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VALUTAZIONE DEL MANAGEMENT DEL PAZIENTE AFFETTO DA LEUCEMIA MIELOIDE CRONICA NELLA PRATICA CLINICA DELL'AZIENDA ULSS 2 MARCA TREVIGIANA, DISTRETTO DI TREVISO

EVALUATION OF THE MANAGEMENT OF PATIENTS AFFECTED BY CHRONIC MYELOID LEUKEMIA IN THE CLINICAL PRACTICE OF THE HEALTH AUTHORITY n.2 MARCA TREVIGIANA, DISTRIC OF TREVISO

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SUMMARY

LIST OF ABBREVIATIONS

ABL= Abelson murine Leukemia

ACA= Additional Chromosomal Alterations

AE= Adverse Effect

AIFA= Italian Agency of Medicines (Agenzia Italiana del Farmaco)

AP= Accelerated Phase

ARA-C = Cytarabine

BC= Blast Crisis

BCR= Breakpoint Cluster Region

CCyR=Complete Cytogenetic Response

CHR=Complete Hematologic Response

CML= Chronic Myeloid Leukemia

CMR= Complete Molecular Response

CP = Chronic Phase

FISH= Fluorescence In Situ Hybridization

FFS= Failure Free Survival

HSCT= Hematopoietic Stem Cell Transplantation

HU= Hydroxyurea

IFNα = Interferon alpha

LHA= Local Health Authority

MCyR= Major Cytogenetic Response

MMR= Major Molecular Response

NHS= National Healthcare System

OCT1= Organic Cation Transporter 1

OS= Overall Survival

PAOD= Peripheral Arterial Occlusive Disease

PFS= Progression Free Survival

Ph= Philadelphia

PPRs= Physicians' Patient Records

RCT= Randomized Control Trial

RQ-PCR= Reverse Transcriptase Quantitative Polymerase Chain Reaction

SE= Standard Error

TKI= Tyrosine Kinase Inhibitor

ABSTRACT

Introduction

Chronic myeloid leukemia (CML) is a myelodysplastic neoplasia accounting for around 15% of all cases of leukemia in adults. CML treatment is mainly based on tyrosine kinase inhibitors (TKIs). Current TKIs approved as first line treatments are imatinib or second-generation TKIs such as dasatinib and nilotinib. Overall, TKIs have modified the natural progression of CML, prolonging survival. As CML has progressively switched from a fatal to a "chronic" pathology, time-limited clinical trials may not evaluate such long-term outcomes adequately. On the brink of the commercialization of generic imatinib, this thesis examines a decade of CML management in the real clinical practice, focusing on treatment effectiveness, therapeutic switches, and adverse effects (AEs).

Methods

A retrospective cohort study was performed on all CML patients followed up in the Local Health Authority of Treviso (Region of Veneto, Italy) between 2005 and 2015. Data were captured integrating both administrative databases and physicians' patient record. The therapeutic pattern was evaluated separately according to CML phase at diagnosis, considering frontline treatments, occurrence of treatment switches and their causes. For patients diagnosed in chronic phase (CP), the effectiveness of different frontline TKIs was assessed using an intention-to-treat approach, considering the achievement of complete hematologic, cytogenetic and molecular response. Occurrence of AEs among different frontline TKIs treatments was compared. All data and statistical analysis were performed using the software STATA version 14.

Results

A total cohort of 119 CML patients was examined; the majority of them were diagnosed in CP (n=97). 60% of subjects were asymptomatic at diagnosis; even when present, symptoms were mainly unspecific. Imatinib was the most common first line treatment for CP subjects (n=73); among second generation TKIs, only nilotinib was used as first line. Nilotinib proved more effective than imatinib, both considering achievement of responses and treatment switches; 28 CP-CML patients with frontline imatinib needed to switch, mainly due to intolerance but there were no therapeutic switches in patients with frontline nilotinib. AEs were common; in particular, osteoarticular pain was significantly more frequent for imatinib compared to nilotinib (50 out of 73 vs 2 out of 8, respectively; p=0.02).

Conclusion

Although based on a small population, this study shows the importance of choosing the most appropriate frontline treatment, in order to allow rapid disease control. Results indicate a superiority of nilotinib as first line therapy for CP-CML, both in terms of effectiveness and of treatment switches and AEs occurrence. While this might be seen as an argument to use nilotinib first line, it might also argue strongly for the continued use of imatinib first line, reserving nilotinib for imatinib intolerant or resistant patients. AEs remain a major concern, highlighting the importance of close monitoring of patients. A full health economic evaluation is required to determine the most cost effective care pathways using these expensive drugs.

1. INTRODUCTION

1.1. Definition of Chronic Myeloid Leukemia

The term leukemia indicates a group of clonal bone marrow malignancies characterized by an increase in the numbers of abnormal white blood cells [1].

Different forms of leukemia exist, according to the type of white blood cells involved. The four main types of leukemia include acute lymphoblastic leukemia, acute myeloid leukemia, chronic lymphocytic leukemia and chronic myeloid leukemia (CML); other less common kinds of leukemia are known, as well [2].

In particular, CML is characterized by a deregulated overproduction of myeloid cells by the bone marrow, with a subsequent increase in the concentration of both mature granulocytes -neutrophils, eosinophils and basophils- and their precursors in the blood circulation.

1.2. Epidemiology of CML: incidence, prevalence and survival

CML accounts for around 15% of all cases of leukemia in adults [3,4], and presents a crude annual incidence of 0.7 – 1.0 cases per 100,000 people [5]. The incidence is far lower among children, and is estimated to be around 0.6–1.2 cases per 1,000,000 children/year [5].

Data coming from different European registries show a correlation between increasing age and CML incidence, with a median age at onset estimated around 57- 60 years and a male/female ratio of 1.2 – 1.7 [5] **(figure 1.1)**.

No variation in CML incidence has been detected over time; on the other hand, the prevalence of CML has significantly increased and is now estimated to be 10 –

12 cases/100,000 people [5-7] **(figure 1.2)**. Such an increase in prevalence is mainly attributable to changes in therapeutic management of CML, that have massively prolonged patients' overall survival (OS). Before the '90s, in fact, only 20-40% of patients affected by CML remained alive at 5 years from diagnosis [8], with more than 85% of patients dying within the first 8 years from CML onset [9].

With the advent of interferon-α (IFNα)-based therapy and allogeneic hematopoietic stem cell transplantation (HSCT) in the '80s, the 8-years survival rapidly improved, reaching 65% in 2000 [9].

However, it was the introduction of Tyrosine Kinase Inhibitors (TKI)-based therapies, in 2001, that allowed the greatest improvement in CML prognosis, reaching an 8-year survival that is now estimated around 87% and that continues to improve, thanks to the use of second- and third-generation TKIs [9].

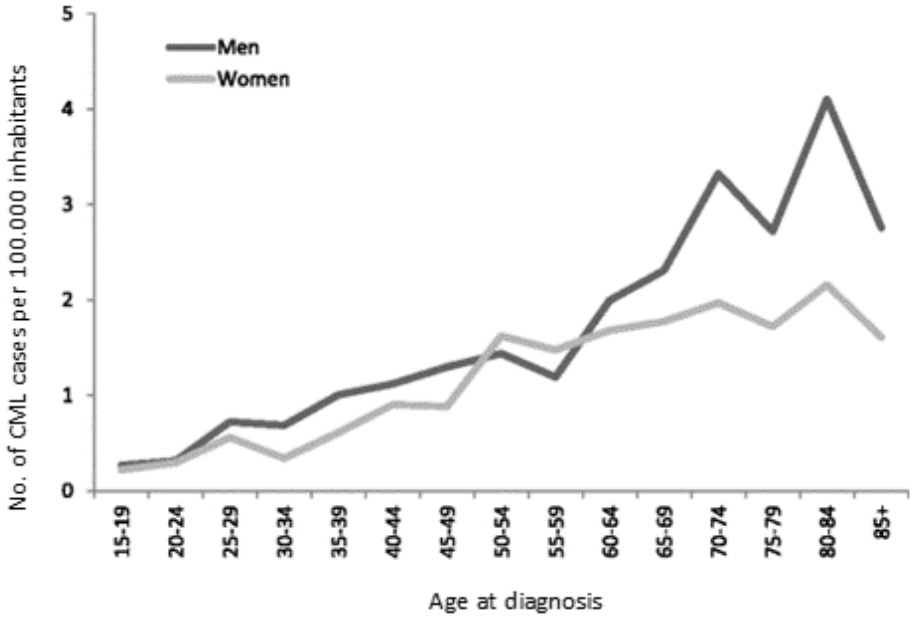

Figure 1.1: Trend of annual incidence of CML among different age groups. Data are obtained from the Swedish Cancer Registry for the years 2002 – 2012 (www.socialstyrelsen.se/register/halsodataregister/cancerregistret/inenglish) [5]

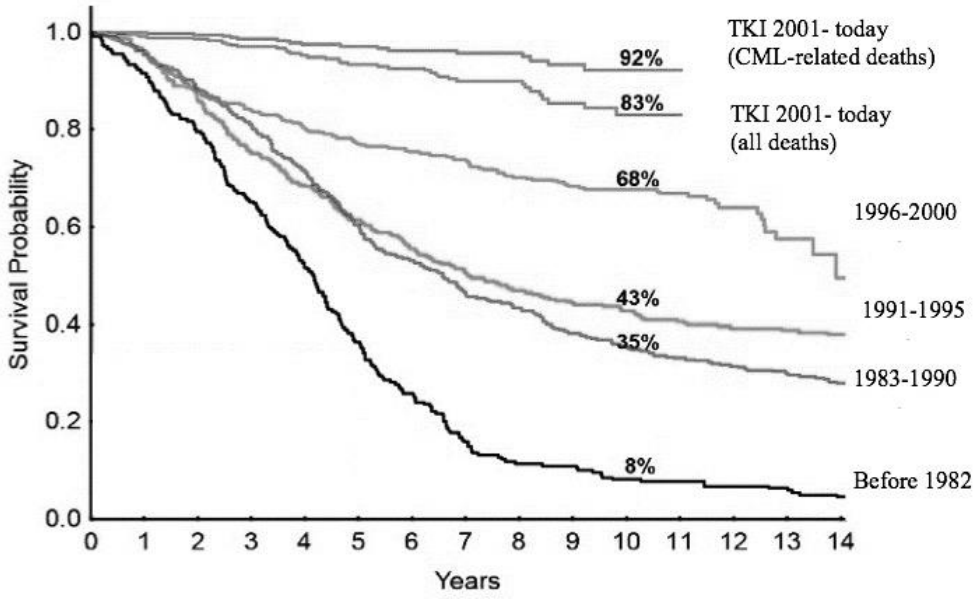

Figure 1.2: Changes in survival of patients with CML over years: the German CML-Study Group experience [10]

1.3. Pathogenesis and risk factors

Both patients' related and environmental risk factors have been associated with the onset of CML. Among unmodifiable patient's related characteristics, male sex is a well-known risk factors, although the clear reason for its association with CML onset is still unclear [11]. History of previous malignancies has been associated to an increased risk of CML, as well, probably in force of a personal enhanced susceptibility towards neoplastic disorders [12]. Considering compartmental modifiable risk factors, attitude and intensity of smoking, as well as poor physical activity, have been positively associated with higher risk of CML onset [13]. Among environmental factors, exposition to ionizing radiation has been detected as one of the major causes of CML, based on observations on survivors of the nuclear bombing [11].

Independently for the causal risk factor, unlike many other malignancies that can originate from the accumulation of different mutations, CML is invariably related to a unique type of mutation, i.e. the BCR-ABL fusion gene on the abnormal Philadelphia (Ph) chromosome. The Ph chromosome was first discovered and described in 1959 by David A. Hungerford from Fox Chase Cancer Center (then the Institute for Cancer Research) and Peter Nowell from the University of Pennsylvania School of Medicine and was therefore named after the city in which both facilities are located.[14].

This abnormal chromosome originates from the reciprocal translocation t(9;22)(q34;q11) between chromosome 9 and 22 [15]. In particular, the upstream part of the BCR gene located on chromosome 22 fuses to the downstream part of the ABL gene located on chromosome 9 [16], therefore creating a new fusion gene named BCR-ABL **(figure 1.3)**.

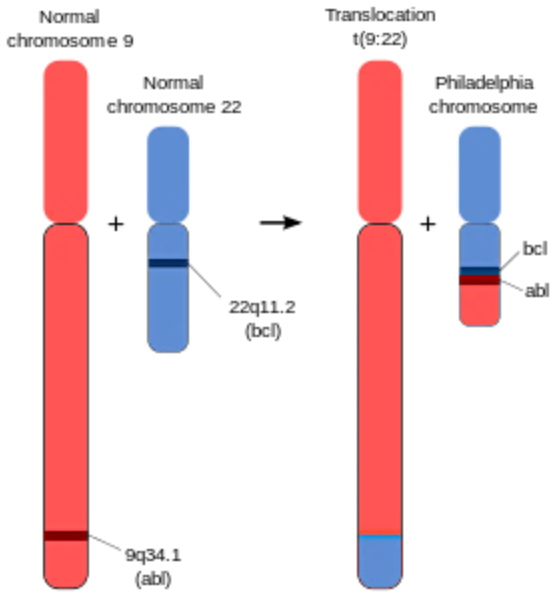

Figure 1.3: The chromosomal translocation and the generation of the Philadelphia chromosome [17]

1.3.1. Physiological role of Abl and Bcr proteins

Physiologically, the ABL gene encodes for a non-receptor tyrosine kinase, whose activity is carried by the SH1 domain [18]. This kinase can have both cytoplasmic and nuclear localization. In the cytoplasm, Abl mediates functions including integrin signaling and cytoskeletal molding, whereas in the nucleus it is involved in the regulation of the cell cycle and in responses to genotoxic stress [19-22].

Normally, the kinase activity of Abl is strictly controlled, through motifs in the N-terminal region of the protein that tightly regulate its phosphorylation. Loss of this N-terminal region – as occurs in the Bcr-Abl fusion protein- results in an uncontrolled constitutively activated kinase activity with a strong oncogenic potential [23-26].

Like the Abl protein, also the Bcr protein physiologically has both cytoplasmic and nuclear localization [27], and it possesses a variety of active motifs involved in phosphorylation and guanosine triphosphate binding [28-32].

In particular, Bcr interacts with G proteins and with xeroderma pigmentosum gene products, therefore being probably involved in intracellular signaling, cytoskeletal organization, cell growth, and normal development, as well as in DNA repair mechanisms [29-31].

The first exon of the BCR gene encodes for one of the most important parts of the Bcr protein. This exon encodes for a serine and threonine kinase, which can mediate both auto- and allo-phosphorilation, therefore, being involved also in signal propagation. This exon appears to be of extreme importance, since it is the only

exon that is always present in the Bcr-Abl proteins and it is considered pivotal for the oncogenic activity of this fusion protein [31,33,34].

1.3.2. The structure of the Bcr-Abl fusion protein

Following a chromosomal reciprocal translocation, the juxtaposition of the BCR and ABL genes occurs, therefore generating a fusion gene, which consists of the 5' end of the BCR gene fused to the 3' end of the ABL gene.

Of note, the position of the BCR and ABL genomic breakpoints is extremely variable **(figure 1.4)**. In particular, the BCR gene presents three main breakpoints: the major breakpoint cluster region (M-bcr), located between exon 6 and 12; the minor one (m-bcr) in the first intron of the gene; and the micro-bcr (μ-bcr), a third breakpoint cluster further upstream in the BCR gene. Therefore, three different fusion proteins may occur, named p210Bcr/Abl and p190Bcr/Abl and p230Bcr/Abl, respectively. In addition, other rare breakpoint cluster region on BCR gene have been observed [35-37]. Instead, on chromosome 9, only one breakpoint in the ABL gene is usually observed. This breakpoint, located between exon 1 and 2, generates a constant ABL-portion, that can associate with one of the three BCR-portions, therefore leading to different BCR-ABL fusion genes [38].

Around 95% of patients present the M-bcr breakpoint, encoding for the b2a2 or b3a2 transcripts (also referred to as e13a2 or e14a2) and producing the p210 fusion protein [39,40]. On the other hand, less than 5% of CML patients present the m-bcr breakpoint, which results in the e1a2 transcript encoding for the p190 protein [39]. The μ-bcr breakpoint producing the e19a2 transcript and the p230 fusion protein is instead much more infrequent, and was initially associated only with neutrophilic

CML [41]; however, some studies revealed that μ -bcr is present also in few cases of classical CML and acute myeloid leukemia [42,43].

Other transcripts such as e2a2, e1a3, e6a2, e13a3 (b2a3), and e14a3 have been reported as well, although they occur quite rarely [44].

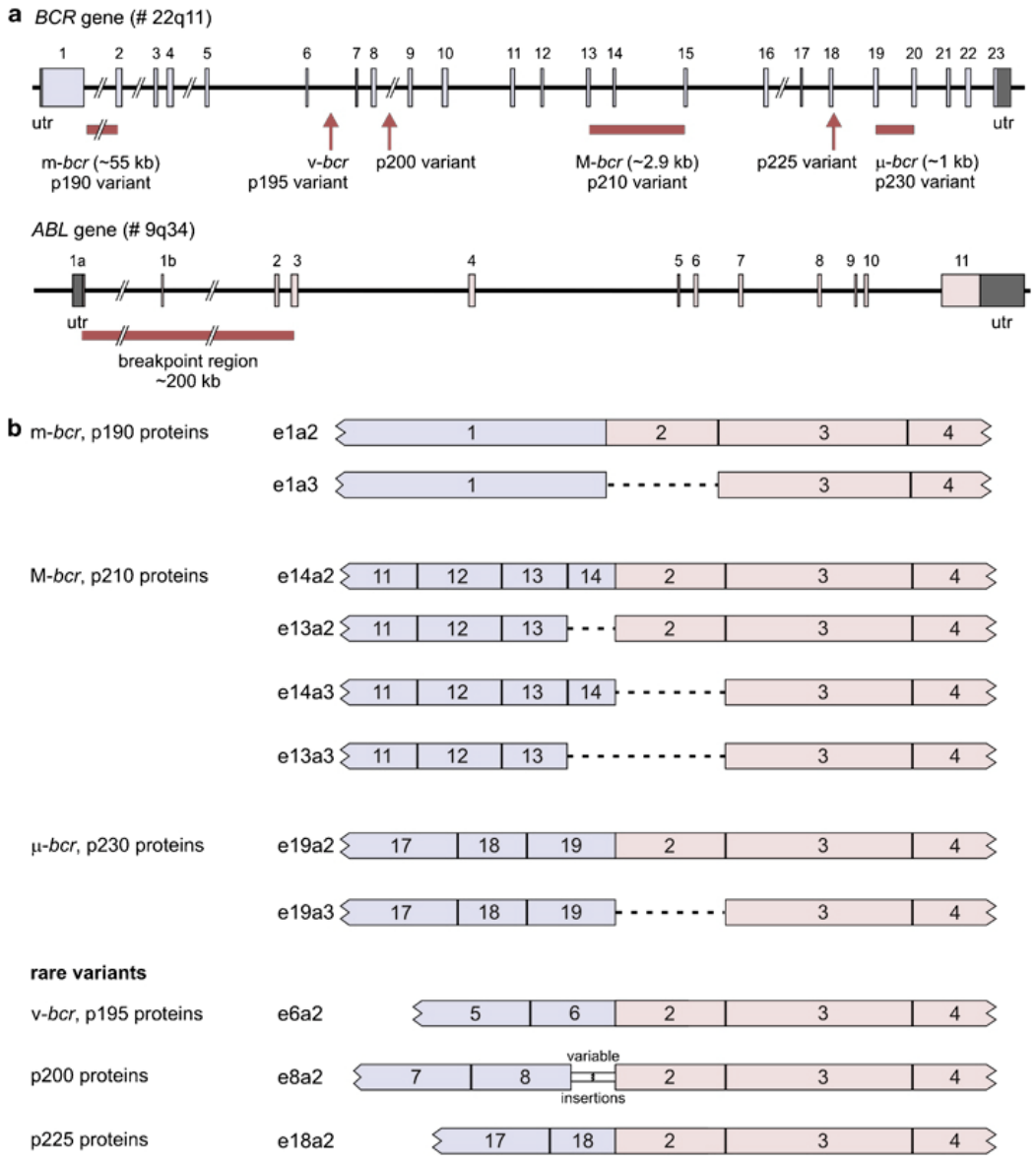

Figure 1.4: The BCR and ABL genes, the main breakpoint regions and the corresponding BCR-ABL transcripts [39]

1.3.3. Pathogenic mechanisms mediated by the Bcr-Abl fusion protein

The Bcr-Abl fusion protein is reported to be involved in a variety of cellular pathways among which changes in growth factor dependence, apoptosis, proliferation and cell adhesion, therefore sustaining granulocytes hyper-proliferation and mediating leukemia pathogenesis [45].

One of the most important pathway involved in CML pathogenesis is the JAK/STAT signalling pathway. Bcr-Abl kinase activity enhances in fact JAK2/STAT activation, therefore promoting cell growth and cell survival [46].

Besides the JAK/STAT pathway, Bcr-Abl can stimulate PI3K proteins signalling, therefore influencing the activities mediated by these proteins among which modulation of transcription factor activation, regulation of cell growth and survival, and inhibition of cell death [47]. In addition, PI3K activation also stimulates the mTOR pathway, which is involved in controlling protein synthesis, cell growth and autophagy [48].

Cell growth is further promoted also by the Bcr-Abl mediated stimulation of the Ras GTPases/MEK kinases [49,50].

Besides promoting cell proliferation, Bcr-Abl can also prevent cell death, mainly by increasing the expression of the anti-apoptotic proteins Bcl2 and Bcl-XL [51]. Furthermore, Bcr-Abl mediates the phosphorylation of the pro-apoptotic protein Bad, thereby restricting Bad to the cytoplasm and preventing its pro-apoptotic action [52].

1.4. How to diagnose CML

CML is a subdual disease, since around 40% of subjects are asymptomatic and are diagnosed through a laboratory test performed by chance [53]. Even if present, symptoms are mainly unspecific, and include fatigue, weight loss, abdominal fullness, bleeding, purpura, splenomegaly, leucocytosis, anaemia, and thrombocytosis [3,54].

The suspect of CML usually arises after an abnormally high granulocyte count coming from a full blood cell count analysis. In this case, the identification of the Ph chromosome through karyotyping and the detection of the BCR-ABL gene by quantitative PCR are used to confirm the diagnosis of CML [40,54].

However, what's worth noting is that the presence of the BCR-ABL transcript can and has been detected also in healthy subjects not affected by CML [55].

A possible explanation is that the generation of certain fusion genes, such as those implicated in CML pathogenesis, is not rare in hematopoietic cells: however, in the majority of cases, these cells do not develop the additional mutations that are necessary for the neoplastic process sustaining CML pathogenesis [56].

However, it must be specified that the BCR-ABL signal coming from the eventual detection of such fusion gene in healthy patients is very low, with the possibility of false-positive diagnosis being therefore practically irrelevant [56].

1.5. Progression of the pathology

CML staging includes three distinct phases. Around 85% of patients with CML are diagnosed in chronic phase (CP), which is the mildest phase [3]. Without therapeutic intervention, CML progresses from CP to the accelerated phase (AP) and to the final blast crisis (BC) [40,54].

The progression from one phase to another is linked with increase in severity and decrease in life expectancy. In fact, following advancement of the disease, the leukemic cells progressively lose their ability to differentiate into mature granulocytes, therefore resulting in the overexpression of primitive immature cells[40].

The massive presence of immature cells and the lack of proper differentiation further leads to acute events of infection, thrombosis and anaemia, which nearly inevitably leads to patient's death [57].

Disease progression can be monitored through the analysis of the blast cell count in the peripheral blood.

In particular, according to the European LeukemiaNet (ELN) guidelines,

"AP is defined by 15 to 29 % blast cells or by 30 to 49 % blast cells plus promyelocytes in blood or marrow or by platelet count <100 × 109/L or by a clonal chromosome abnormality in Ph + cells. On the other hand, BC is defined by a blast cells percentage ≥30 % in blood or marrow or by blast cells involvement of non hematopoietic tissues, excluding liver and spleen" [58,59].

The ELN criteria are slighlty different from those indicated by the World Health Organisation, which defines AP with blast cells percentage of 15-19% and BC with percentages ≥20 % [60] **(figure 1.5)**.

In addition, the detection of additional clonal chromosomal abnormalities (ACA) - a phenomena known as clonal evolution- is recognized as a sign of therapeutic failure and of disease progression [59].

It must be specified that both myeloid and lymphoid phenotypes can be present in BC, with myeloid-BC being two times more frequent compared to lymphoid-BC [61].

Accelerated phase	Definition
ELN criteria	Blasts in blood or marrow 15-29%, or blasts plus promyelocytes in blood or marrow >30%, with blasts <30%
	Basophils in blood ≥20%
	Persistent thrombocytopenia (<100×10^9/L) unrelated to therapy
	Clonal chromosome abnormalities in Ph+ cells (CCA/Ph+), major route, on treatment
WHO criteria	Blasts in blood or marrow 10-19%
	Basophils in blood ≥20%
	Persistent thrombocytopenia (<100×10^9/L) unrelated to therapy
	CCA/Ph+ on treatment
	Thrombocytosis (>1000×10^9/L) unresponsive to therapy
	Increasing spleen size and increasing white blood cell count unresponsive to therapy
Blast phase	
ELN criteria	Blasts in blood or marrow ≥30%
	Extramedullary blast proliferation, apart from spleen
WHO criteria	Blasts in blood or marrow ≥20%
	Extramedullary blast proliferation, apart from spleen
	Large foci or clusters of blasts in the bone marrow biopsy

The ELN criteria are those that were used in all main studies of TKI. The use of TKI may require a change of the boundaries between CP, AP, and BP and modify to some extent the classic subdivision of CML in 3 phases, but the data are not yet sufficient for a revision.

CCA/Ph+, clonal chromosome abnormalities in Ph+ cells.

Figure 1.5: Definition of accelerated phase (AP) and blast crisis (BC) according to the European LeukemiaNet (ELN) criteria vs the World Health Organisation (WHO) criteria [59]

1.6. Assessment of patient's risk of progression

As previously said, CML phase at entry is a fundamental prognostic factor for patient's survival.

Besides phase at entry, patient's specific risk factors at baseline are extremely important, since they seem to impact even more than therapeutic treatment on CML prognosis [62].

To assess them, three main risk scores are available, which have been developed through years according to the availability of different therapeutic treatments and to the on-going knowledge of the pathology **(table 1.1)**.

In particular, the first two scores -Sokal and Euro- [63,64] were developed in the "pre-TKI" era, when only IFNα and/or hydroxyurea (HU) /busulfan were available, and the link between BCR-ABL gene and CML pathogenesis were still unknown.

These scores have represented an extremely important tool in the estimation of prognosis and survival of patients treated with traditional chemotherapy or interferon, respectively.

The only score elaborated after the advent of TKIs is the EUTOS [65]. However, even in the era of TKIs, the Sokal score is still considered an extremely predictive tool in clinical practice, since it resulted to significantly correlate with response to TKIs, with survival, and also with treatment-free durable molecular response [66].

According to the ELN recommendations, all three scores are useful to assess patient's risk [59]. In particular, their combinatorial use in clinical practice may be

crucial to identify those patients who would benefit the most from a first line treatment with a second-generation TKI [67].

Score and study	Calculation	Risk definition
Sokal **Sokal et al. 1984**	Exp 0.0116 × (age − 43.4) + 0.0345 × (spleen − 7.51) + 0.188 × [(platelet count ÷ 700) − 0.563] + 0.0887 × (blast cells − 2.10)	Low risk: <0.8 Intermediate risk: 0.8-1.2 High risk: >1.2
Euro **Hasford et al. 1998**	0.666 when age ≥50 y + (0.042 × spleen in cm) + 1.0956 when platelet count >1500 × 10^9L + (0.0584 × blast cells) + 0.20399 when basophils >3% + (0.0413 × eosinophils) × 100	Low risk: ≤780 Intermediate risk: 781-1480 High risk: >1480
EUTOS **Hasford et al. 2011**	Spleen in cm × 4 + basophils × 7	Low risk: ≤87 High risk: >87

Table 1.1: Calculation and stratification of the Sokal, Hasford and EUTOS risk scores [63-65]

Besides these three risk scores, the presence of ACAs at diagnosis is clearly recognized as an important prognostic factor [68-70]. In a study on imatinib-treated patients, subjects carrying ACAs at diagnosis had a significantly lower 5-year failure free survival (FFS) rate compared to patients with no ACAs (52% vs 84%)[71]. Similarly, presence of ACAs at baseline has been associated with lower rates of treatment responses [69]. In particular, deletion and variant translocations of the chromosome 9 seem to have no significant impact on prognosis, whereas abnormalities including trisomy 8, trisomy Ph (+der(22)t(9;22)(q34;q11)), isochromosome 17 (i(17)(q10)), trisomy 19, and ider(22)(q10)t(9;22)(q34;q11) have been associated with adverse prognosis [68,69,72].

The development of ACA during treatment- named clonal evolution- is instead considered a sign of disease progression, as reported in the previous paragraph.

Furthermore, also the variability in BCR-ABL transcripts seems to affect CML prognosis. Focusing on the most common transcripts generated by the major breakpoint, the e14a2 transcript appears to be associated with a better response to imatinib compared to the e13a2 transcript [73-75]. This could be attributable to the differences in term of secondary structure elements and amino acidic sequences between the two transcripts that could exert different activities in mediating signal transduction pathway [76]. The e1a2 transcript coding for the p190 protein, although infrequent in patients, was found to be associated with poor prognosis and poor response to TKI therapies. Patients carrying this transcript are therefore identified as high-risk patients, for whom close monitoring and evaluation of HSCT eligibility are recommended [44]. Similarly, variability in hematological and cytogenetic responses to TKIs and aggressiveness in clinical course have been described for e19a2 and e6a2 transcripts [77-82].

Besides these well-recognized risk factors, the role of several other baseline factors is under investigation. Among them, the BCR-ABL level at diagnosis could represent another prognostic factor, although its real clinical impact is still unclear [83]. According to a study conducted on patients treated with imatinib, higher levels of BCR-ABL transcripts at diagnosis were associated with lower rates of FFS and progression free survivals (PFS) [84]. However, other studies did not support this correlation [85].

In addition, the levels of expression of certain cellular protein involved in the drug transport, such as the organic cation transporter 1 (OCT1), have been associated with CML prognosis. In fact, OCT1 is the major active influx pump for imatinib; for patients treated with this TKI, high levels of expression of OCT1 have been connected to higher response and better survival [86,87]. However, at the moment no test is clinically available for the assessment of OCT1 expression levels in CML patients, and the real clinical impact of OCT1 on responses to first and second generation TKIs is still unclear.

Furthermore, also the gene expression profile, the polymorphisms of genes coding for proteins involved in drug metabolism and transport, and in apoptosis, and the presence of low-level BCR-ABL mutations, have been considered as potential risk factors, whose real clinical impact must be clarified [70].

1.7. Treatment

1.7.1. History of CML therapeutic approach

First reports of cases of splenomegaly associated with abnormally high leukocyte count date back to the 1840s. These cases were probably examples of the later-discovered CML. During the 19th century, the only well-documented therapy in use was arsenic, which displayed good efficacy in reducing leucocytosis but had poor benefits on patient's OS [88].

Therapeutic strategies moved then to radiotherapy, and later on to busulfan, hydroxycarbamide, or IFN-α, which represented the most common therapeutic

strategies during the 20th century. These therapies however accounted for poor long-term survival, with more than 85% of patients dying within the first 8 years from diagnosis [9,88].

The discoveries of the Ph chromosome in 1960, of the (9;22) translocation in 1973, and of the breakpoint cluster region on chromosome 22 in 1984, deeply modified the therapeutic approach of CML [88]. From 1980 allogenic HSCT became the first-choice treatment for eligible patients, with a great improve on the 8-years OS, that reached 65% in 2000 [9,88].

However, it was the advent of TKIs that deeply modified both the therapeutic approach and the prognosis of CML, overcoming all other therapeutic options and accounting for a long-term survival that is now comparable to that of the general population [9,88].

1.7.2. The advent of Tyrosine Kinase Inhibitors (TKIs)

1.7.2.1.First generation TKI: imatinib

In the late 1980, using a high throughput screening techniques, scientists of actual Novartis pharmaceutical company discovered a lead molecule – a 2-phenylaminopyridine-based ATP competitive inhibitor, known as ST1-571 -, that displayed a strong activity as ABL kinase inhibitor.

In fact, this compound resulted to competitively bind the ATP binding site of the inactive conformation of the ABL protein tyrosine kinase, therefore preventing its switch to the active conformation and strongly inhibiting the ABL kinase activity [89-91] **(figure 1.6)**.

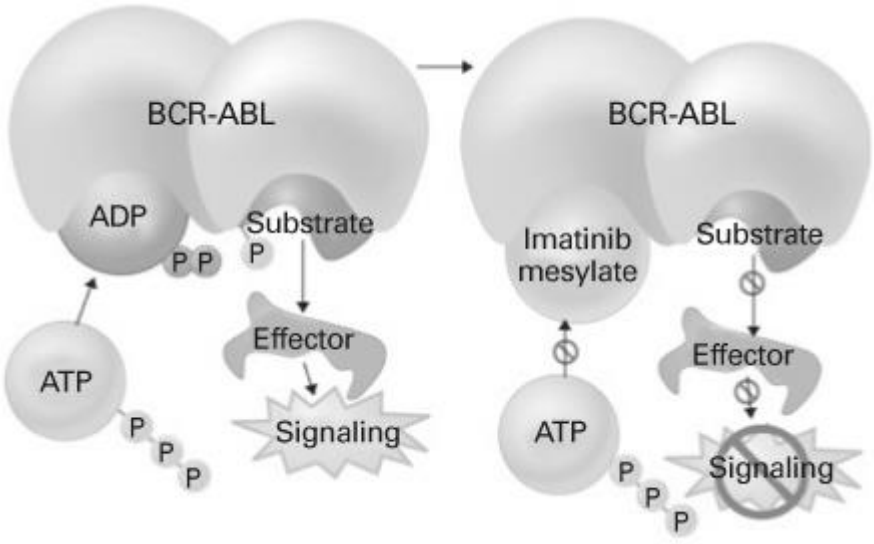

Figure 1.6: Mechanism of action of imatinib [92]

This molecule, named imatinib, was proved to inhibit cellular proliferation in an apoptosis-independent pathway; furthermore in vitro studies showed that imatinib decreased the growth of Ph positive cells colonies of about 92-98%, with no significant alteration on the growth of normal cells colonies [93]. Besides ABL inhibition, this molecule resulted to be active also against other kinases (such as the c-kit) and against the platelet-derived growth factor receptors (PDGFR), which can be involved in the oncogenic pathway [91,94,95]. In addition, imatinib was seen to play immunomodulatory effects on T cells and antigen-presenting cells [96]. Following the promising results of phase I and II randomized control trials (RCTs) performed on AP and BC CML patients treated with imatinib 600mg/day [97-100], imatinib was firstly approved by the FDA (Food and Drug Administration) with the commercial name of Glivec, for the treatment of AP and BC CML patients. Later on, it was the phase III clinical trial IRIS that first demonstrated the great benefit coming from TKI treatment with imatinib also in CP CML [101]. In this trials, imatinib at the dosage

of 400 mg daily was compared with the combination of IFNα and low-dose cytarabine -i.e. the gold standard treatment at the time- for the treatment of newly diagnosed CP CML patients. After a median time of 19 months of imatinib treatment, 96%, 87% and 76% of patients had reached complete hematologic response (CHR), major cytogenetic response (MCyR) and complete cytogenetic response (CCyR), respectively.

The efficacy of imatinib was further confirmed in a 6-years follow-up study, reporting an estimated event-free survival of 83% and a percentage of intolerance-related treatment discontinuations of 5% [102].

According to the current ELN recommendations, imatinib should be used at an initial daily dosage of 400mg [59]. No supporting data exist in fact for the use of high-dose imatinib as first line treatment. The prospective TOPS (Tyrosine kinase inhibitor OPtimization and Selectivity) trial compared the efficacy of high dose (800mg/daily) vs standard dose (400mg/daily) imatinib. Although percentages of achievement of major molecular response (MMR) at 3 and 6 months following treatment beginning were higher among patients treated with high dose imatinib, no difference emerged in the achievement of 12-months MMR [103]. Similarly, a study by the ELN did not show better responses among patients treated with high-dose [104].

Despite the efficacy of imatinib, the development of either treatment resistance or intolerance still represents a major concern in the treatment of CML. In the IRIS trial, 31% and 5% of patients were unable to achieve CCyR within 1 and 5 years of treatment, respectively. What's more, 3-years treatment failure occurred in 3-7% of

patients, and 5% of patients discontinued imatinib because of severe intolerance [101,105].

To overcome this problem, second-generation TKIs were developed.

1.7.2.2.Second generation TKIs: nilotinib and dasatinib

Second-generation TKIs were developed based on the chemical and crystal structure of imatinib–ABL complex **(figure 1.7)**. In particular, a similar ATP-competitive phenylaminopyrimidine molecule named nilotinib (brand name Tasigna) was designed by Novartis.

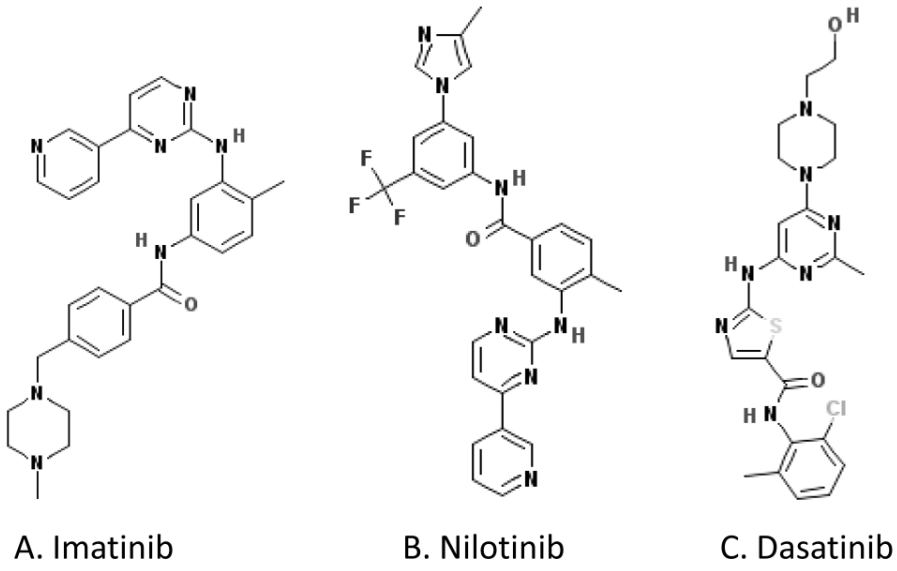

Figure 1.7: Chemical structure of the three TKIs

Like imatinib, nilotinib binds and blocks the ABL kinase in its inactive form, therefore preventing the substrate binding and the consequent catalytic action [106].

Compared to imatinib, crystallographic data indicate a higher affinity of nilotinib for the ABL kinase, therefore resulting in increased selectivity and in an 30-times higher in vitro efficacy on inducing Ph positive cells lysis [106-109].

In addition, nilotinib showed good efficacy in a phase II trial conducted on imatinib-resistant CP patients, with percentages of 6-months MCyR and CCyR achievement of 48% and 31% [110]. Similarly, a phase II trial performed on imatinib-resistant AP patients showed rates of hematologic response, CHR, MCyR and CCyR of 56%, 31%, 32% and 20%, respectively, with a median time to hematologic response of 1 month [111]. What's more important, nilotinib resulted to be effective in 32 out of 33 mutations leading to imatinib resistance; however, no efficacy was seen against the T351I mutation [107-109].

In light of these results, the FDA finally approved nilotinib in 2007 for the treatment of CP and AP patients resistant or intolerant to imatinib.

In the ENESTnd (Evaluating Nilotinib Efficacy and Safety in Clinical Trials–Newly Diagnosed Patients) phase III trial, nilotinib was further evaluated as a possible frontline treatment for newly diagnosed CP patients [112,113]. In this trial, nilotinib 300mg or 400mg twice daily was compared to imatinib 400mg once daily. At 12 months, the rates of MMR were significantly higher among nilotinib-treated patients (44% for 300mg twice daily nilotinib, 43% for 400 mg twice daily nilotinib and 22% for 400 mg once daily imatinib, respectively). Similarly, a 4-years follow-up of the ENESTnd study indicated significantly higher efficacy and lower rates of disease progression among patients treated with frontline nilotinib compared to imatinib, as well as fewer cases of development of BCR-ABL mutations [114].

Nilotinib is therefore currently approved also for the treatment of newly diagnosed adult patients with Ph positive CP CML.

Besides nilotinib, another ATP-competitive, non-phenylpyrimidine-based compound was designed to inhibit BCR-ABL tyrosine kinase. This molecule, named dasatinib (brand name Sprycel, produced by Bristol-Myers Squibb), was actually developed as a Src-family kinases (SFKs) inhibitor and it resulted to inhibit different tyrosine kinases, including Src, Lck, YES, EPH receptor A 2 (EPHA2) A2 and PDGFR. Dasatinib was also seen to bind both the active and inactive conformations of the ABL domain [115-117], and in vitro studies revealed a higher potency of dasatinib compared to imatinib in inhibiting both wild-type and imatinib-resistant BCR-ABL kinase, except in presence of the T315I mutation [107].

A phase I study conducted on imatinib-intolerant or resistant patients further demonstrated the efficacy of dasatinib in inducing significant clinical responses among all BCR-ABL genotypes, excluding the T315I mutation [118].

In light of these phase I results, different phase II studies, known as the SRC/ABL Tyrosine Kinase Inhibition Activity Research Trials (START), were designed to evaluate the efficacy of dasatinib in Ph positive imatinib-intolerant or resistant patients. According to the START-C trial, dasatinib accounted for long-lasting major or complete responses in high percentages of patients, independently from the presence of mutations leading to imatinib resistance [119]. Similarly, the comparison of dasatinib 70mg/twice daily with imatinib 800mg in the START-R trial reported significantly higher percentages of CHR, MCyR and MMR among

dasatinib-treated patients (93% vs. 82% of CHR; 52% vs. 33% of MCyR; 16% vs. 4% for MMR) [120].

The START-A, the START-B and START-L trials further proved the efficacy of dasatinib also for AP and BC CML patients [121-123].

Based on the results of these RCTs, the initial dose of dasatinib approved for CML patients with resistance or intolerance to imatinib was of 70 mg twice daily. However, data coming from a long-term follow-up of the phase I trial showed fewer adverse effects among patients treated once daily compared to patients treated twice-daily [118]. Therefore, two phase III trials, named CA180-034 and CA180- 035, were further conducted to identify the optimal dosage [124,125]. In the CA180-034 trial, four dosages of dasatinib (100 mg once daily, 50 mg twice daily, 140 mg once daily, or 70 mg twice daily) were compared for the treatment of CP CML. Efficacy resulted to be similar among the 4 groups, although the 100 mg once daily treatment was associated with lower rates of toxicity and of dose interruptions [124]. The CA180-035 trial focused instead on AP CML, and compared the 140 mg once daily regiment vs the 70 mg twice-daily regimen. The once-daily dose of 140 mg dasatinib demonstrated comparable efficacy but lower toxicity [125].

Based on these trials, the dosage of dasatinib currently approved by the FDA is of dasatinib 100 mg once daily for CP patients and 140 mg once daily for AP or BC CML patients.

A subsequent phase III trial named DASISION (DASatinib versus Imatinib Study In treatment-Naive CML patients) was performed to compare the treatment with dasatinib 100 mg vs imatinib 400 mg among newly diagnosed CP CML

patients [126]. Dasatinib resulted to be associated with significantly higher percentages of CCyR (83% vs 72%), and MMR (46% vs 28%) at 12 months. The efficacy and safety of dasatinib as first line therapy were further confirmed during a 24 months' follow-up [127].

In light of these RCTs, dasatinib is currently approved also as first line treatment for newly diagnosed CP-CML.

1.7.2.3.Other TKIs approved as second or third line therapies

As previously reported, imatinib, dasatinib and nilotinib are all approved both as first or second line treatments. Despite their well-documented efficacy, development of resistance or intolerance to all these treatments can occur.

Bosutinib (brand name Bosulif, produced by Pfizer Inc.) is a second-generation TKI that acts as an inhibitor of both ABL and Src kinases. It is currently approved for the treatment of Ph positive CP, AP and BC CML patients, with intolerance or resistance to other TKIs. In fact, RCTs demonstrated that bosutinib is effective in inducing durable responses, as well as in accounting for high rates of PFS and OS, in either second, third or fourth line treatment [128,129].

On the other hand, no superiority of bosutinib compared to imatinib was found in the treatment of newly diagnosed CML patients; therefore, bosutinib is not indicated as first line CML treatment [130,131].

Similarly to nilotinib and dasatinib, bosutinib resulted to be effective in most cases of mutations providing resistance to imatinib; however, it showed no efficacy towards the T315I or the V299L [128].

To overcome the resistance to first and second generation TKIs provided by the T315I mutation, a new third generation TKI named ponatinib (trade name Iclusig) was developed by ARIAD Pharmaceuticals. The efficacy of ponatinib in T315I-carrying CP CML patients was first demonstrated in a phase I trial in 2010.

The phase II PACE (Ponatinib Ph+ ALL and CML Evaluation) trial further confirmed the efficacy of ponatinib for the treatment of Ph positive CML or acute lymphoblastic leukaemia with resistance or unacceptable intolerance to dasatinib or nilotinib or with the presence of the T315I mutation [132]. Furthermore, another phase II trial was performed to evaluated the efficacy of ponatinib as frontline treatment in newly diagnosed CP CML patients. Ponatinib confirmed its efficacy; however, due to the high risk of vascular thrombotic events and the availability of other therapeutic options for these patients, ponatinib has not been approved as first line treatment in T315I-free patients [133].

In light of this study, ponatinib is currently approved at the dosage of 45 mg daily for the treatment of CP, AP or BC CML in patients with intolerance or resistance to dasatinib or nilotinib, or in patients carrying the T315I mutation.

1.7.3. Current guidelines for pharmacological treatment of CML

As previously discussed, the TKIs currently approved for first line CML therapy are the first generation TKI imatinib (Glivec; recommended dosage of 400 mg once daily), or the second generation TKIs nilotinib (Tasigna; recommended dosage of 300 mg twice daily) and dasatinib (Sprycel; recommended dosage of 100 mg once daily) **(figure 1.8)**. The choice of the TKI must be done according to drug

tolerability and safety, as well as patient's characteristics such as age, comorbidities, and pattern of mutations on the BCR-ABL gene [70].

In particular, second generation TKIs displayed higher potency and efficacy in RCTs; however, no solid evidence exists for the preferential choice of these therapies as frontline treatment of CP non-high risk patients. According to the most recent ELN recommendations, high-risk patients and patients diagnosed in AP or BC could be the groups of patients benefiting the most from a second-generation TKI therapy [70].

Treatment recommendations

Line	Event		TKI, standard dosage[1]					Transplantation				
Chronic phase												
								Search for		alloSCT		
			Imatinib 400 mg/qd	Nilotinib 300 mg/bid	Dasatinib 100 mg/qd	Bosutinib 500 mg/qd	Ponatinib 45 mg/qd	HLA type + sibs	unrelated donor	consider	recommended	Chemotherapy
1st	Baseline		X	X	X			X[2]				
2nd	Intolerance to 1st TKI		Any other TKI approved 1st line									
	Failure 1st line of	imatinib		X[8]	X	X	X	X				
		nilotinib			X	X	X	X	X	X		
		dasatinib		X[8]		X	X	X	X	X		
3rd	Intolerance to/failure of two TKI		Any remaining TKI								X	
Any	T315I mutation						X	X	X	X		
Accelerated or blast phase												
In newly diagnosed, TKI naïve patients		start with	X[3]		X[4]			X	X			
		no optimal response, BP									X[7]	X[5]
TKI pre-treated patients			Any other TKI				X[6]				X[7]	X[5]

[1]choice of the TKI consider tolerability and safety, and patient characteristics (age, comorbidities), [2]only in case of baseline warnings (high risk, major route CCA/Ph+), [3]400 mg/bid, [4]70 mg/bid or 140 mg/qd, [5]may be required before SCT to control disease and to make patients eligible to alloSCT, [6]in case of T315I mutation, [7]only patients who are eligible for alloSCT, not in case of uncontrolled, resistant BP, [8]400 mg bid in failure setting qd: Once daily bid: Twice daily

References: 1. Baccarani M, Deininger M, Rosti G, et al. European LeukemiaNet recommendations for the management of chronic myeloid leukemia: 2013. Blood 122:872-884, 2013. 2. Baccarani M, Cortes J, Pane F, et al. Chronic myeloid leukemia. An update of concepts and management Recommendations of the European LeukemiaNet. J Clin Oncol. 27:6041-51, 2009. 3. Baccarani M, Sagio G, Goldman J, et al: Evolving concepts in the management of chronic myeloid leukemia: recommendations from an expert panel on behalf of the European LeukemiaNet. Blood 108:1809-1820, 2006.

Figure 1.8: Treatment recommendations according to the ELN guidelines [59]

In case of failure or intolerance to first-line treatment, patients should be switched to a second-line treatment.

Available second-line treatments include the above-mentioned TKIs, that can be used at the standard or at a higher dose (400mg twice daily for imatinib; 400 mg twice daily for nilotinib; 70 mg twice daily or 140 mg once daily for dasatinib), and bosutinib (500 mg once daily) [59].

If the cause of switch is intolerance, any other available TKI can be chosen; in this case, a second line therapy with imatinib can be started after a frontline treatment with a second-generation TKI [70].

On the other hand, imatinib should not be use as second line treatment in case of resistance to first line therapy. Due to the lack of studies comparing the different second-line TKIs, the therapeutic choice should be based on patient's characteristics (age, comorbidities, side effects of first TKI), as well as on the presence and type of BCR-ABL kinase mutations, that can impact on the sensitivity towards each TKIs. In particular, the T315I mutation is sensitive only to the third-generation TKI ponatinib (recommended dosage of 45 mg once daily) [70].

Ponatinib can be used also in absence of this mutation, in case of failure of both first- and second-line treatment.

In these cases, ELN guidelines recommend an accurate analysis of patient's karyotype and of eventual BCR-ABL mutations, as well as a close monitoring of the patient in preparation of HSCT [70].

On the other hand, use of non-TKI chemotherapy is not recommended and is limited to few particular circumstances. In particular, the association of IFNα with a TKI treatment could potentially represent a valid strategy, which is still under investigation.

Treatment with HU can be used for a short time before starting a TKI, until confirmation of CML diagnosis. Similarly, cytotoxic chemotherapy can represent a valid control for BC while preparing patients for HSCT [59].

1.7.4. The role of Hematopoietic Stem Cell Transplantation

HSCT is primarily considered as a salvage intervention: current recommendations advise that HSCT should be reserved for BC or for subjects who are resistant to at least one second-generation TKI because the chances of a sustained response to another TKI are negligible [134]. In addition, recent studies have provided evidence on the superiority of HSCT compared to both dasatinib and nilotinib in increasing survival in adult patients with AP CML [135].

The recommendations of limiting HSCT as salvage procedure were initially based on the early results of imatinib compared to previously available therapies, and from studies reporting an increased early mortality after HSCT [136-139]. Nowadays mortality connected to HSCT has drastically decreased and risk score for transplant outcome have been implemented. Among them, the European Group for Blood and Marrow Transplantation (EBMT) risk score system is based on 5 variables: donor type, disease phase, recipient age, donor/recipient sex combination, and interval from diagnosis to transplantation [140]. In addition, inflammatory levels and comorbidities have been proposed as independent

predictors of lower survival [141]. Therefore, the decision for transplantation involves careful balancing of the risks for HSCT against the risk for disease progression in each individual patient [134].

After HSCT, patients should be monitored by Reverse Transcriptase Quantitative Polymerase Chain Reaction (RQ-PCR); treatment with donor lymphocyte infusion and/or TKI is recommended as well [59].

1.7.5. Monitoring of responses

According to the ELN recommendations,

"the response to TKI is the most important prognostic factor". In fact, current guidelines "do not recommend which TKI should be used but which response should be achieved, irrespective of the TKI that is used" [59].

Patient's response to treatment is evaluated at three different levels of response, i.e. hematological, cytogenetic, and molecular response [142]. Hematological response is assessed through hematochemical blood tests, considering the count of white blood cells, platelets and circulating blast cells [7].

In particular, cytogenetic monitoring is based on the identification and the count (in percentage) of Philadelphia chromosome positive nuclei during metaphase, using chromosome banding analysis or fluorescence in situ hybridization (FISH) analysis. FISH of blood interphase cell nuclei can be used as well for the evaluation of cytogenetic responses [59].

Molecular analysis is performed instead using qualitative and reverse transcriptase quantitative PCR, to estimate the ratio of BCR-ABL mRNA compared

to an internal reference gene, such as ABL, GUSB or BCR [143]. Molecular results are indicated using the International Scale (IS) as a BCR-ABL percentage, with 100% BCR-ABL IS corresponding to the International Randomized Study of Interferon and STI571 (IRIS) study standardized baseline [59]. In particular, BCR-ABL percentages of 10%, 1%, 0.1%,0.01%, 0.0032%, and 0.001% indicate a reduction of 1, 2, 3, 4, 4.5, and 5 logs, respectively, below the standard baseline used in the IRIS study [59].

Both cytogenetic and molecular tests should be performed at different time-milestones, and timing should be adapted according to the patient's response to treatment **(figure 1.9)**.

Timing of Cytogenetic and Molecular Monitoring

At diagnosis	CBA, FISH in case of Ph- (for cryptic or variant translocations), qualitative PCR (transcript type)
During treatment	RQ-PCR every 3 months until MMR has been achieved, then every 3 to 6 months and/or CBA at 3, 6, and 12 months until CCyR has been achieved, then every 12 months. Once CCyR is achieved, FISH on blood cells can be used.
Failure, progression	RQ-PCR, mutational analysis, and CBA. Immunophenotyping in blast phase.
Warning	Molecular and cytogenetic tests more frequently. CBA in case of myelodysplasia or CCA/Ph-

CBA: Chromosome banding analysis of marrow cell metaphases at least 20 metaphases analysed

Figure 1.9: Timing of cytogenetic and molecular evaluations, according to the ELN recommendations [59]

The best response is defined as the achievement of both CHR, CCyR and complete molecular response (CMR), as reported in **table 1.2**.

Response	Assessment
Complete hematologic response	Normalization of leukocyte count (white blood cell count <10 cells×109/L) and platelet count (platelets <10 cells×109/L), no immature cells or blasts in the peripheral blood, no signs or symptoms of disease with the disappearance of palpable splenomegaly
Cytogenetic response	% Philadelphia chromosome positive metaphases: (with a minimum of 20 metaphases examined) Minor: 35%–90% Partial: 1%–34% Major: 0%–35% (complete+partial) Complete: 0%
Molecular response	Major: ≥3 log reduction of BCR-ABL1 mRNA expression Complete: BCR-ABL1 mRNA expression undetectable by reverse-transcriptase polymerase chain reaction

BCR, breakpoint cluster region; ABL, Abelson murine leukemia; mRNA, messenger ribonucleic acid.

Table 1.2: Assessment response to CML treatment [7]

Although CHR, CCyR and MMR/CMR are the gold standard to be achieved, definition of optimal response and of therapeutic failure varies according to the time and the therapeutic line **(figure 1.10)**.

Different studies have independently demonstrated that achievement of optimal response in the earliest phases is associated with more favourable long-term outcomes.

In the first study supporting this correlation, levels of BCR-ABL transcript < 10% at 3 months from treatment beginning resulted to be significantly associated with higher rates of future MCyR [144]. Similarly, a later study supported the correlation between 5-years CCyR and levels of cytogenetic response at 3 and 6 months [145].

Besides correlation with MCyR or CCyR, earlier cytogenetic and molecular responses have been found to be associated with OS, PFS and FFS [146,147].

Response definitions for any TKI first line, and 2nd line in case of intolerance, all patients (CP, AP, and BC)

Time	Optimal response	Warning	Failure
Baseline		High risk Major route CCA/Ph+	
3 mos.	BCR-ABL^{IS} ≤10%* Ph+ ≤35% (PCyR)	BCR-ABL^{IS} >10%* Ph+ 36-95%	No CHR* Ph+ >95%
6 mos.	BCR-ABL^{IS} <1%* Ph+ 0% (CCyR)	BCR-ABL^{IS} 1-10%* Ph+ 1-35%	BCR-ABL^{IS} >10%* Ph+ >35%
12 mos.	BCR-ABL^{IS} ≤0.1%* (MMR)	BCR-ABL^{IS} 0.1-1%*	BCR-ABL^{IS} >1%* Ph+ >0%
Then, and at any time	MMR or better	CCA/Ph- (-7, or 7q-)	Loss of CHR Loss of CCyR Loss of MMR, confirmed** Mutations CCA/Ph+

Response definitions to 2nd line therapy in case of failure of imatinib (can be used provisionally, NOT for the response to 3rd line treatment).

Time	Optimal response	Warnings	Failure
Baseline		No CHR Loss of CHR on imatinib Lack of CyR to 1st line TKI High risk	
3 mos.	BCR-ABL^{IS} ≤10%* Ph+ <65%	BCR-ABL^{IS} >10%* Ph+ 65-95%	No CHR, or Ph+ >95%, or New mutations
6 mos.	BCR-ABL^{IS} ≤10%* Ph+ <35% (PCyR)	BCR-ABL^{IS} ≤10%* Ph+ 35-65%	BCR-ABL^{IS} >10%* Ph+ >65%* New mutations
12 mos.	BCR-ABL^{IS} <1%* Ph+ 0 (CCyR)	BCR-ABL^{IS} 1-10%* Ph+ 1-35%	BCR-ABL^{IS} >10%* Ph+ >35%* New mutations
Then, and at any time	MMR or better	CCA/Ph- (-7 or 7q-) or BCR-ABL^{IS} >0.1%	Loss of CHR, or Loss of CCyR or PCyR New mutations Loss of MMR** CCA/Ph+

*and/or **in 2 consecutive tests, of which one ≥1% IS: BCR-ABL on International Scale

Figure 1.10: Definition of the responses to first and to second line TKI treatment, according to the ELN recommendations [59]

Intermediate suboptimal results are instead considered as a warning; in this case, closer monitoring is recommended to detect eventual early signs of therapeutic failure [59].

Therapeutic failures can be either primary, i.e. the patient does not achieve a given response at a given time, or secondary, i.e. a loss of response occurs at a certain time after its achievement. In both cases, the therapeutic treatment should be changed to minimize the risk of progression and of mortality [59].

Failure is a frequent event: for imatinib, the most common first-line treatment, primary failure is assessed in around 40% of patients.

Patients who fail imatinib must switch to second-generation tyrosine kinase inhibitors. However, 37 to 52% of patients do not respond neither to these treatments [148,149], and 23 to 26% lose the initial MCyR within 2 years [149].

According to literature data, the most common cause is connected to drug resistance; in addition, unacceptable side effects are commonly associated with therapeutic failure, as well [150].

1.7.6. Resistance to treatment

10 to 20% of newly diagnosed CP-CML patients exhibit primary or acquired resistance to frontline TKI therapy, and responses in patients with BP CML are usually transient [145,151].

Resistance to TKIs can occur due to the several mechanisms, which can be either Bcr-Abl dependent of independent.

Among Bcr-Abl mechanisms, point mutations of the BCR-ABL kinase can be detected in around 50% of patients who experience treatment failure or disease progression [152-155]. These mutations are probably the most clinically relevant mechanism for resistance, since they are responsible for changes in the conformation of the kinase domain which can interfere with the TKI binding [91].

These mutations are independent from the transcript and kinase polymorphisms, and have been associated with a higher genetic instability and an increased risk in progression, due to a loss of response to TKI treatment [156].

In particular, different mutations can lead to the resistance to different TKIs. Using the Sanger sequencing technique, more than 80 different amino acidic substitutions have been detected and associated with the development of imatinib resistance [152,155]. For dasatinib, instead, the mutation which are more frequently connected to resistance are the V299L, F317L/V/I/C, T315A, or T315I, whereas patients developing resistance to nilotinib are usually detected with the Y253H, E255K/V, F359V/C/I, or T315I mutations [154,155]. Of note, patients carrying the T315I mutation are resistant to imatinib, dasatinib, nilotinib and bosutinib, and the only TKI effective for this mutation is ponatinib [157-159].

Besides point mutation, the amplification of the Bcr-Abl can be involved in TKI resistance, as well. The association between Bcr-Abl gene amplification and the activity of the BCR-ABL kinase has been reported by different studies [160,161]. However, the real impact of this mechanism is still under investigation.

Among Bcr-Abl independent mechanisms, instead, different proteins involved in drug efflux and influx could be involved in TKI resistance. Among them, the

overexpression of the Pgp efflux transporter has been suggested as a possible mechanism, due to the role of the Pgp in the transport of TKIs outside the cell; however, up to now this mechanism has not been detected in patients resistant to imatinib [162,163]. Similarly, the uptake transporter hOCT1 could be involeved in imatinib resistance, as well [91].

In addition, several signalling molecules have been considered as possibly implicated in TKIs resistance, including STAT3,11 PP2A30,47 and β-catenin [164-166]. Among them, β-catenin is worth of note, since it resulted to be involved in several steps of CML progression and resistance to treatments [164-167]. In particular, β-catenin results to be stabilized by BCR-ABL kinase [168], and increased concentration of nuclear β-Catenin have been found in imatinib-resistant patients [166].

1.7.7. Intolerance to treatment

Beside development of TKI resistance, occurrence of severe intolerance to the treatment represents an important concern in CML management. Severe intolerance can in fact invalidate the durability of treatment, while increasing the risk of morbidity and mortality [169].

According to phase III RCTs, 6-11% of imatinib-treated patients discontinued the treatment because of adverse events [113,131,170]. In particular, according to the IRIS RCT the lead to the approval of imatinib [105], the most common adverse events reported during imatinib treatment were oedema and nausea (both of which involving more than 50% of patients), followed by osteoarticular cramps or pain, diarrhoea, dermatologic manifestations, and asthenia.

Compared to imatinib, second and third generation TKIs showed some variability in term of induction of side effects. In particular, compared to imatinib, nilotinib has been associated with higher risk of dermatologic manifestations, headache, pancreatitis, and cardiovascular events. On the other hand, dasatinib resulted to be associated with a lower risk of oedema, but with an increased risk of gastrointestinal toxicity and of pleural effusion [169].

The reasons sustaining this variability among TKIs risk profiles are still debatable. A possible explanation could be based on the inhibition of non-BCR-ABL kinases (such as FGFR1, FLT3, KIT, PDGFR, or SRC) differently mediated by each TKI [105,113,131,170,171].

Ponatinib instead resulted to be associated with particularly high frequency of cardiovascular toxicity, including arterial thrombosis (8 %), myocardial infarction (5 %), peripheral arterial occlusive disease (PAOD) (2 %), and cerebrovascular events (2 %) [172]. Serious arterial thrombotic events and cardiovascular toxicities were reported in 5-7% of ponatinib-treated patients enrolled in the EPIC and PACE trials, respectively [132]. What's more, results from another RCT showed that around 50% of patients treated with frontline ponatinib experienced either cardiac or vascular events, which lead to the early stop of this trial [133].

Cardiovascular toxicity, however, is not limited to ponatinib treatment, and still represents one of the major concerns during CML treatment, independently from the chosen therapy. Cases of QT prolongation have been reported during treatment with all TKIs. In particular, nilotinib has been associated with the highest risk of cardiac arrhythmias, which led to cases of sudden death during trials and to the

subsequent recommendation of close electrocardiogram evaluations before and during treatment [109,110].

A retrospective cohort analysis by Giles et al. [173], further reported a higher incidence of PAOD among nilotinib-treated patients compared to imatinib. However, in a study conducting adopting a SCORE risk assessment tool from the European Society of Cardiology to distinguish the personal cardiovascular risk of each patient, no cardiovascular events associated with nilotinib treatment were found among low-risk patients [174]. The use of risk assessment tools in both RCTs and real life treatment could therefore help to identify which group of patients could experience the worse adverse events, as well as to estimate the true cardiovascular impact of nilotinib on each subset of patients.

Development of heart failure is considered instead to be quite uncommon for all TKIs treatments [97,175-177].

A possible mechanism responsible for TKI cardiovascular toxicity could be connected to the TKI-dependent inhibition of non- BCR-ABL kinases involved in the vascular endothelial regulation, such as the VEGF [178,179].

In addition, nilotinib was reported to be associated with increased levels of plasma glucose and with alterations in the lipid profile, which on their turn could lead to cardiovascular disorders [112,180].

Similarly, also dasatinib was found to be associated with increased cholesterol levels, comparable to that of nilonib-treated patients and significantly higher than those of patients in imatinib [181].

In light of this, a careful evaluation of cholesterol, glycated haemoglobin and glycaemia levels should be performed before treatment beginning, in order to identify subjects at higher risk of developing glycometabolic disorders and to guide the choice of the most appropriate TKI.

Besides cerebrovascular complications, all BCR-ABL TKIs have been associated with the occurrence of pleural or pericardial effusions, with dasatinib being associated with the highest risk [169]. In the DASISION trial, in particular, 20% of patients needed to discontinue dasatinib treatment due to occurrence of pleural effusion, and 5% of patients were found with pulmonary arterial hypertension, a severe pulmonary toxicity [182].

Data from medical literature have further reported a not-well-established risk of secondary malignancies developed following TKIs treatment. In particular, a study conducted in 2005 suggested an increased risk of malignancies among imatinib-treated patients, with the occurrence of prostate cancer being four times higher compared to the general population [183]. According to a recent population study based on The Surveillance, Epidemiology, and End Results (SEER) database, the risk of secondary malignancies is the highest in the first year of TKI treatment [184].

Imatinib, in fact, plays immune-regulatory effects through the inhibition of T-cells activation and proliferation and through the reduction of primary T-cell responses mediated by dendritic cells [185,186]. In addition, in vitro studies shew an association of imatinib with genetic instability [185-187], which was seen to significantly affect the development as well as the progression of many cancers [188,189].

1.7.8. The gold standard: the stable discontinuation of treatment

According to the current recommendations of both the National Comprehensive Cancer Network (NCCN) and the ELN, TKI treatment for CML patients should be continued indefinitely [59,190].

Despite the great effectiveness of these drugs, however, a life-long treatment with TKIs can be associated with an increased risk of the above mentioned side effects, deeply compromising patients' quality of life [191,192]. In particular, treatment discontinuation may be particularly relevant for fertile women who have achieved an optimal response, because TKI treatment is contraindicated during conception and pregnancy. In these patients, when the optimal response is stable for at least 2 years, TKI discontinuation can be considered, after informed consent and with very frequent molecular monitoring [58] .

Therefore, attempts have been made to evaluate whether therapeutic treatment can be stably and safely discontinued following the achievement of a deep response.

Cases of successful discontinuation have been reported in the past year in patients with CCyR following IFNα treatment [193,194]. Later on, several studies have been conducted to evaluate the effects of stable imatinib discontinuation [195-197]. According to these studies, up to 50% of patients who discontinued imatinib treatment after having achieved deep responses, maintained MMR in median follow-up of 7.5 years [198]. The probability of relapsing after the first 6 months of discontinuation was estimated at 10% [199]. What's more, relapsing patients could re-start treatment and re-obtain deep responses with the same TKI [199], therefore providing assurances on the safety of treatment discontinuation.

Up to now, data on discontinuation of second-generation TKIs are instead poor. Preliminary results of a French pilot study showed that around 60% of patients discontinuing a second-generation TKI maintained MMR after 12 months [200].

As for imatinib, cases of relapse following dasatinib discontinuation are more frequent within the first 7 months, with re-initiation of treatment allowing to re-gain deep molecular responses within 6 months of re-treatment [201].

The possibility of stable and safe treatment discontinuation represents also an important economic challenge. In fact, due to the long-term survival provided by current therapeutic options and to the increasing trend in prevalence, life-long continuation of therapy currently represents a consistent burden for the National Healthcare System (NHS) [202].

Identifying predictive factors for successful discontinuation of TKI remains a key issue. Several independent study correlate longer durations of response, especially of deep MR before stopping, with lower rates of relapses [192]. In the STIM study, imatinib therapy duration and Sokal risk score have been identified as independent prognostic factors for molecular relapse after imatinib cessation: in particular, patients treated for more than 50 months and at lower risk according to the Sokal score, had significantly lower rates of relapse compared to patients treated with imatinib for fewer than 50 months or at high risk of progression[196].

So far, data available from literature indicate that all patients who have relapsed remain sensitive to TKI re-treatment, therefore providing evidence of the safety of treatment discontinuation [192].

1.7.9. Regulatory aspects and costs of TKI treatment in Italy

1.7.9.1.TKIs prescription and delivery

Imatinib (Glivec) was the first BCR-ABL TKI that entered the Italian market, in 2001. Dasatinib (Sprycel) and nilotinib (Tasigna) became available a few years later, in 2006 and 2007, respectively. In 2017, following the expiration of Glivec, generic imatinib entered the market, as well.

In Italy, prescription of all TKIs is limited to haematologist physicians; at time of TKI prescription, compilation of a therapeutic plan is compulsory.

The therapeutic plan is a module, filled by the hospital specialized doctor, that since 1994 has been declared necessary by the Italian Agency of Medicines (AIFA) and by the Health Ministry for the prescription of some drugs, among which TKIs. Therapeutic plans have been introduced for the definition of the reimbursement of the considered drugs, as well as for the assurance of the therapeutic appropriateness.

Data reported in the therapeutic plan include: data of the authorized centre prescribing the drug; patient's personal data; name of the patient's general practitioner; diagnosis of the pathology; drug prescribed, doses, and time of administration; duration of therapy.

Three copies of the therapeutic plan must be completed; one remains within patient's medical records, one is for the patient's general practitioner, and the last one is for the pharmaceutical service of the Local Health Authority (LHA) of patient's residence.

Following prescription by the haematologist, TKIs are delivered to patients directly by the hospital pharmacy. For second and third generation TKIs, the delivery of the drug must be accompanied by the compilation of a specific register created by AIFA. AIFA registers represent a tool introduced in 2005 to assure the prescriptive appropriateness, as well as to control the pharmaceutical expenditure.

Information requested by AIFA register include data of the authorized centre prescribing the drug; patient's personal data; pathology for which the drug is used; drug prescribed, doses, and time of administration; line in which the treatment is administered; eventual causes of switch to a further therapeutic line.

1.7.9.2.Costs of TKIs treatment in Italy

In Italy, pharmacological management of CML in entirely reimbursed by the NHS, i.e. patients do not have to pay for these medications.

Retail prices for a treatment, as reported in the Italian "Informatore del Farmaco" [203], are shown in **table 1.3**. However, real prices at which each hospital buys TKIs may vary according to internal tenders with pharmaceutical companies, as well to private discounting practices.

Focusing on drugs approved in first line, costs per day of treatment with imatinib (assumed at the dose of 300 mg/die) range from 11.67 euros for the generic imatinib to 104.93 euros for the brand Glivec (120 capsules, 100 mg). Costs per day for Tasigna and Sprycel are instead of 165.33 and 220.90 euros, respectively.

	Active principle	Pharmaceutical industry	Dosage	Originator (O) / Generic (G)	Daily dose	Price (€)	Cost (€) per day
Glivec	Imatinib mesilato	Novartis farma spa	120 capsules; 100 mg	O	400 mg	3147.80	104.93
Imanivec	Imatinib mesilato	KRKA Farmaceutici Milano	120 tablets; 100 mg	G	400 mg	944.34	31.45
Imatinib Accord	Imatinib mesilato	Accord Healthcare Italia	120 tablets; 100 mg	G	400 mg	944.34	31.45
Imatinib Accord	Imatinib mesilato	Accord Healthcare Italia	30 tablets; 400 mg	G	400 mg	911.04	30.37
Imatinib AHCL	Imatinib mesilato	Dr Reddy's Laboratories Ltd	120 tablets; 100 mg	G	400 mg	944.34	31.45
Imatinib Aurobindo	Imatinib mesilato	Aurobindo Pharma Italia	120 capsules; 100 mg	G	400 mg	350.00	11.67
Imatinib DOC	Imatinib mesilato	DOC generic	120 capsules; 100 mg	G	400 mg	300.00	11.67
Imatinib EG	Imatinib mesilato	EG	120 capsules; 100 mg	G	400 mg	300.00	11.67
Imatinib Fresenius Kabi	Imatinib mesilato	Fresenius Kabi Italia	60 tablets; 100 mg	G	400 mg	455.52	30.37
Imatinib Mylan	Imatinib mesilato	Mylan	120 tablets; 100 mg	G	400 mg	944.34	31.45
Imatinib Mylan Pharma	Imatinib mesilato	Mylan	120 capsules; 100 mg	G	400 mg	944.34	31.45
Imatinib Ranbaxy	Imatinib mesilato	Ranbaxy Italia	120 tablets; 100 mg	G	400 mg	944.34	31.45
Imatinib Sandoz	Imatinib mesilato	Sandoz	120 tablets; 100 mg	G	400 mg	944.34	31.45
Imatinib Teva	Imatinib mesilato	Teva Italia	120 capsules; 100 mg	G	400 mg	400.00	13.33
Tasigna	Nilotinib cloridrato monoidrato	Novartis farma	112 capsules; 150 mg	O	600 mg	4629.36	165.33
Tasigna	Nilotinib cloridrato monoidrato	Novartis farma	112 capsules; 200 mg	O	600 mg	6172.47	165.35
Sprycel	Dasatinib	Bristol-Myers Squibb	30 tables; 100 mg	O	100 mg	6626.92	220.90
Sprycel	Dasatinib	Bristol-Myers Squibb	30 tables; 140 mg	O	140 mg	6626.92	220.90
Sprycel	Dasatinib	Bristol-Myers Squibb	60 tables; 50 mg	O	100 mg	6626.92	220.90
Sprycel	Dasatinib	Bristol-Myers Squibb	30 tables; 80 mg	O	80 mg	6626.92	220.90
Iclusig	Ponatinib	Incyte Biosciences Italy	60 tables; 15 mg	O	15/30 mg	8862.55	147.71/ 295.42
Iclusig	Ponatinib	Incyte Biosciences Italy	30 tables; 45 mg	O	45 mg	8862.55	295.42
Bosulif	Bosutinib	Pfizer	28 tables; 100 mg	O	500 mg	1543.12	275.56
Bosulif	Bosutinib	Pfizer	28 tables; 500 mg	O	500 mg	6172.47	220.44

Table 1.3: Retail prices for TKI treatments, taken from www.codifa.it

1.8. Rational and Aim

As extensively described in the introduction to this thesis, TKIs have deeply revolutionized the prognosis of CML, proving to be very effective and accounting for survivals comparable to those of the general population.

As CML has progressively switched from a fatal to a "chronic" pathology, standards for the management of CML have moved from short-term survival to long-term outcomes, such as achieving of early deep responses, reducing persistent treatment intolerance, and potentially stopping treatment in stable patients [204].

Data coming from RCTs have therefore progressively become inadequate to assess this, due to their relative short times of follow-up compared to the overall real-life duration of the pathology [204].

Besides "temporary" limitations, populations carefully selected for enrolment in RCTs appear to be quite different from those found in everyday practice. According to a German study, for example, patients enrolled in a CML RCT were in average 11 years younger than patients excluded from it [205]. Such a difference in age appear to be crucial in the assessment of clinical outcomes, with significant differences being reported in the occurrence of adverse events for older patients compared to those enrolled in RCTs [206].

In light of this,

"*a shift in focus from data provided by RCTs to more longitudinal, patient-centric outcomes, such as those addressed in observational studies, may be helpful*" [204].

This thesis aims therefore to take a picture of the management of CML over a decade of real clinical practice in the LHA n.2, Region of Veneto, District of Treviso (Italy), addressing long-term outcomes such as treatment switches, TKI effectiveness and occurrence of adverse events.

2. MATERIALS AND METHODS

2.1. Consulted administrative databases

A retrospective cohort study was performed on the population affected by CML and followed up at the Department of Haematology of the LHA n.2, Region of Veneto, District of Treviso (Italy).

Data were collected from local administrative databases and included:

- demographic data on NHS beneficiaries;
- prescriptions of medicines reimbursed by the NHS and delivered either by territorial or hospital pharmacies:
- therapeutic plans, i.e. specific forms filled in by the physician for the prescription and delivery of some drugs, among which TKIs;
- AIFA national registers, i.e. registries created by the Italian Agency of Medicines (AIFA) and filled in at time of drug delivery for the monitoring of the use of specific drugs, among which second and third generation TKIs:
- hospital discharge records and emergency room attendances, with primary or secondary diagnosis fields coded through ICD9-CM codes;
- blood laboratory tests with their results.

Record linkage among databases was performed using the individual patient identification code (Regional Health Code-RHC) attributed by the NHS to each beneficiary.

In accordance with the Italian rules on privacy, the original RHC was replaced by an anonymous unique alphanumeric identifier.

Besides administrative databases, data coming from the physicians' patient record (PPR) were available as well. The PPR is an internal registry completed by the haematologist at time of each physician visit, and contains information on patient's anamnesis, CML phase at entry, symptoms at diagnosis, risk scores of CML progression, type of BCR-ABL transcript, eventual presence of ACAs, therapeutic treatments, treatment switches and causes, adverse effects (AEs) of treatments, as well as results of haematochemical, cytogenetic and molecular tests.

2.2. Identification of the cohort: inclusion and exclusion criteria

The cohort of patients affected by CML was identified in the period from January 1st 2005 to June 30th 2015.

Patients matching at least one of the following criteria were included in the study:

- hold of a therapeutic plan for a TKI drug, with CML as indication for use;
- registration in the AIFA registers for the monitoring of use of second or third generation TKIs, with CML as indication for use;
- hospitalization(s) with a diagnosis of CML (ICD9-CM code 205.1) in primary or secondary diagnosis field.

According to these inclusion criteria, 163 patients were identified as affected by CML **(figure 2.1)**.

Of them, all patients with no available information on their therapeutic treatment (n=31) were excluded: these patients had been occasionally hospitalized for CML in the Hospital of Treviso, but were then followed up in other Italian hospitals. Similarly, patients that were diagnosed with CML before TKI availability and that prosecuted non-TKIs treatments during the whole study period (n=13) were excluded from the study.

The final examined cohort was therefore composed of 119 patients affected by CML. For 103 of them, PPR was accessible, as well.

For each patient, the real date of diagnosis was captured from his/her PPR. If unavailable, the date of diagnosis was estimated as proxy, considering the first date among first TKI prescription or the first hospitalization with a diagnosis of CML.

From the date of diagnosis, all patients accumulated person-time until the occurrence of the first of i) HSCT transplantation, ii) death, or iii) end of data availability (December 31st 2015). The first date among these events was considered as exit date.

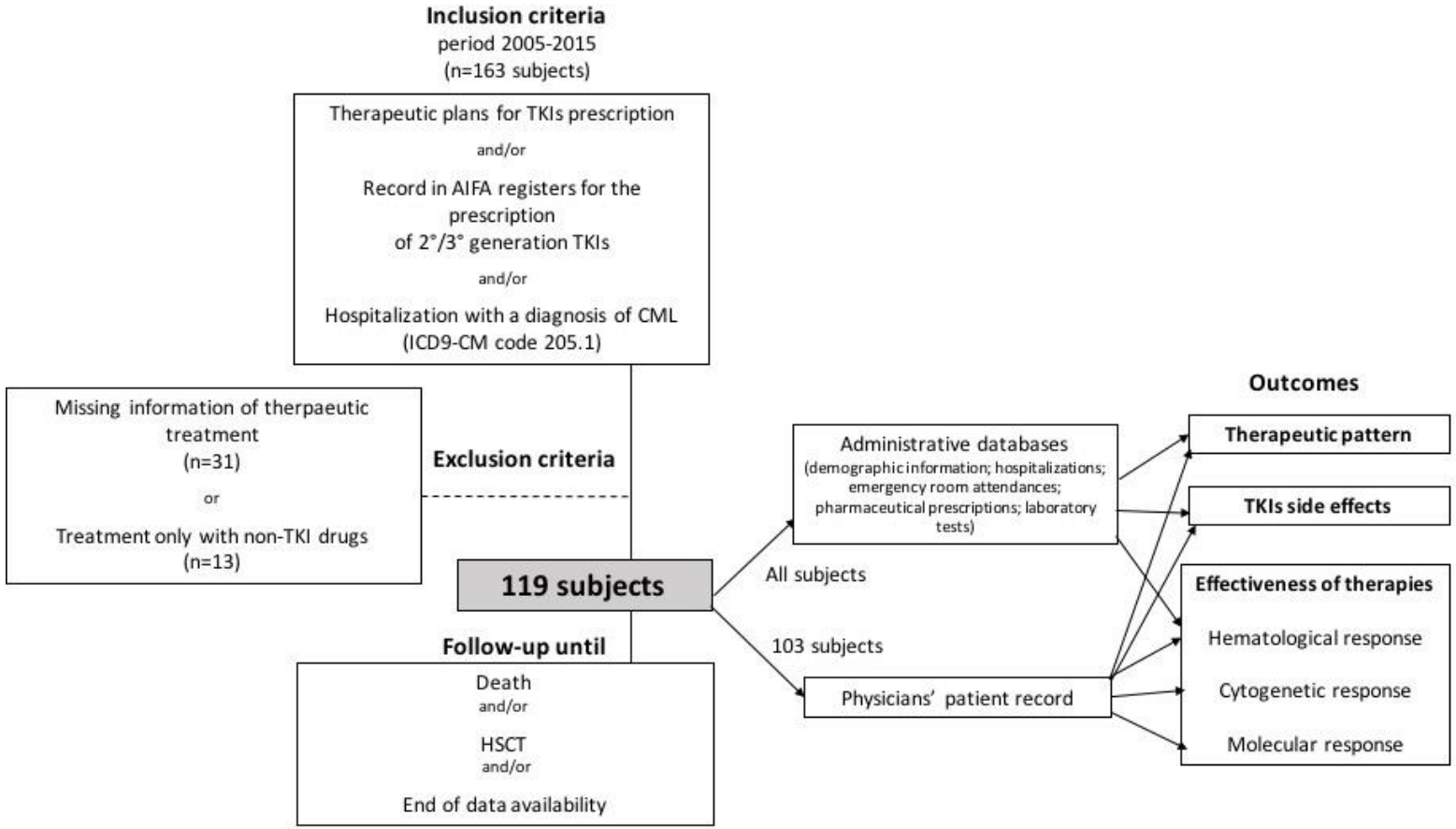

Figure 2.1: Study design and examined outcomes

2.3. Characterization of the cohort

All patients were characterized based on demographic information, i.e. gender and age at diagnosis. When available, patients were characterized also by further information taken from PPR, i.e. i) CML phase at onset, ii) Sokal-Hasford-EUTOS risk scores, iii) symptoms reported at time of diagnosis, iv) BCR-ABL transcript type, and v) presence of ACAs at time of CML diagnosis.

Besides the above mentions characteristics, the presence of other comorbidities at baseline was evaluated as well. Data concerning comorbidities were captured from either:

- anamnestic information reported in the PPR;
- hospitalizations occurred at any time before CML diagnosis, considering ICD-9 CM codes reported in **table 2.1** in either primary or secondary diagnosis fields;
- therapies used at time of diagnosis, captured from the databases of territorial and hospital drug deliveries, considering ATC codes reported in **table 2.1**.

Comorbidities	ICD9-CM codes	ATC codes
Autoimmune pathologies	2420* or 2452* or 555* or 556* or 696* or 710* or 714* or 720*	L04*
Cardiovascular pathologies	402*-404* or 410*-414*or 427*-438* or 7850	C01*
Diabetes	250*	A10*
Disorders of thyroid gland	240* - 246*	H03*
Dyslipidaemia	272*	C10*
Gastrointestinal diseases	530* - 535*	-
Hypertension	401*	C02* or C03* or C07* or C08* or C09*
Mental disorders	290*-319*	N05* or N06*
Neurologic pathologies	330*-337* or 340*-345*	N03* or N04*
Obesity	278*	A08*
Osteoarticular pathologies	715* or 733*	M05*
Previous cancer	104*-208*	-
Renal diseases	584* or 585*	-
Chronic respiratory pathologies	491* or 492* or 493* or 496	R03*

Table 2.1: ICD9-CM and ATC codes considered for the evaluation of comorbidities

2.4. Evaluation of therapeutic pattern

The pharmacological treatment of CML was evaluated starting from the date of diagnosis. Data on active drug, length of treatment, therapeutic switches and reason for switch (where available), were collected from both PPR and administrative databases of territorial and hospital drug deliveries. Of note, therapies were evaluated up to the third line of treatment, i.e. after the occurrence of two therapeutic switches; treatments prescribed after the third line were not included in the analysis.

In addition, variations in drug dosage and temporary interruption of treatment (for up to 2 consequent physician visits) without changes of the drug were not taken into account. These patients were not considered as switchers, and were therefore counted in the same therapeutic line.

Baseline characteristics were compared between CP CML patients prescribed with first vs second generation TKIs, to evaluate whether these factors influenced treatment choice as well as to consider their impact on responses to treatment.

2.5. Assessment of treatment effectiveness

The effectiveness of therapeutic treatments was evaluated based on the achievement of:

- CHR, defined as platelet count $<450x10^9$L and white blood cells count $<10x10^9$L;
- CCyR, defined as the non-detectability of the Ph chromosome through cytogenetic FISH analysis;
- MMR, defined as BCR-ABL transcript levels <0,10 IS detected through RQ-PCR;
- CMR, defined as the non-detectability of the BCR-ABL transcript through RQ-PCR.

The definitions of the above mentioned responses were based on the ELN guidelines [59].

Results of blood tests were captured from both PPR and the administrative database. Instead, results of cytogenetic and molecular analysis were captured only from PPR.

Responses were evaluated both in the overall period, i.e. independently from the time of their achievement, and at specific time milestones, i.e. 3 - 6 - 9 -12 and 24 months after treatment beginning.

Effectiveness of TKIs was assessed overall. In addition, a comparison of the effectiveness of the different TKIs as frontline treatment for CP patients was performed. Time of response achievement was calculated from the first date of TKI prescription to the date of achievement of the considered response. An intention-to-treat approach was used, i.e. achievement of responses was arbitrarily attributed to the frontline treatment used, although a therapeutic switch might have occurred later. The choice of using this approach rather than a per-protocol analysis was driven by the difficulty of estimating the contribution of previous treatments on the achievement of responses.

2.6. Evaluation of the development of additional chromosomal alterations

Development of ACAs was evaluated according to CML phase at entry. In addition, the occurrence and the time to development of ACAs was compared among different frontline TKIs. For this comparison, an intention-to-treat approach was used, i.e. occurrence of ACAs was attributed to the frontline TKI, although a therapeutic switch might had occurred and a different TKI was currently used at time of ACAs development.

2.7. Evaluation of adverse effects

Occurrence of AEs was evaluated at any time following treatment beginning, based on adverse manifestations reported in the PPR, hospitalizations and emergency room attendances.

AEs were evaluated in the overall cohort of patients, independently from CML phase and therapeutic treatment. Furthermore, for patients in CP, occurrence of AEs during frontline treatment was compared among different TKIs.

2.8. Statistical analysis

Descriptive statistic was used to summarize demographics and baseline characteristics.

Continuous variables were reported as median values and range (min-max values), and were compared using a Kruskal-Wallis test. Percentages were compared using Fisher exact test.

A Cox univariate regression model was fitted, with patients accumulating time from treatment beginning to the date of achievement of the considered response (either CHR, CCyR, MMR or CMR). Achievement of responses among different TKIs was compared using a Kaplan Meier model.

Missing data were randomly distributed; cause for missing data was the loss of the records of some laboratory and clinical examinations during the study period. In each analysis, subjects with no available data for the considered variable were excluded. In the analysis of treatment effectiveness, missing data at specific time milestones were imputed equal to those of the previous measure.

Statistical analysis was performed using the software STATA version 14. Statistical significance was considered with p-values <0.05.

3. RESULTS

3.1. Incidence and prevalence of CML

A total cohort of 119 patients affected by CML was identified from January 1st 2005 to June 30th 2015. For 93 of these patients, first diagnosis of CML had actually occurred during this period, with a median incidence of 9 cases/year [range: 2 cases in 2007 -13 cases in 2010]. On the other hand, 26 patients were diagnosed between 1997 and 2004 **(figure 3.1)**.

Of note, also people resident outside the District of Treviso but followed up in the hospital of Treviso were enrolled in this study; therefore, these results do not allow to calculate an incidence and prevalence rate of CML in the considered District.

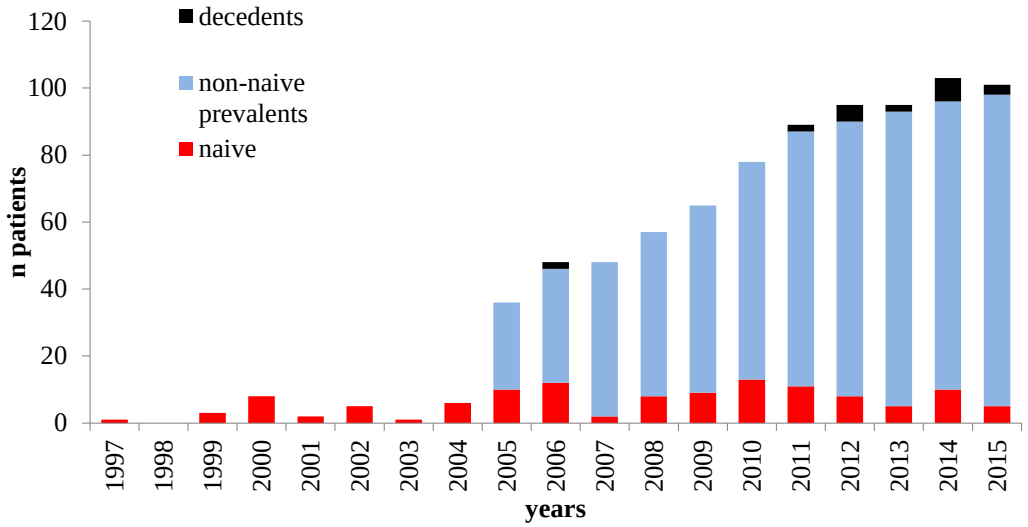

Figure 3.1: prevalence to CML over years

Overall, the median observational time [range] for these patients was 2190.5 days [85 – 6848]. 21 of these patients exited the study because of death, after a

median time of 2164 days [85 – 6186]. The median rate of mortality was of 2 deaths/year [0 – 7]. 3 patients exited the study because of HSCT, after a median time of 350 ± 69 years. All other patients were follow-up until December 31st 2015.

3.2. Baseline characteristics of the cohort

3.2.1. General patients-related characteristics

The baseline characteristics of the cohort are described in **table 3.1**. Information on CML phase at entry was available for 103 out of 119 patients. 97 patients were diagnosed in CP, 1 in AP, and 5 in BC.

Considering sex, 63 patients were men and 56 were women, with a men : women ratio of 1.13. Sex had equal distribution among CML phases at diagnosis.

The mean age ± SE at diagnosis was of 60.38 ± 1.31 years, with more than 50% of patients being 55 or older at diagnosis. No significant variation in mean age was found among patients diagnosed in CP, AP or BC.

Considering CML-related information, the majority of patients were considered at low risk of progression. 11 out of 61 patients with Sokal score, 3 out of 24 subjects with measurement of Hasford score, and 5 out of 28 subjects with EUTOS score, were classified at “high risk”.

Characteristics	n	Chronic phase (n)	Accelerated phase (n)	Blast crisis (n)	p-value
Phase at entry	103	97	1	5	
Sex					
N obs	119	97	1	5	
Men	63	52	1	3	1.00
Women	56	45	0	2	
Age at diagnosis					
N obs	119	97	1	5	
Median age [range]	62 [25-95]	61 [25-95]	79	61 [40-75]	0.31
<35 years	7	6	0	0	0.84
35-54 years	34	29	0	2	
55-74 years	61	50	0	2	
>74 years	17	12	1	1	
Sokal score					
N obs	61	61	0	0	
Low	31	31	-	-	n.c.
Intermediate	19	19	-	-	
High	11	11	-	-	
Hasford score					
N obs	24	24	0	0	
Low	15	15	-	-	n.c.
Intermediate	6	6	-	-	
High	3	3	-	-	
EUTOS score					
N obs	28	28	0	0	
Low	23	23	-	-	n.c.
High	5	5	-	-	

n.c.= not calculable

Table 3.1: Baseline patient's-related characteristics, overall and stratified according to CML phase at entry

3.2.2. CML-related characteristics

General CML-related characteristics are reported in **table 3.2**.

Information on the type of BCR-ABL transcript and on the presence of ACA at diagnosis was available for 77 out of 119 patients.

The e14a2 or the e13a2 transcripts, coming from the major BCR breakpoint, were the most common (41 and 30 patients, respectively). 2 patients carried instead both e14a2 and e13a2 transcripts. The less common e19a2 transcript, coming from

the minor BCR breakpoint, was detected in another 2 patients. The rare e6a2 and e1a2 transcripts were present instead in 1 patient each.

Of note, the type of BCR-ABL transcript resulted to significantly correlate with CML phase at entry (p =0.01). In particular, the majority of patients diagnosed in CP carried either the e14a2 or e13a2 transcripts; this latter transcript was found also in the patient diagnosed in AP. On the other hand, patients carrying either the e6a2 or the e1a2 transcript were all diagnosed in BC, with these two transcripts accounting for 2 out 3 of cases of BC with information on BCR-ABL transcript.

Considering other chromosomal abnormalities, 12 out of 77 patients presented ACAs at diagnosis; 11 of them were diagnosed in CP, whereas 1 patient was in BC.

Characteristics	n	Chronic phase (n)	Accelerated phase (n)	Blast crisis n	p-value
BCR-ABL transcript					
N obs	77	73	1	3	
e14a2	41	40	0	1	0.01
e13a2	30	29	1	0	
e14a2/e13a2	2	2	0	0	-
e19a2	2	2	0	0	-
e6a2	1	0	0	1	-
e1a2	1	0	0	1	-
Presence of ACAs					
Positive for ACA	12	11	**0**	1	0.14

Table 3.2: Baseline CML-related characteristics, overall and stratified according to CML phase at entry

3.2.3. Evaluation of symptoms at diagnosis

Symptoms diagnosis were available for the 103 patients with PPR (**figure 3.2**).

60 of them reported no symptom at diagnosis, with leucocytosis and consequent research for Ph chromosome being detected by chance during routinely laboratory tests.

Even when present, symptoms resulted to be broadly unspecific. In particular, weight loss, asthenia and fever were the most common (12, 10 and 7 patients).

In addition, 23 out of 103 patients were found with hepatosplenomegaly at the first physician visit.

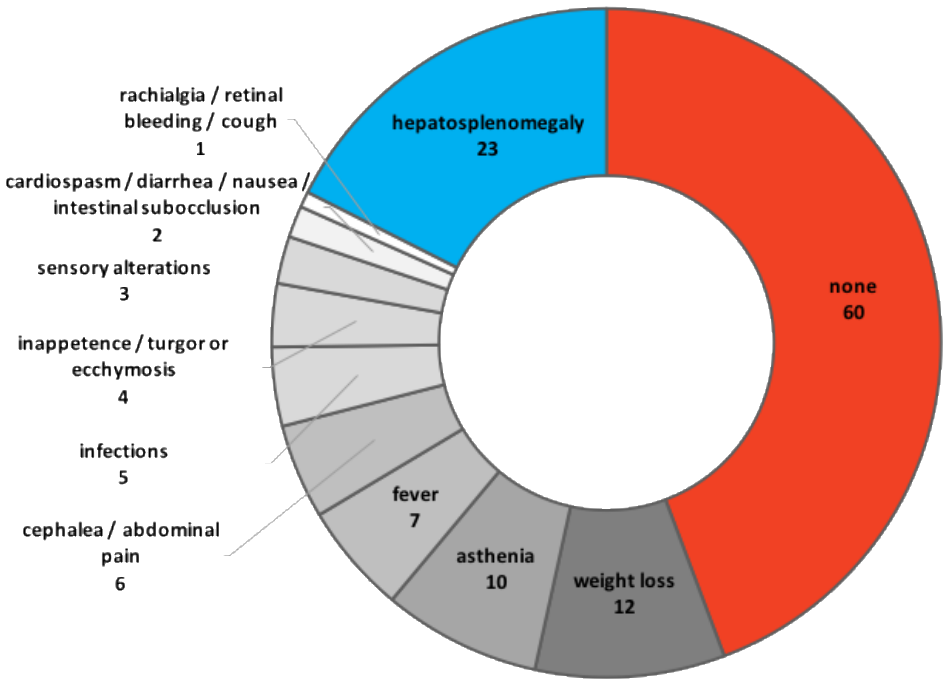

Figure 3.2.: Evaluation of symptoms at diagnosis (n out of 103 patients)

3.2.4. Assessment of comorbidities at baseline

Information on additional comorbidities at baseline was available for all 119 patients (**figure 3.3**).

Cerebro-cardiovascular pathologies were the most common comorbidity (n=63), followed by arterial hypertension (n=59), and chronic respiratory pathologies (n=42).

Furthermore, 26 patients had history of other malignancies. In particular, breast cancer represented 23.08% of cases of previous cancers (n=6 out of 26), followed by hematologic neoplasia and colorectal cancers (n=4, 15.38% each). Other forms of previous malignancies included lung and bladder cancer (n=3 cases each), brain, cutaneous, prostate and uterine cancer (n=2 cases each), and laryngeal, kidney, stomach, and thyroid cancer (n=1 case each).

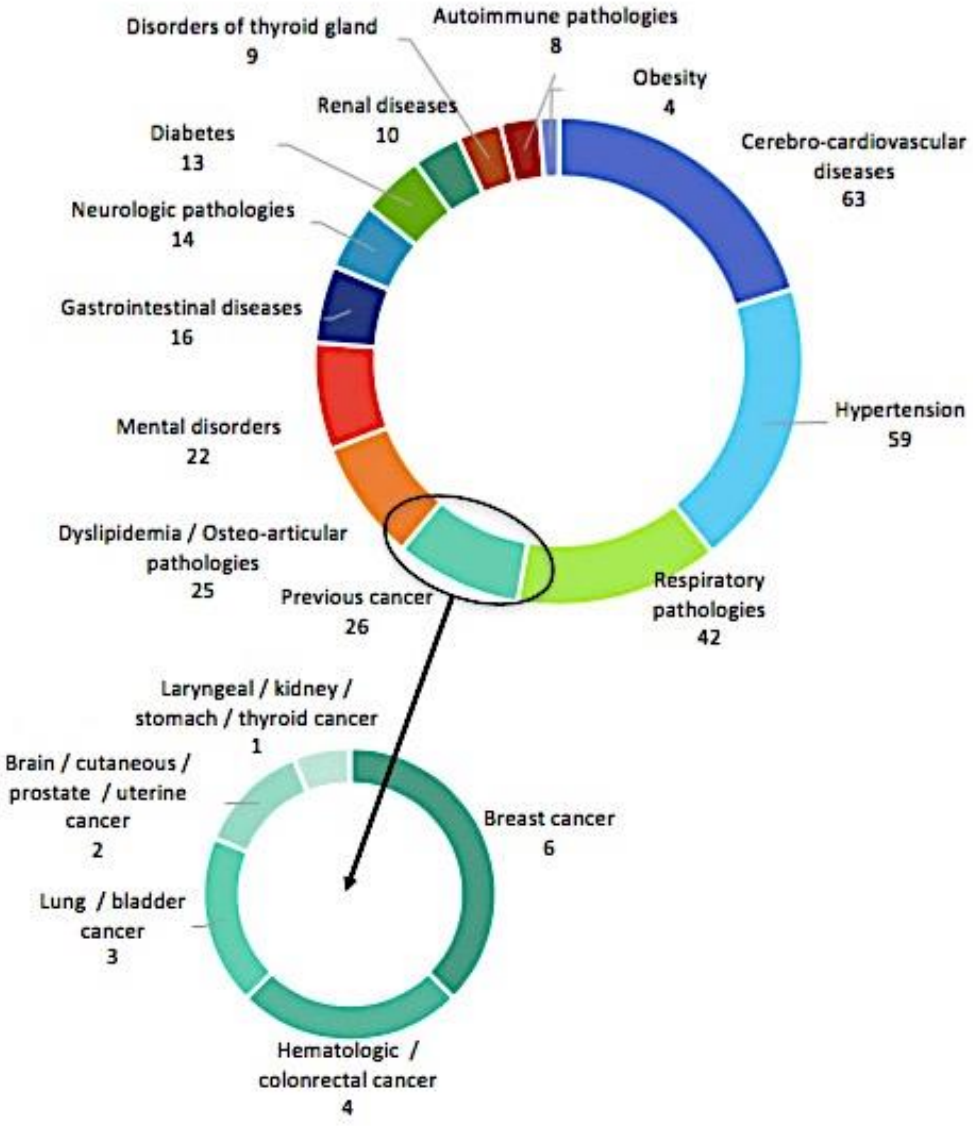

Figure 3.3: Evaluation of comorbidities at diagnosis

3.3. Evaluation of therapeutic pattern

Therapeutic treatment was evaluated separately according to CML phase at entry.

Out of the 97 patients diagnosed in CP, 73 started a first line therapy with imatinib, whereas 8 were prescribed with nilotinib. First-line non-TKIs treatments with HU or IFN were found in 9 and 7 patients, respectively (**figure 3.4.a, table 3.3**).

Median length of treatment was comparable in the imatinib vs nilotinib groups (1663 [13-4106] vs 1313 [281-1805], respectively; p=0.40) (**table 3.3**).

However, the percentage of switches resulted to be different in the two groups: among imatinib-treated patients, therapeutic switches to a second line drug occurred in 28 out of 73 patients; on the other hand, all patients treated with frontline nilotinib prosecuted this therapy without switches. Switches from imatinib were mainly related to intolerance (19 out of 28 cases), but 8 cases were attributable to resistance.

Patients interrupting imatinib mainly switched to a second generation TKI (15 to dasatinib and 12 to nilotinib). Of note, one patient interrupted imatinib and remained off-therapy for 593 days, because of severe intolerance (**figure 3.5.a**).

Considering second line TKI therapies, imatinib, dasatinib and nilotinib were used in 14, 15 and 12 patients, respectively. The median duration of treatment for imatinib resulted to be significantly longer compared to that of dasatinib or nilotinib (4505 [193 – 5520], 1127 [349 – 2183] and 760 [28 – 2450] days for imatinib, nilotinib, and dasatinib, respectively; p-value=0.01). However, the three

treatments resulted to be comparable in term of switches (5 patients in each group; p-value=0.92) (**table 3.3**).

Patients switching from a second line treatment mainly moved to a different second generation TKI. Of note, 2 patients moved to ponatinib, a third generation TKI. In addition, 5 patients discontinued therapy without starting a new treatment; of them, 3 patients interrupted the treatment because of severe toxicity, whereas 2 patients discontinued the therapy following the achievement of a stable complete response after 5291 and 6805 days, respectively.

One patient was diagnosed in AP. This patient was treated with imatinib in first line for 2247 days, with no switches to other drugs (data not shown).

Among the 5 patients diagnosed in BC, dasatinib resulted to be the most common first line treatment (3 patients), whereas imatinib and dasatinib were used in one patient each (**figure 3.4.b**). The patient initially treated with imatinib switched to dasatinib after 9 days, because of treatment intolerance. On the other hand, the patient treated with frontline nilotinib stably continued this treatment for 336 days (**table 3.3**). Among the 3 patients treated with dasatinib, instead, 2 subjects needed to switch to cytarabine (ARA-C), i.e. a pre-HSCT treatment, either in second or in third line.

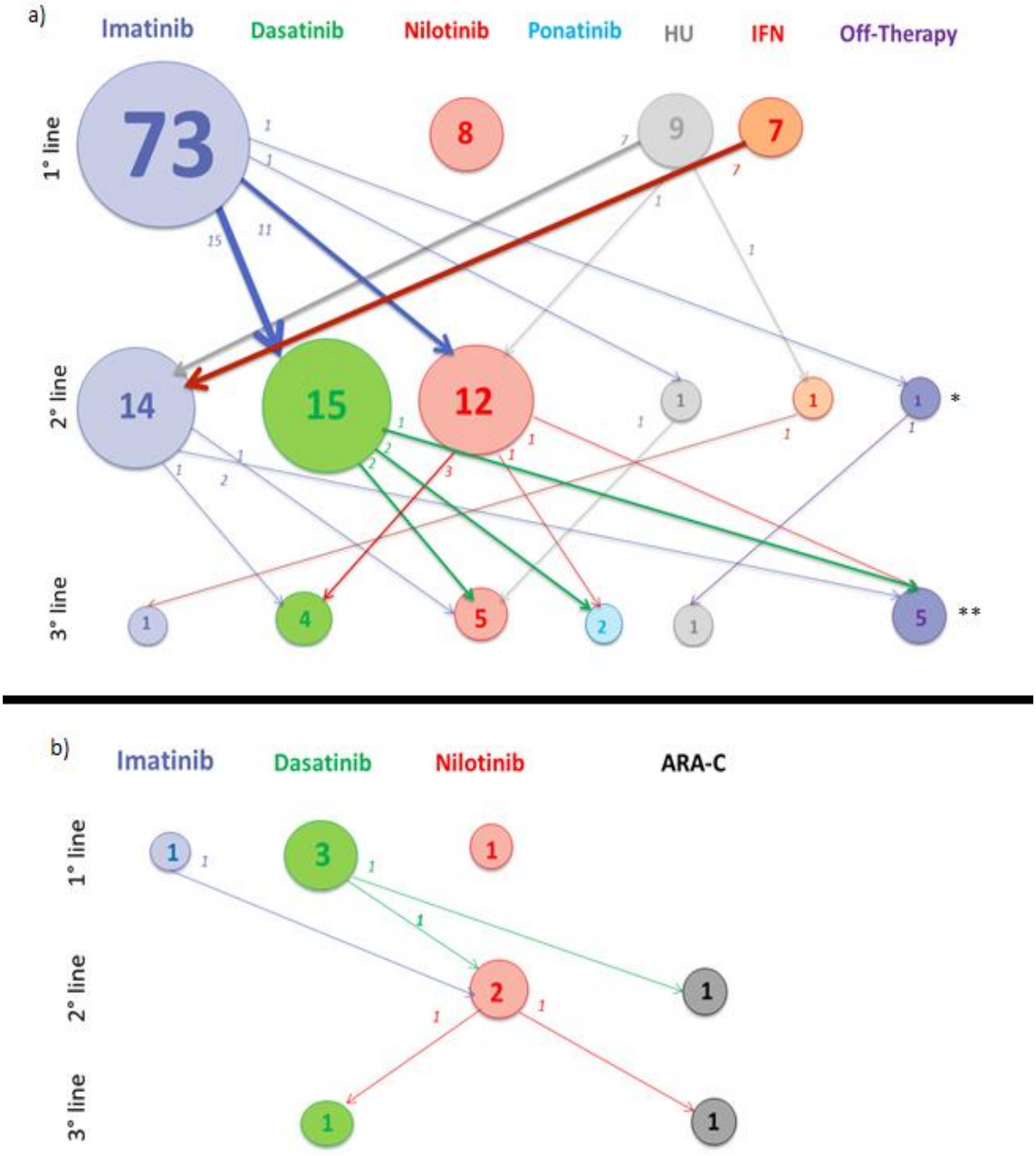

Figure 3.4: Evaluation of the therapeutic pattern for patients diagnosed a) in chronic phase or b) in blast crisis.

*1 patients off-therapy after 1st line treatment due to intolerance.
** 5 patients off-therapy after 2nd line treatment: 3 patients due to extra-haematological toxicity; 2 patients due to achievement of stable complete response.

Treatment line	Phase at diagnosis	Examined outcome	Imatinib (N: Tot)	Nilotinib (N:Tot)	Dasatinib (N:Tot)	p-value
First line	**Chronic phase**	Length of therapy *	1663 [13-4106]	1312.5 [281 – 1805]		0.40
		Time to switch*	533 [13 – 4010]	-		n.c.
		Occurrence of Switch	28:73	0:8	-	n.c.
		Cause of switch				
		Intolerance	19:28	-	-	n.c.
		Resistance	8:28	-	-	-
		Unknown	1:28	-	-	-
Second line		Length of therapy *	4505 [193 – 5520]	1127 [349 – 2183]	760 [28 – 2450]	0.01
		Time to switch*	3835 [193 – 4704]	529 [349 – 1331]	917 [760 – 1595]	0.14
		Occurrence of Switch	5:14	5:12	5:15	0.92
		Cause of switch				
		Intolerance	1:5	1:5	3:5	0.74
		Resistance	1:5	3:5	2:5	
		Unknown	3:5	1:5	-	
First line	**Blast crisis**	Length of therapy *	9	336	160 [42 – 185]	0.20
		Time to switch*	9	-	113.5 [42 – 185]	0.22
		Switch occurrence	1:1	0:1	2:3	1.00
		Cause of switch				
		Intolerance	1:1	-	0:2	n.c.
		Resistance	-	-	1:2	-
		Unknown	-	-	1:2	-

n.c.= not calculable

*Median value [min-max]

Table 3.3: Comparison of length of treatment and occurrence of switches among the different TKI therapies in first or second line, stratified according to patients' phase at entry

Comparisons of baseline characteristics of CP CML patients prescribed with first vs second generation TKIs as frontline treatment are reported in **table 3.4**. Imatinib and nilotinib groups resulted to be comparable in terms of all considered characteristics.

Characteristics	Frontline imatinib N (%)	Frontline nilotinib N (%)	p-value
Tot patients	73	8	
Gender			
Men	38 (52.05)	5 (62.50)	0.72
Women	35 (47.95)	3 (37.50)	
Age at diagnosis			
Median age [range]	62 [25-87]	60 [39-69]	0.28
Risk scores of progression			
Sokal score (tot)	49	6	
Low	26 (53.06)	2 (33.33)	0.46
Intermediate	15 (30.61)	2 (33.33)	
High	8 (16.33)	2 (33.33)	
Hasford score (tot)	20	2	
Low	13:20	1 (50.00)	0.30
Intermediate	5:20	0 (0.00)	
High	2:20	1 (50.00)	
EUTOS score (tot)	23	1	
Low	21:23	0 (0.00)	n.c.
High	2:23	1 (100.00)	
Genetic characteristics			
Type of BCR-ABL transcript (tot)	57	6	
e14a2	30 (52.63)	3 (50.00)	1.00
e13a2	23 (40.35)	3 (50.00)	
e14a2/e13a2	2 (3.51)	0 (0.00)	
e19a2	2 (3.51)	0 (0.00)	
Presence of ACAs (tot)	57	6	
Yes	9 (15.79)	1 (16.67)	0.98
No	48 (84.21)	5 (83.33)	

n.c.= not calculable

Table 3.4: Comparison of baseline characteristics among CP CML patients treated with first vs second generation TKIs as frontline treatment

3.4. Evaluation of treatment effectiveness

3.4.1. Achievement of complete and major responses

The effectiveness of CML treatments was initially evaluated overall, considering the time and the proportion of patients achieving CHR, CCyR, MMR and CMR independently from therapy and CML phase (**figure 3.5**).

Considering hematologic responses, the majority of patients already reached CHR within the first 3 months from treatment beginning (n=87 out of 95 patients; 92%). Percentages of CHR achievement continued growing over time, with 115 out of 116 patients (99%) reaching CHR at any time following treatment beginning.

Similarly, CCyR was reached in 91% of patients (n=89 out of 97 patients), with more than 50% of patients achieving it within the first 6 months from treatment beginning (n=39 out of 67 subjects, 58%).

On the other hand, achievement of MMR and CMR resulted to more difficult, especially at early time milestones. In fact, only 2 out of 49 patients (4%) reached MMR and CMR within the first 3 months from treatment beginning, and less than 50% of patients reached MMR in the first year of treatment. Overall, however, 85% of patients reached MMR (86 out of 101 patients), with 59% of subjects reaching also CMR (60 out of 101).

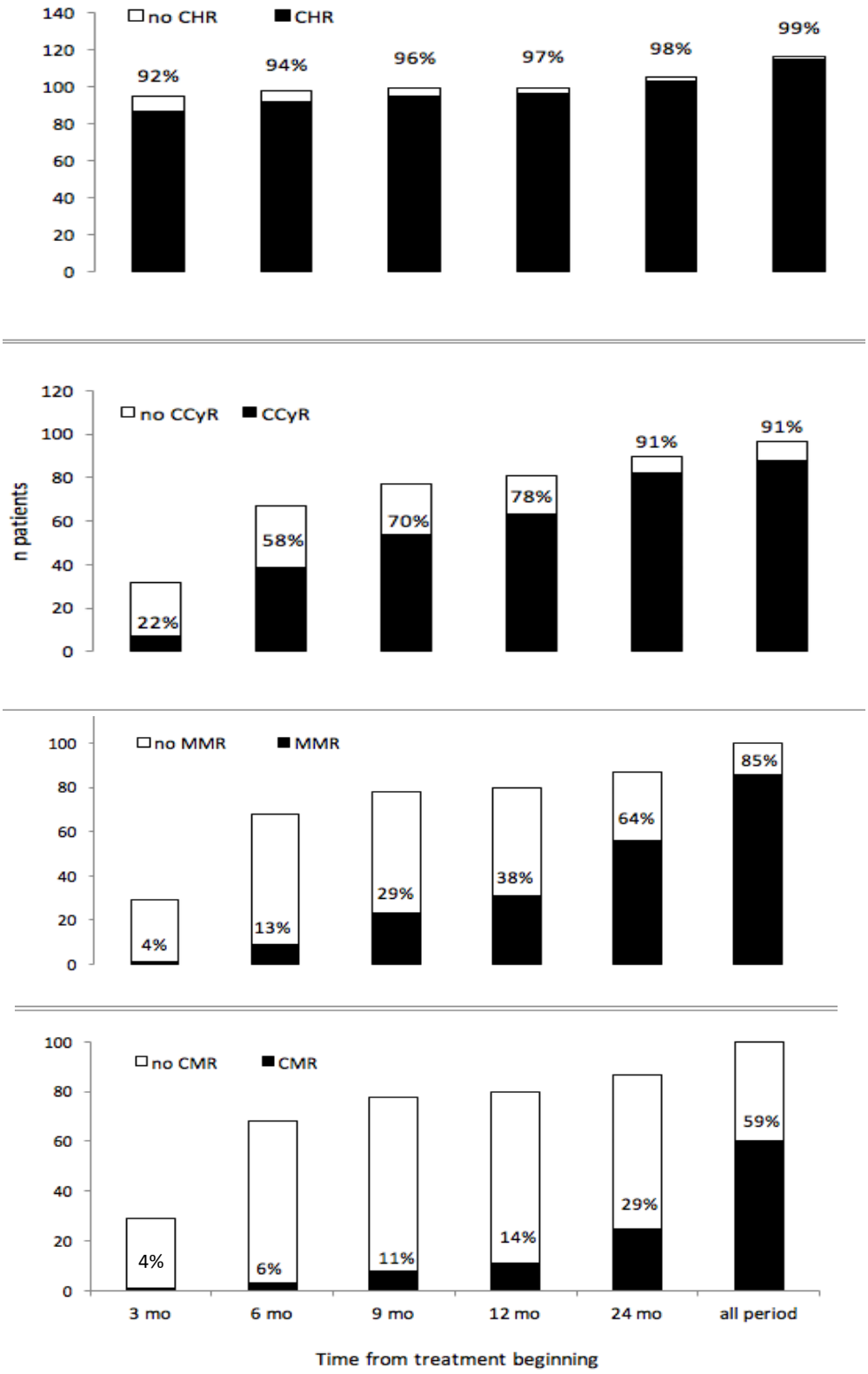

Figure 3.5: Evaluation of the proportion of responses, overall and at fixed time milestones

Beside this overall analysis, achievement of responses was analysed separately according to frontline TKI treatment; for this analysis, only patients diagnosed in CP and treated with TKIs in first line were included.

Nilotinib accounted for higher rate of CHR compared to imatinib **(figure 3.6)**; in fact, all eight patients treated with nilotinib reached CHR within the first 3 months following the start of treatment. On the other hand, only 61 out of 70 patients treated with frontline imatinib reached CHR in the first 3 months, and a further seven later.

Similarly, nilotinib displayed higher effectiveness measured by CCyR and MMR **(figure 3.7 and 3.8)**. In fact, all patients treated with nilotinib reached both CCyR and MMR within 24 months from treatment initiation (7 out of 7 and 8 out of 8 patients where this outcome was recorded). Only 65 out of 69 imatinib-treated patients reached CCyR at any time from imatinib treatment beginning, and only 51 out of 68 patients reached MMR.

With regard to CMR **(figure 3.9)**, imatinib had higher rates of response than nilotinib. 16 out of 68 imatinib-treated patients reached CMR within 24 months from treatment initiation, compared to only 1 out of 8 nilotinib-treated patients. Overall, 4 out of 8 patients treated with frontline nilotinib eventually achieved CMR, compared to 43 out of 68 imatinib-treated subjects.

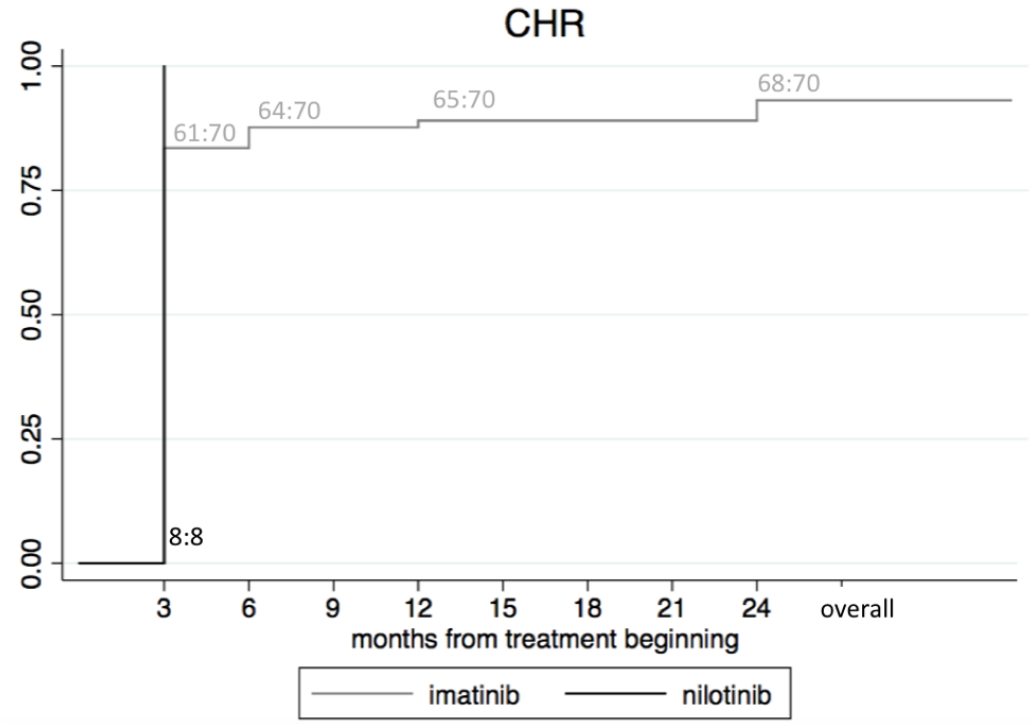

Figure 3.6: Achievement of CHR among CP patients treated with frontline imatinib vs nilotinib

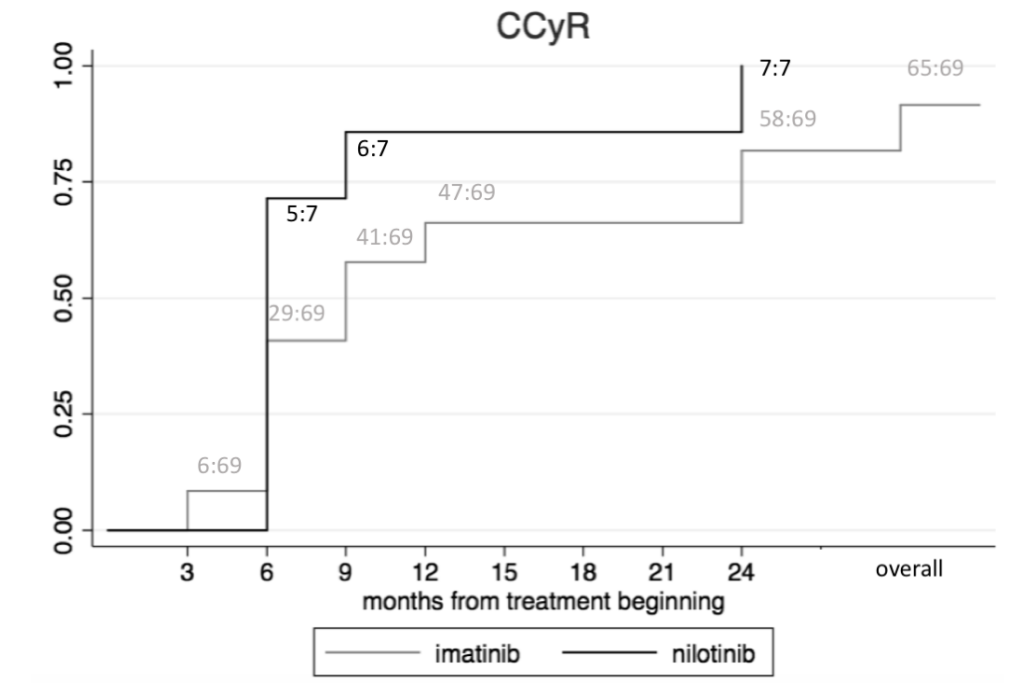

Figure 3.7: Achievement of CCyR among CP patients treated with frontline imatinib vs nilotinib

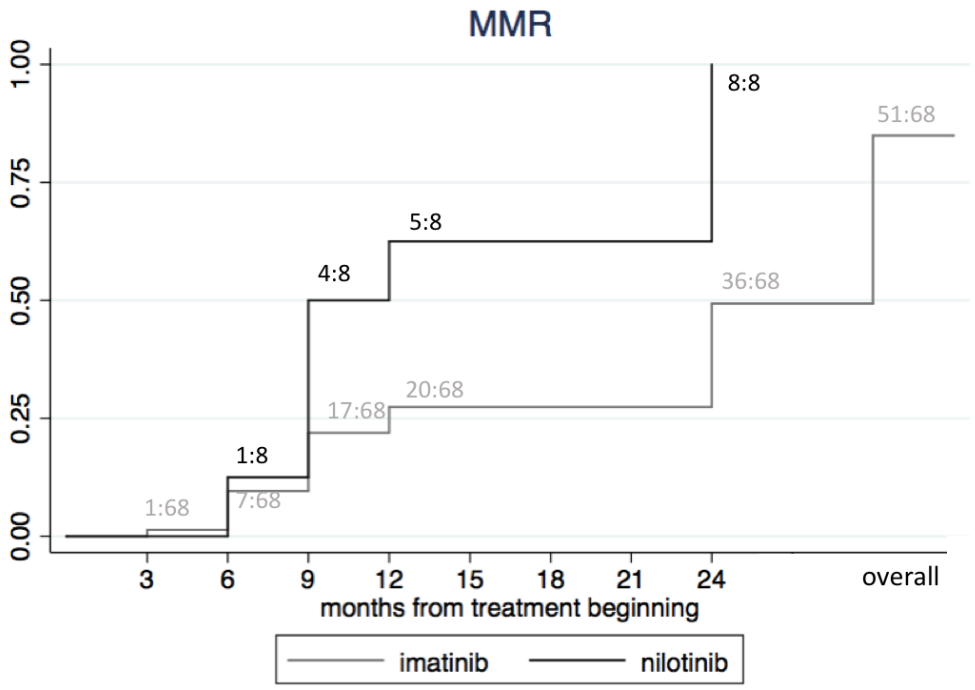

Figure 3.8: Achievement of MMR among CP patients treated with frontline imatinib vs nilotinib

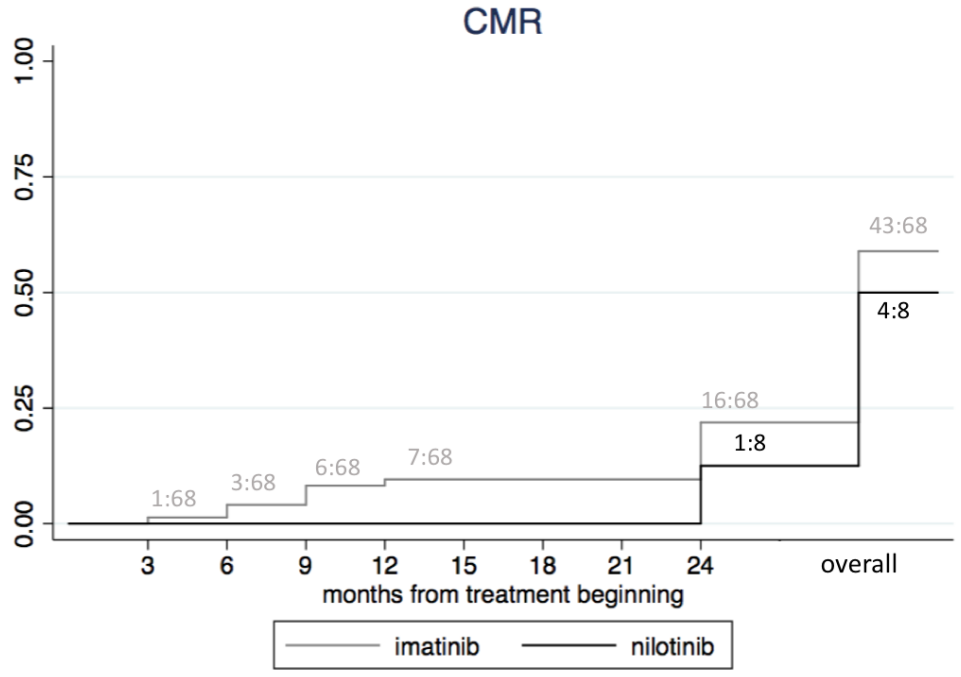

Figure 3.9: Achievement of CMR among CP patients treated with frontline imatinib vs nilotinib

3.4.2. Evaluation of the development of ACAs

Beside achievement of responses, development of ACAs in patients free from ACA at diagnosis was evaluated.

Overall, 10 out of 88 patients developed ACAs at any time following CML diagnosis. Occurrence of ACAs significantly differed according to CML phase ($p < 0.05$). In fact, ACAs were more frequent among patients in AP (1 out of 1 patient) and in BC (1 out of 4 patients); among patients diagnosed in CP, instead, 8 out of 75 subjects developed ACAs (**figure 3.10**).

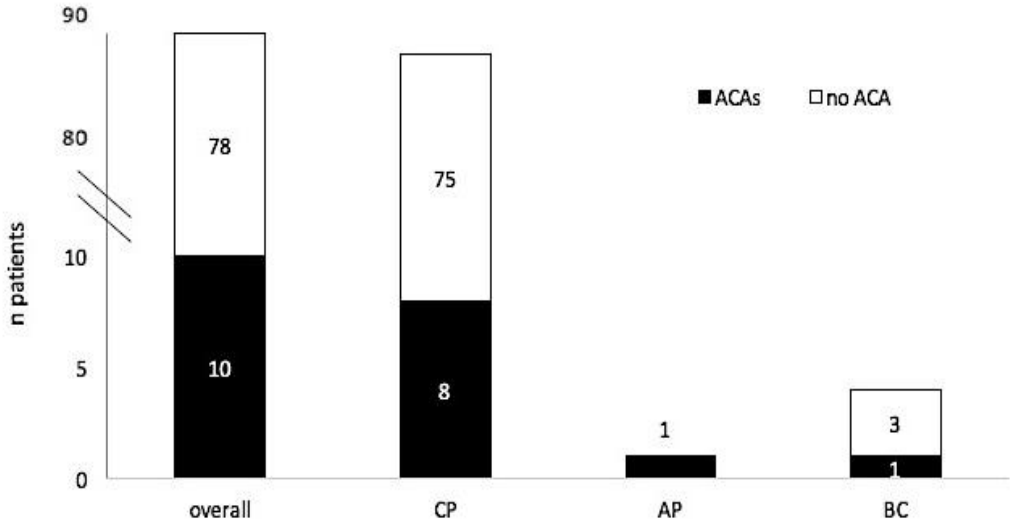

Figure 3.10: Assessment of the development of ACAs in patients with no ACA at diagnosis, overall and stratified according to CML phase at entry

The possible correlation between the development of ACAs and the type of frontline TKI treatment was evaluated (**table 3.5**). Among patients treated with imatinib in frontline, 5 out of 65 subjects where this outcome was recorded

developed ACAs, after a median time of 1640 days [168 – 2801]. Among nilotinib and dasatinib-treated patients, 2 out of 6 and 1 out of 3 patients developed ACAs, after a median time of 110 [103 – 117] days and 158 days, respectively.

	Frontline imatinib N:Tot	**Frontline nilotinib N:Tot**	**Frontline dasatinib N:Tot**	**p-value**
Occurrence of ACAs	5:65	2:6	1:3	0.11
Median time of ACA development [min-max]	1640 [168 – 2801]	110 [103 – 117]	158	0.07

Table 3.5: Occurrence of ACAs according to the different frontline TKIs treatments, overall and stratified according to CML phase at entry

3.4.3. Evaluation of the adverse effects

AEs occurred after treatment initiation are reported in **figure 3.11**.

Osteoarticular pain and dermatologic manifestations were the most common AEs, occurring in 73 and 71 out of 119 subjects, respectively. Other AEs involving a significant number of patients included asthenia (n=48), alterations of cardiac rhythm (n=35), diarrhoea (n=32), epigastric pain, fever and virus reactivation (n=30 each).

Focusing on the most serious AEs, 20 patients were found with heart failure, 14 with pleural effusion, 7 with stroke or ischemic diseases, 4 subjects with acute myocardial infarction, 2 with pericarditis, one patient had an aneurism and another patient had subarachnoid haemorrhage. 14 patients developed benign or malignant neoformations; of them, 3 subjects had colon cancer and, 2 women developed mammary nodules, 2 had thyroid adenoma, 2 had gastric cancer, 1 developed prostate cancer, 1 developed basocellular carcinoma,1 had intestinal adenoma, 1 had uterine cancer, and 1 had a pulmonary nodule.

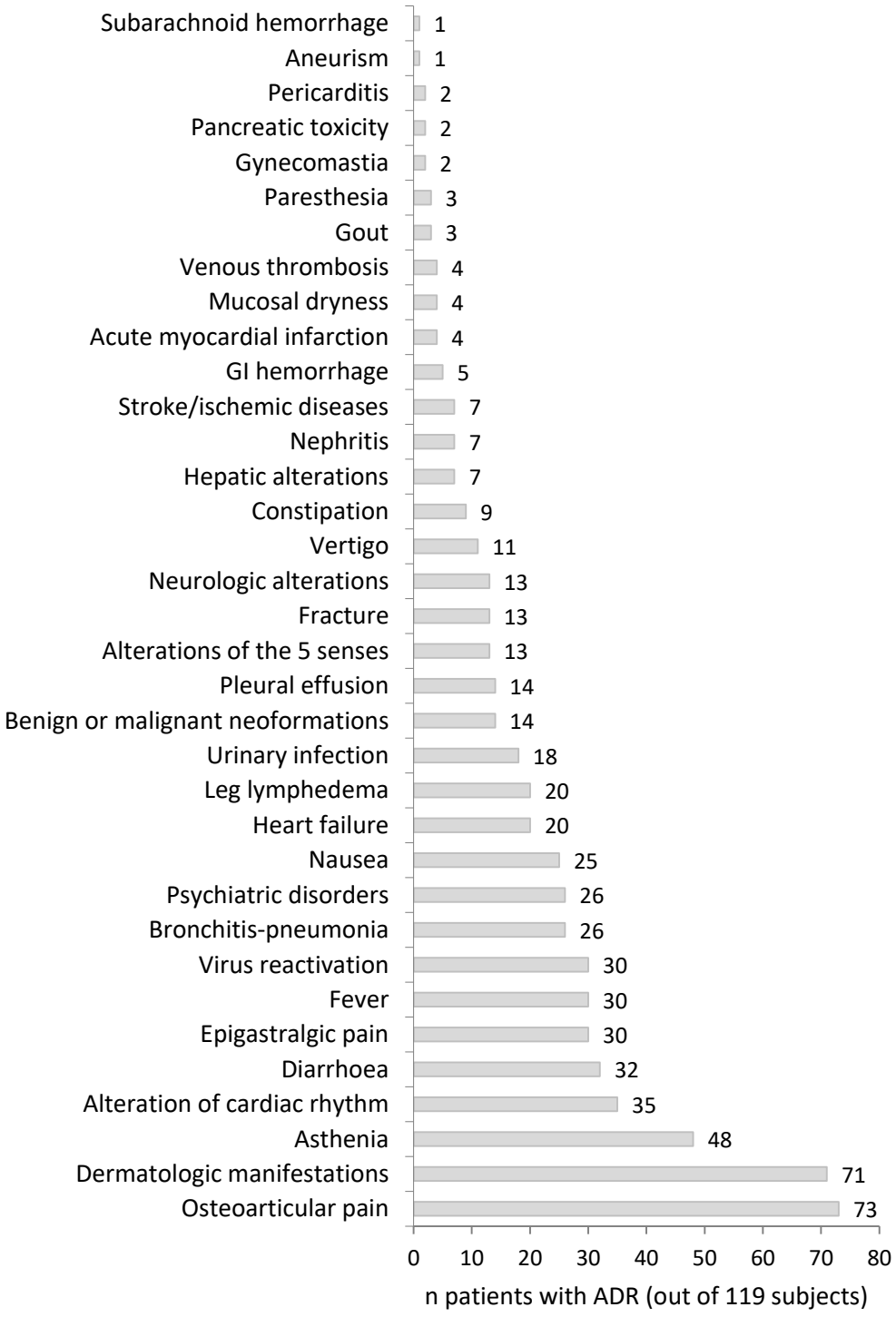

Figure 3.11: Occurrence of adverse effects following CML treatment beginning

To evaluate whether AEs differed among TKIs, occurrence of AEs during frontline CP-CML treatment was compared in imatinib vs nilotinib groups.

Osteoarticular pain resulted to be the most common AE (**table 3.6**), significantly more frequent among imatinib-treated patients (50 out of 72 imatinib-treated vs 2 out of 8 nilotinib-treated patients, p=0.02). Occurrence of all other AEs were similar between imatinib and nilotinib.

Of note, four patients developed other malignancies: one colon cancer, one gastric cancer, one prostate cancer, and another uterine cancer.

Adverse effects (AEs)	Imatinib n=73 (%)	Nilotinib n=8 (%)	p-value
Most frequent AEs			
Osteoarticular pain	50 (68.49)	2 (25.00)	0.02
Dermatologic manifestations	49 (67.12)	3 (37.50)	0.13
Asthenia	29 (39.73)	2 (25.00)	0.70
Diarrhoea	21 (28.77)	2 (25.00)	1.00
Epigastric pain	19 (26.03)	2 (25.00)	1.00
Fever	12 (16.44)	1 (12.50)	1.00
Virus reactivation	12 (16.44)	1 (12.50)	1.00
Alterations of cardiac rhythm	3 (4.11)	0 (0.00)	n.c.
Most serious AEs			
Stroke/Ischemic diseases	4 (5.48)	0 (0.00)	n.c.
Malignancies	4 (5.48)	0 (0.00)	n.c.
Pleural effusion	2 (2.74)	1 (12.50)	0.27
Heart failure	2 (2.74)	1 (12.50)	0.27
Acute myocardial infarction	1 (1.37)	0 (0.00)	n.c.
Aneurism	1 (1.37)	0 (0.00)	n.c.
Pericarditis	0 (0.00)	1 (12.50)	n.c.

Table 3.6: Evaluation of the most common adverse events occurring during first line TKI treatment in CP patients

4. DISCUSSION

This observational study considered the long-term management of CML with TKIs in an Italian real world setting.

The examined cohort, although very limited in size, was typical of patients seen in the real world, in term of both sex and age.

A slightly higher prevalence was found among men compared to women, confirming epidemiological literature [5,207].

The median age at diagnosis was of 61 years, i.e. 14-15 years older compared to that of patients enrolled in the nilotinib vs imatinib ENESTnd RCT, and 13-16 years older than that of the dasatinib vs imatinib DASISION RCT [112,208]. Furthermore, the present population included also patients aged 80 or more at time of CML onset, who are usually excluded from RCTs and whose management can be particularly critical. The discrepancy in term of age between real-life and RCTs populations has been discussed [204,205], and how such RCTs might be proof predictors of response in real world populations has been highlighted [204,206,209].

Most patients in the examined cohort were diagnosed in CP, in line with literature data reporting that around 90% of CML cases are detected during CP [3,5]. Diagnosis of CML in the examined cohort resulted to be mostly fortuitous, with the majority of patients being diagnosed by chance. Even when present, symptoms resulted to be extremely unspecific, with weight loss, asthenia and fever being the most frequently reported.

CML phase at entry resulted to be significantly correlated to the type of BCR-ABL transcript. In accordance with literature [40], the e14a2 and e13a2 transcripts resulted to be the most common, especially among CP patients. Unexpectedly, within this small cohort of patients, we reported the presence of the rare e6a2 and e1a2 transcripts. Of note, both transcripts were found in BC patients, thereby confirming the well-known higher aggressiveness associated with these transcripts[77,210,211].

Furthermore, ACAs at diagnosis were found in 12 patients, with no significant difference among CML phases. CP-patients carrying ACAs or uncommon BCR-ABL transcripts were classified at high risk of CML progression. Among ACAs-free patients, most were considered at low risk of progression, independently from the risk score used. Of note, the Sokal score, although developed before TKI advent, was the most frequently reported in PPR, confirming how this score is still routinely used in clinical practice and is still considered an extremely predictive tool for treatment response and patients' survival [212].

Although being mostly identified as low-risk patients, the majority of patients resulted to be clinically compromised by other comorbidities. In particular, cerebro-cardiovascular pathologies occurred in more than half of the cohort, and one out of five subjects had history of other malignancies. Of note, patients with severe or uncontrolled cardiovascular diseases were excluded from both the ENEST, ENESTnd and DASISION trials [213-215] and little is known on the best management of CML for patients with previous malignancies.

In light of this, this study provides important information on the pharmacological management of CML in the real clinical practice.

Our results clearly show a central role of imatinib as first line treatment for CP patients, compared to a marginal number of patients prescribed with second generation TKIs. Baseline patients' and CML related characteristics were comparable among CP-CML subjects treated with frontline imatinib vs nilotinib, indicating that the choice of frontline treatment was not influenced by these factors.

Of note, nilotinib was the only second-generation TKI prescribed as first line for CP CML, although the indications for its use are comparable to those of dasatinib, and there are no specific recommendations on the choice between the two molecules [59]. Dominance of nilotinib could be motivated by its lower cost per day of treatment compared to dasatinib (165.33 vs 220.90 euros per day); however, considering that real prices at which hospitals buy these drugs might vary according to internal private tenders with pharmaceutical companies, this hypothesis cannot be validated in practice.

This pattern of choice of first line treatments for CP patients is adherent to international recommendations, that recommend second-generation TKIs in patients diagnosed in AP or BC, while leaving to physicians the choice between first and second generation TKIs in low-risk CP patients [59].

Despite the discrepancy in term of patients' baseline characteristics between this study and RCTs, our analysis of the comparative effectiveness of TKIs confirms data from RCTs [112,113], highlighting higher rates of early and overall achievement of CHR, CCyR and MMR among patients treated with first line nilotinib compared

to imatinib. However, unexpectedly, rates of CMR were higher in the imatinib group. Although measured baseline characteristics were comparable in imatinib and nilotinib groups, these finding may suggest a possible selective use of nilotinib as first line in more clinically complex patients.

2 out of 97 CP-CML patients stably discontinued TKI therapy following the achievement of a stable response with imatinib treatment. No case of treatment discontinuation and subsequent relapse was reported. This result suggests that to date long-term management of CML in clinical practice is mainly based on stable prosecution of TKI treatment, in accordance with ELN recommendations [59]. Although still experimental, stable treatment discontinuation is gaining increasing attention in clinical practice, given its crucial impact on patients' quality of life, reduction of adverse effects, as well as on the economic burden of the pathology for the NHS [192]. Moreover, our study finds a difference in the occurrence of therapeutic switches between these two TKIs. In fact, we found no switches in patients treated with frontline nilotinib, compared to 28 out of 73 imatinib-treated patients who switched to a second-line treatment. This result must be taken carefully, given the very small and unbalanced number of patients in the imatinib vs nilotinib group, and the different availability of the two TKIs over the observed period (imatinib was available in the whole period, whereas nilotinib entered the Italian market in 2007). Nevertheless, two recent National and International observational studies by Castagnetti et al. and Machado-Alba et al. [216,217] found similar results, reporting significantly higher proportion of switches in patients treated with imatinib compared to second-generation TKIs, mainly because of resistance.

On the other hand, in our study, most switches from imatinib were due to severe treatment intolerance.

In fact, our results clearly highlight as AEs remain a critical concern during CML management. 61% of examined patients suffered osteoarticular pain following CML treatment beginning, with a significantly higher frequency among imatinib-treated subjects. The association of imatinib with muscle cramps is well known in literature [10]; this AE, although not serious, can deeply compromise a patient's quality of life, leading to poor compliance to treatment and to need of treatment changes. Other frequent AEs included dermatological manifestations, asthenia, diarrhea and epigastric pain.

This study claims the necessity of monitoring of patient's overall clinical condition and of developing a strategy to manage AEs. From this perspective, also the ELN recently published recommendations for the management and avoidance of AEs of treatment in CML [218]. According to them, efforts should be made to predict and manage AEs without reducing or interrupting CML treatment. Particular attention must be given to comorbidities and drug interactions, and to new events unrelated to TKIs that are inevitable during such a long-lasting treatment [218]. In addition, wider studies on the development of secondary malignancies following CML treatment are needed.

This study has limitations. First, the cohort was small, and confined to one Italian district. Evaluation of demographic and disease-related characteristics revealed that this cohort was representative of CML population, as described in National as well as International literature [5,206,207]. Nevertheless, the limited size of this study

compromises the generalizability of these results. Second, there was disproportion in size between imatinib and nilotinib groups. Third, despite baseline characteristic were comparable among CP patients treated with frontline imatinib and nilotinib, unmeasured confounding by indication could be present, i.e. nilotinib could have been mainly likely in patients with clinical characteristics different from imatinib-treated subjects. Fourth, this study covers a prolonged period (2005-2015), and aspects related to treatment might have changed over this time: in particular, while imatinib was available during all this period, nilotinib entered the Italian market only in 2007. Fifth, an intention-to-treat approach was used in the analysis of treatment effectiveness and ACAs development. The choice of adopting this approach was connected to the difficulty in attributing these events to the right treatments, given the high rate of treatment changes and the potential contribution of previous therapies on response achievement. However, this approach could limit the lifelikeness of the obtained results. Sixth, adherence to treatments could not be evaluated. A systematic review by Noens et al [219] reported that non-adherence is common among CML patients, mainly due to drug-related AEs and forgetfulness, and is associated with critical outcomes; however, data on comparative adherence to different TKIs are lacking. Therefore, our results on treatment effectiveness may have been influenced by a poor and different adherence to imatinib and nilotinib. Seventh, underreporting and underestimation of milder AEs could be present, since only acute events leading to hospitalizations or emergency room attendances, or patients' self-reported AEs recorded by physicians were captured. Eighth, patients may be co-followed up by different hospitals and physicians at the same time; therefore, examined PPR and local administrative databases might not be complete,

due to information recorded in other hospital districts. Finally, this study could not take into account the quality of life experienced by patients on different treatments, given that data on patients' perceptions and satisfaction with treatment were not recorded on PPR. Considering that few data on patients' quality of life are available in literature and given the long term duration of CML treatment, further research is worth of been conducted in this area.

5. CONCLUSION

Progress in treatment of CML has been going on so rapid, that any recommendation on the management of CML can quickly become obsolete [70].

Nevertheless, increasing knowledge from the routine clinical practice represents a key strategy in the life-long fighting of this disease, which has now become chronic.

Despite the above-mentioned limits, this study represents an unbiased reference on the long-term management of CML in an Italian clinical setting, providing important real-world evidence on therapeutic patterns and effectiveness of TKIs. Our results indicate a superiority of nilotinib as first line therapy for CP-CML, both in terms of effectiveness and of treatment switches and AEs occurrence. While this might be seen as an argument to use nilotinib first line, it might also argue strongly for the continued use of imatinib first line, reserving nilotinib for imatinib intolerant or resistant patients. This is particularly true as the first generic imatinib is about to be launched, thereby increasing the already high difference in costs for the NHS between imatinib and nilotinib. A full health economic evaluation is required to determine the most cost effective care pathways using these expensive drugs.

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