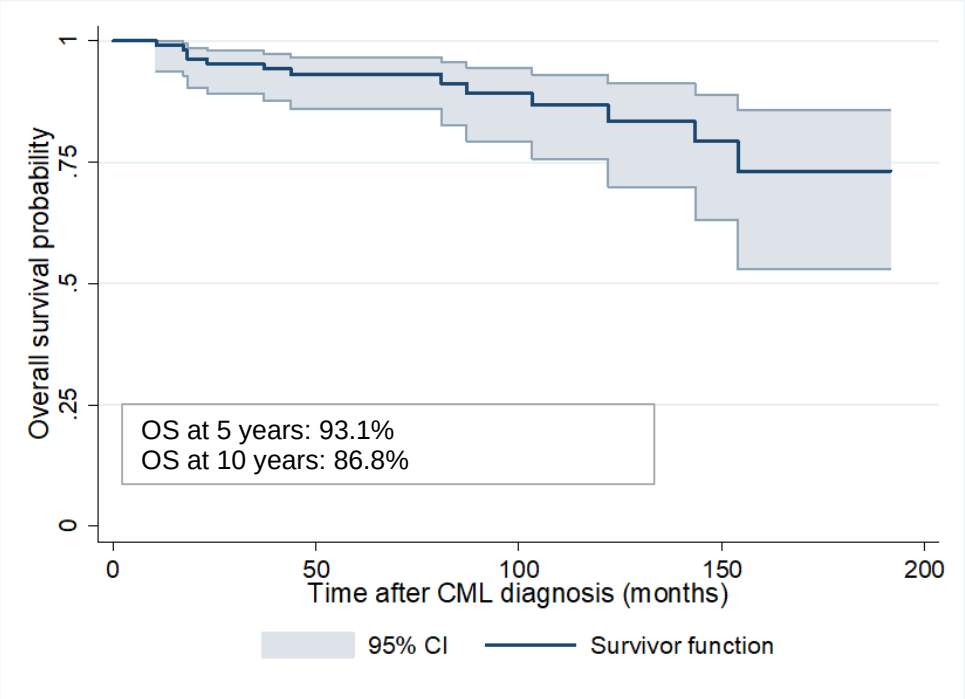


Figure 3 - Overall Survival of the studied CML population

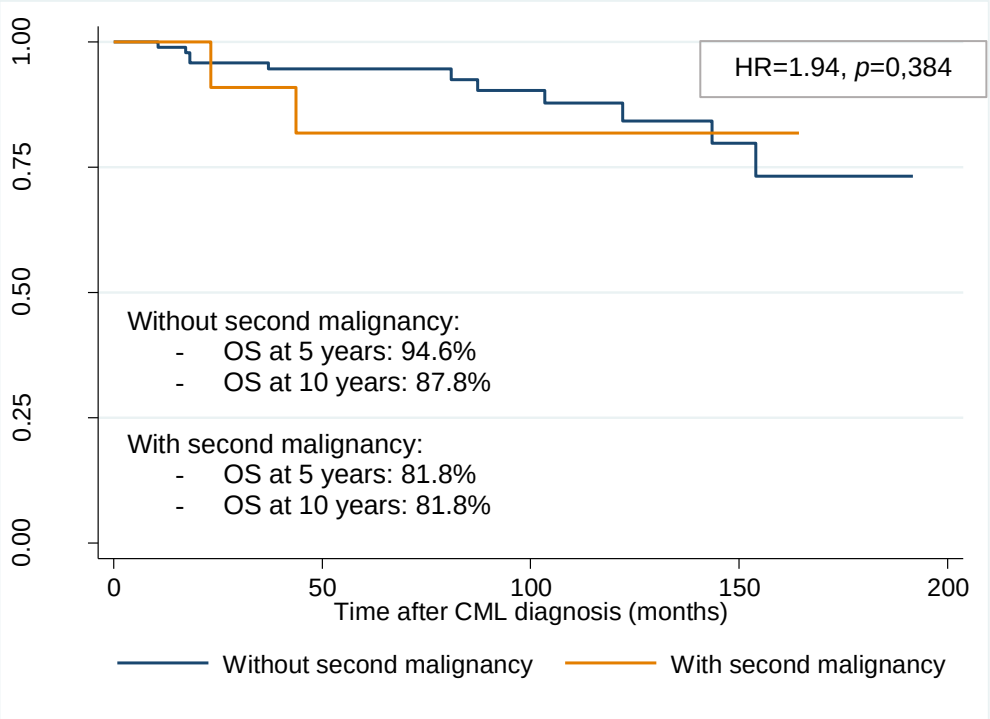


Figure 4 - Overall survival of CML patients with (Blue lines) and without (Yellow lines) second malignancies

IV. Discussion

The introduction of TKIs as first line treatment for Chronic Myeloid Leukemia has revolutionized CML's prognosis, significantly prolonging the overall survival of CML patients. However, given the fact that CML patients receive treatment with TKIs for long periods of time, it becomes extremely relevant to assess the potential long-term side effects of this group of drugs. The development of second malignancies in CML patients has been studied as one of the potential complications of TKIs. Although increased incidences of SMs were found in some studies, the results are controversial and it has not yet been possible to demonstrate a direct causal relationship with the TKIs treatment [17]. Further, what is known to us, this study has not yet been carried out in Portugal. In the present study, we assessed the incidence of second malignancies in 108 CML patients in CP treated only with TKIs. After a median follow-up of 6.4 years we found that these patients have a 2.35-fold higher risk of developing SMs as compared to the general Portuguese population (SIR=2.35; 95%CI: 2.06-2.67, *p*=0.001). For this calculation we excluded the nonmelanoma skin cancer found in our study.

Several previous studies have also reported increased incidences of second malignancies in CML patients. In conformity with the present study, Xiu-Feng Yin *et al.* reported that the incidence rate of SMs in 223 patients with CML treated with TKIs was 2.45-fold higher than in the general Chinese population. Larger population reports also demonstrated a higher risk of

SM's development, but they showed a lower incidence of SMs when compared to the present study [6,7]. The reason for these discordant results may be related to the methodology used to calculate the SIR and to the sample size of each study. Gunnarsson N *et al.* reported a 1.52-fold increased risk of SMs occurrence, based on a sample of 868 patients followed by a median time of 3.7 years. Similarly, Kumar V *et al.* studied 9,200 CML patients with a median follow-up of 4.2 years and obtained a 1.30-fold higher risk of developing SMs. One the other hand, other reports did not demonstrate higher incidences of SMs in CML patients compared to the expected ones in the respective general population [9,18]. In fact, Verma D *et al.* even suggested a lower than expected rate of second malignancies in CML patients treated with TKIs (SIR=0.60). Interestingly, they found that the incidences of some malignancies were higher than the expected when SIR was assessed by cancer sites [19]. Nevertheless, the follow-up periods of all studies mentioned above were different than ours, which may explain the divergence between the results obtained. It should also be considered that none of the studies used the same exclusion criteria.

Considering the limited number of individual cancers, we could not separately compare the relative incidences for each type of malignancy found with the expected incidences for those malignancies in the general population. However, colorectal and nonmelanoma skin cancer were the most common types found in our study, accounting for 27.3% each. It must be considered that these two types of cancers are among the most prevalent types of malignancies in Portugal and both are directly related with age [16,21]. This result is concordant with other reports, which also showed high incidences of these two types of malignancies [17,20]. In contrast, several studies reported prostate cancer as one of the most incidents in CML patients [6,8]. However, our study detected only one patient developing this malignancy. These conflicting results on the pattern of SMs can be explained by differences in several personal factors, which influence the risk of developing certain types of malignancies, such as ethnicity and lifestyle.

A higher age at diagnosis of CML and an intermediate or high risk Sokal Score were the only two factors in our analyses proven to be associated with the development of SMs. In their study, Gugliotta G *et al.* [22] also reported a significant association between these two factors and the development of second malignancies. Regarding age at diagnosis of CML (with SMs vs. without SMs: 62.0 vs 54.0 years), we can justify its influence on the development of SMs since age by itself increases the risk of cancer development. Moreover, the low odds ratio value obtained (OR=1.05) shows, with a high probability, that this factor will not meaningfully influence SMs' development. As for Sokal Score (OR=3.08), its calculation is based on clinical and laboratory parameters: spleen size, platelet count, percentage of myeloblasts in peripheral blood and age at diagnosis of CML. A higher age at CML's diagnosis can help to justify the predictive value acquired from this relative score [22]. Also, the wide confidence interval

obtained, reflecting the small sample size, gives us little reliability on the predictive effect of this score. However, one must consider that this 3.08-fold increased risk obtained might suggest that the development of SMs is more associated with disease's or patient's related factors, rather to be associated with TKIs.

In order to assess if TKI confers a higher risk of SMs occurrence, some studies compared the incidence of SMs between patients treated in pre and post-TKI eras, and found significantly higher rates of second cancers within the post-TKI population [23]. However, since the overall survival of CML patients treated in the pre-TKI era is significantly lower than the OS observed in patients treated in the post-TKI era, it is difficult to establish a reliable comparison of the incidence of SMs between these two groups of patients. Although we have not collected data from CML patients treated in the pre-TKI era, we report some results on therapeutic features that do not point to a relationship between TKIs and SMs. If treatment with TKIs increased the risk of development of second cancers, we would expect our study to find significant differences in duration of treatment with TKIs between patients with and without SMs, which did not occur (76 vs 73 months; p=0.509). Moreover, we reported a cumulative incidence of SMs that stabilized over the follow-up time, instead of increasing, as would be expected since our follow-up time corresponds roughly to TKI exposure time. This result is consistent with other studies. Gunnarsson N *et al.* showed that the SIR was numerically similar (1.58 vs. 1.47), before and after the patients had reached 2 years since CML's diagnosis. However, in this study, the median time from CML's diagnosis to SMs' diagnosis was lower than the one found in our study (2.0 vs 3.9 years, respectively).

On the other hand, some studies propose that CML itself, due to its genomic instability and, consequently genetic susceptibility, might play a role in the increased risk of SMs [5,17]. Several reports have already proven that *BCR/ABL1* fusion oncogene induces an overproduction of reactive oxygen specimens, leading to higher rates of DNA damage in CML cells. Since DNA repair mechanisms also appear to be impaired by *BCR/ABL1*, this DNA damage will likely turn into mutations and cytogenetic abnormalities, which consequently will enhance genomic instability [2,24]. However, in our study, patients with SMs did not show a higher rate of CCAs or percentage of BCR/ABL1 at the diagnosis of CML, when compared with patients that did not develop SMs.

There are multiple other factors that may contribute to the increased risk of SMs, namely external factors (habits, diet, environmental exposures, etc.), host factors (genetic, immune, etc.) or a combination of innate and acquired risk factors. Although not statistically significant, we did report a positive predictive effect of the personal history of cancer in the development of SMs, which might be explained by an increased inborn genetic susceptibility to develop malignancies. Personal history of chemotherapy also showed a positive, but not statistically significant, effect on the development of SMs. However, this association is already well-known

and proven in literature [25]. Ultimately, one must also consider that the close monitoring of these patients throughout their lives probably contributes to the increased incidence of second malignancies, and even chance might play a role in this increase in CML patients.

The overall survival curves obtained in our study verify the previously reported high survival rates of CML patients treated with TKIs, with an overall survival of 91.3% (95%CI 86.0-96.7%) at 5 years and of 86.8% (95%CI 75.7-93.1%) at 10 years [26]. The OS obtained at 5 and 10 years after CML diagnosis, for patients with SMs, was lower than the one found for patients without SMs. However, these results were not statistically significant, which may be due to the small sample size. In their study, Miranda MB *et al.* did not show an increased incidence of SMs, but reported an unfavorable impact of second malignancies on overall survival and progression-free survival. [8] Gugliotta G *et al.* also reported a particularly high death rate among patients with SMs (53%), with a short median OS (18 months since CML's diagnosis).

We are thoroughly aware of the potential limitations of our study. The lack of a comparative group of patients treated for CML in the pre-TKI era prevents us from establishing a causal relationship between this group of drugs and the development of second malignancies. In addition, due to the small sample size of patients, particularly those who developed second malignancies, we were unable to find relevant connections between second malignancies and the potential risk factors analyzed, as well as to determine the SIRs for each specific malignancy that occurred in our sample. Therefore, a study with a larger sample, and that includes patients treated in the pre-TKI era, is required in order to better clarify the incidence of second malignancies in CML patients as well as its potential risk factors.

To our knowledge, this is the first study to evaluate the risk of SMs' development in patients with CML, and treated exclusively with TKIs, in Portugal. Strengths of the present study include the population-based design, and the long-term follow-up of our sample.

In conclusion, our study suggests that patients with CML treated with TKIs are at a higher risk of developing second malignancies. However, the cause of this increased risk remains unclear. The development of second malignancies in CML patients can have significant clinical implications, including early death. Therefore, we suggest that doctors who follow CML patients should be encouraged to pay attention to signs and symptoms that might suggest the presence of other malignancies.

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