THERAPEUTIC ADVANCES in
Hematology

Review

Twenty years of evolution of CML therapy: how the treatment goal is moving from disease to patient

Ther Adv Hematol

2023, Vol. 14: 1–16

DOI: 10.1177/20406207231216077

Domenico Russo, Michele Malagola, Nicola Polverelli, Mirko Farina, Federica Re and Simona Bernardi

***Abstract*:** The introduction of imatinib in 2000 opened the era of tyrosine kinase inhibitors (TKIs) for CML therapy and has revolutionized the life expectancy of CML patients, which is now quite like the one of the healthy aged population. Over the last 20 years, both the TKI therapy itself and the objectives have undergone evolutions highlighted and discussed in this review. The main objective of the CML therapy in the first 10 years after TKI introduction was to abolish the disease progression from the chronic to the blastic phase and guarantee the long-term survival of the great majority of patients. In the second 10 years (from 2010 to the present), the main objective of CML therapy moved from survival, considered achieved as a goal, to treatment-free remission (TFR). Two phenomena emerged: no more than 50–60% of CML patients could be candidates for discontinuation and over 50% of them molecularly relapse. The increased cumulative incidence of specific TKI off-target side effects was such relevant to compel to discontinue or reduce the TKI administration in a significant proportion of patients and to avoid a specific TKI in particular settings of patients. Therefore, the treatment strategy must be adapted to each category of patients. What about the patients who do not get or fail the TFR? Should they be compelled to continue the TKIs at the maximum tolerated dose? Alternative strategies based on the principle of minimal effective dose have been tested with success and they are now re-evaluated with more attention, since they guarantee survival and probably a better quality of life, too. Moving from treating the disease to treating the patient is an important change of paradigm. We can say that we are entering a personalized CML therapy, which considers the patients' age, their comorbidities, tolerability, and specific objectives. In this scenario, the new techniques supporting the monitoring of the patients, such as the digital PCR, must be considered. In the present review, we present in deep this evolution and comment on the future perspectives of CML therapy.

Keywords: CML, digital PCR, discontinuation, intermittent, MRD monitoring, personalized treatment, pregnancy, response, TKI, treatment-free remission

Received: 26 May 2023; revised manuscript accepted: 19 October 2023.

Correspondence to:
Domenico Russo
Unit of Blood Diseases and Cell Therapies, Department of Clinical and Experimental Sciences, ASST-Spedali Civili Hospital of Brescia, Piazzale Spedali Civili 1, 25123, Brescia, University of Brescia, Viale Europa 11, 25123, Brescia, Italy
domenico.russo@unibs.it

Michele Malagola
Nicola Polverelli
Mirko Farina
Unit of Blood Diseases and Cell Therapies, Department of Clinical and Experimental Sciences, ASST-Spedali Civili Hospital of Brescia, University of Brescia, Brescia, Italy

Federica Re
Simona Bernardi
Unit of Blood Diseases and Cell Therapies, Department of Clinical and Experimental Sciences, ASST-Spedali Civili Hospital of Brescia, University of Brescia, Brescia, Italy

Centro di Ricerca Emato-oncologico AIL (CREA), ASST-Spedali Civili Hospital of Brescia, Brescia, Italy

Introduction

Imatinib (IM) was the first tyrosine kinase inhibitor (TKI) targeting the constitutively activated p210 *BCR::ABL1* protein, and its introduction in 2000 radically changed the fate of Philadelphia positive Chronic Myeloid Leukemia (Ph+ CML), from fatal to chronic disease.[1]

The first registrative study (IRIS), randomly treating CML patients in chronic phase (CP) with IM *versus* interferon-α (IFNα) + low-dose cytarabine (AraC), reached today a median follow-up of 11 years and showed a 10-year overall survival (OS) of 83.3% for IM-treated patients. The patients achieving the major molecular

response (MMR = *BCR::ABL1* IS ⩽0.1%) and molecular response 4.5 (MR4.5 = *BCR::ABL1* IS ⩽0.0032%) were 93.1% and 63.2%, respectively. Moreover, the 10-year freedom from CML-related death was 100% for those patients with an MMR.[1]

These results were obtained with continuous daily IM treatment, which showed a good toxicity profile since only 9.3% of the patients suffered from grade III–IV therapy-related adverse events (AEs), such as fluid retention, abdominal pain, or liver transaminases. Overall, 39 patients (7.1%) had cardiac AEs of any grade and the incidence of second neoplasms was 11.3%.[1]

Following the registrative study, the clinical research on CML moved forward to the optimization of IM dose and schedule, the combination of IM with other drugs, the development of second- and third-generation TKIs, and the goal of TKI discontinuation aiming to obtain treatment-free remission (TFR) and improve quality of life (QoL). The purpose of this review is to span over the development of CML management, focusing on the main topics that are still a matter of debate.

Imatinib high dose or in combination with IFNα or AraC

From this study onward, other trials tested higher doses of IM or its combination with IFNα or AraC to achieve more, faster, and deeper molecular responses (MR). The pivotal study conducted by the German group (CML-study IV) randomly investigated five different approaches in 1551 patients with CML in CP: IM 800 mg/daily, IM 400 mg/daily + IFNα, IM 400 mg/daily, IM 400 mg/daily + AraC, and IM after IFNα.[2] The OS was greater than 82% at 10 years and was comparable in all the five arms. Molecular responses (in particular MMR and MR4.0) were significantly faster with IM 800 mg, but, inversely, more drug-related AEs occurred with IM 800 mg and IM 400 mg plus IFN. Considering all the patients, the cumulative probability of AEs at 8 years was 76%; in particular, the incidence of grade 3–4 AEs was 22%, of non-hematologic AEs 73%, and of hematologic AEs 28%.[3]

In the same period, the French group investigated a similar strategy in a randomized trial, comparing IM 400 mg/daily *versus* IM 400 mg/daily + AraC *versus* IM 400 mg/daily + Peg-interferon *versus* IM 600 mg/daily. A total of 636 patients were randomized and at 12 months, the rate of MMR was significantly higher for patients receiving IM and Peg-interferon (30%) than for patients receiving 400 mg of IM alone (14%). However, gastrointestinal events were more frequent among patients receiving AraC, whereas rash and depression were more frequent among patients receiving Peg-interferon.[4] As observed also in the German study, no differences were detected in progression-free survival (PFS) between the different treatment arms.[5] The Swedish group also randomized patients with CML in CP at low/intermediate Sokal risk and in complete hematological remission to IM 400 mg/daily *versus* a combination of IM 400 mg/daily + Peg-interferon. The rate of MMR was significantly higher among patients who received the combination of IM and Peg-interferon (82% *versus* 54%), but no differences were observed in PFS; moreover, 61% of the patients had to discontinue Peg-interferon for toxicity.[6] Taken together, these studies provide evidence for a deeper and faster molecular response with the combination of IM and IFNα or a high dose of IM alone. Nevertheless, more patients experienced higher toxicity when IM was combined with IFNα or AraC and when its dose was increased. None of the studies demonstrated an advantage of the PFS or OS for the experimental arms *versus* IM alone at the standard dose of 400 mg/daily (Table 1).

Second-generation TKIs first-line in CP

The goal of achieving more, faster, and deeper MRs was the same objective that guided the introduction of the second-generation TKIs: nilotinib (NIL), dasatinib (DAS), and bosutinib (BOS) which rapidly received approval for first-line CML therapy between 2011 and 2012.

The ENESTnd trial randomly compared NIL 300 mg BID *versus* NIL 400 mg BID *versus* IM 400 mg/daily. Among the 846 patients enrolled, more than half of the patients in each NIL (300 mg twice daily, 54%; 400 mg twice daily, 52%) arm achieved a deep molecular remission (DMR or MR4.5; *BCR::ABL1* ⩽ 0.0032% on the International Scale) compared with 31% in the IM arm. A benefit of NIL was observed across all Sokal risk groups, and each NIL arm resulted in a lower risk of progression to the accelerated phase/blastic phase (AP/BP). Despite that, ENESTnd had a dropout rate of 40% and 50% for NIL and

Table 1. Imatinib in combination with IFNα or AraC.

Study	Pts (N°)	Type of treatment	MMR	MR4.0	OS	PFS	Adverse events Non-hematological	
CML-study IV [1] [2]	1551 *400* *430* *158* *128* *420*	IM 400 mg/daily IM 400 mg/ daily + IFNα IM 400 mg/ daily + AraC IM 400 mg/daily after IFNα IM 800 mg/daily	*@ 1 year* 37% 43% 30% 10% **56%**[a]	*@ 1 year* 8% 16% 6% 1% **20%**[b]	*@ 10 years* 80% 84% 84% 79% 79%	*@ 10 years* 80% 83% 82% 75% 77%	*@ 8 years* 65% (edema, gastrointestinal, myalgia, fatigue) **78%**[c] **(fatigue, neurological, constitutional)** **81%**[c] **(fluid retention, gastrointestinal, myalgia, fatigue, ocular)**	*@ 8 years* 24.7% (pancytopenia) **42.6%**[d] **(pancytopenia)** **29%**[d] **(pancytopenia)**
[3]	636 *159* *160* *158* *159*	IM 400 mg/daily IM 600 mg/daily IM 400 mg/ daily + AraC IM 400 mg/ daily + IFNα	*@ 1 year* 38% 49% 46% **57%**[e]	*@ 1 year* 14% 17% 15% **30%**[f]	*@ 2 years* NS NS NS NS	*@ 2 years* NS NS NS NS	*@ 1 year* More grade 3–4 diarrhea, nausea, vomiting More grade 3–4 rash, depression, asthenia, and edema **High % of pts discontinued INFα in the first year**	*@ 1 year (grade 3–4)* 13% 22% **83% (Neutrop., anemia, Thromb.)**[g] **65% (Neutrop. and anemia)**[g]
[4]	112 56 56	IM 400 mg/daily IM 400 mg/ daily + IFNα	*@ 52 weeks* 54% **82%**[h]	NA NA	NA NA	NS NS	*@ 1 year* 16% grade 3–4 **32% grade 3–4 (musculoskeletal pain, rash, fatigue)** **61% of pts discontinued INFα for toxicity**	*@ 1 year* 14% grade 3–4 **41% grade 3–4 (++Neutrop.)**

[a] p 0.003.
[b] p 0.003.
[c] IM 800 *versus* IM 400 $p < 0.001$; IM 400 + IFNα *versus* IM 400 $p < 0.001$.
[d] IM 800 *versus* IM 400 p 0.006; IM 400 + IFNα *versus* IM 400 p 0.017; in brackets the symptoms with a statistically significant difference.
[e] $p < 0.001$.
[f] p 0.001.
[g] IM 400 + IFNα *versus* IM 400 $p < 0.001$; IM 400 + AraC *versus* IM 400 $p < 0.001$; thrombocytopenia was more common with AraC than with IFNα ($p < 0.001$).
[h] p 0.002.
AraC, cytarabine; IFNα, interferon-α; IM, imatinib; MMR, major molecular response; MR4.0, molecular response 4.0; NA, not applicable; Neutrop., neutropenia; NS, not significative; OS, overall survival; PFS, progression-free survival; Pts, patients; Thromb., thrombocythemia; wks, weeks; ys, years.
The italic refers to the time and guides the reader in understanding the evolution of the side effects.
The bold refers to the main adverse events.

imatinib, respectively. This latter makes comparisons uncertain.[7]

The DASISION trial randomly compared DAS 100 mg ($n = 259$) *versus* IM 400 g ($n = 260$) in newly diagnosed CP-CML patients. The rate of MMR or DMR was higher in the DAS group but no differences in long-term outcomes were recorded.[8]

Finally, in the BFORE trial, 536 patients with treatment-naive CML in CP were randomly assigned to receive BOS 400 mg/daily *versus* IM 400 mg/daily. Again, the rate of MMR was higher and faster in the BOS arm but the percentage of patients who progressed to AP/BP was comparable across the two groups (Table 2).[9]

In summary, in terms of efficacy, these sponsored trials showed that second-generation TKIs induced a DMR more frequently and faster than IM but this did not translate into an improvement of long-term OS, except NIL in the ENESTnd trial. However, they proved to overcome resistance due to additional acquired ABL mutations, with the exception of the T315I. In terms of toxicity, they resulted in different profiles of side effects, suggesting a preferential use in specific categories of patients selected on comorbidity profile or resistance to IM sustained by ABL mutations.

Table 2. Improvement of patients' outcomes observed in clinical trials comparing second-generation TKIs with IM administered at standard dose (400 mg/daily).

Second-generation TKI (dose)	Number of patients treated with second-generation TKI (total number of enrolled patients)	Result obtained with IM	Improvement obtained by second-generation TKI	Clinical trial
Nilotinib (300 mg/twice daily)	282 (846)	31% 5-year DMR	54% 5-year DMR	ENESTnd[5]
Nilotinib (400 mg/twice daily)	283 (846)	31% 5-year DMR	52% 5-year DMR	ENESTnd[5]
Dasatinib (100 mg/daily)	259 (519)	64% 3-month *BCR::ABL1* ⩽ 10% 64% 5-year MMR 33% 5-year MR4.5	84% 3-months *BCR::ABL1* ⩽ 10% 76% 5-year MMR 42% 5-year MR4.5	DASISION[6]
Bosutinib (400 mg/daily)	268 (536)	- 66.4% 12-month CCyR - 57.3% 3-month *BCR::ABL1* ⩽ 10% - 36.9% 12-month MMR	- 77.2% 12-month CCyR - 75.2% 3-month *BCR::ABL1* ⩽ 10% - 47.2% 12-month MMR	BFORE[7]

CCyR, complete cytogenetic response; DMR, deep molecular response; IM, imatinib; MMR, major molecular response; MR4.5, molecular response 4.5; TKI, tyrosine kinase inhibitors.

In particular, for NIL, cardiovascular events are the most frequently observed (20% and 25% at 5 and 10 years, respectively),[10] and a history of coronary heart disease, cerebrovascular accidents, or peripheral arterial-occlusive disease has become a strong contraindication for using NIL. Another frequent toxicity is represented by pancreatitis (5% of treated patients). For DAS, pleuro-pulmonary toxicity, in terms of recurrent pleural effusions (37%), is the most frequent toxic event, and this may occur even after years of previously uncomplicated treatment. For BOS, diarrhea (70%) and transient elevations of transaminases are frequently observed. A summary of the side effects is reported in Table 3.

Guidelines on CML management

In the last 15 years, a series of CML therapy guidelines have been produced by the European Leukemia Net (ELN)[11–14] and by the National Comprehensive Cancer Network (NCCN).[15–19] Table 4 reports a comparison of the last edition of the ELN[12] and the NCCN guidelines.[19] They were mainly focused on the early identification of poor responsive patients and, eventually, on the intensification of therapy by dose adjustment or the switch of TKI. In this context, the switch of TKIs related to early identification of resistant sub-clones is strongly recommended and will help the reduction of resistance and the control of the resistant leukemic cells.

The recommendations of these guidelines continue to be followed to achieve the highest OS and PFS probability for CML patients by managing the treatment according to the best hematological, cytogenetic, and molecular response measured at 3, 6, and 12 months.

Therefore, these guidelines do not take into consideration neither the characteristics of the patients, such as the age and the presence of comorbidity, nor the risk of the disease. Moreover, these guidelines were not generated to select the best candidates for TKI discontinuation and TFR, the new goal of CML therapy that overcomes the goal of survival.

TKI discontinuation and TFR

Obtaining a deeper and faster MR became the 'must have' of TKI therapy when Mahon *et al.*[20] first reported that the TFR could be pursued and

Table 3. Second-generation TKI toxicity.

TKI treatment	Adverse events	Incidence (%)	Number of patients (total number of enrolled patients)	Clinical trial
Nilotinib (300 mg/ twice daily)	Medically severe fluid retention Cardiovascular events Hypertension Significant bleeding Second malignancies Hepatotoxicity Pancreatitis Symptomatic QT prolongation (syncope or convulsion)	11.1 16.5 10.4 3.6 4.7 1.8 1.8 1.8	282 (846)	ENESTnd[5,10]
Nilotinib (400 mg/ twice daily)	Medically severe fluid retention Cardiovascular events Hypertension Significant bleeding Second malignancies Hepatotoxicity Pancreatitis Symptomatic QT prolongation (syncope or convulsion)	14.4 23.5 8.3 5.4 3.2 5.4 2.9 2.5	283 (846)	ENESTnd[5,10]
Dasatinib (100 mg/ daily)	Pleural effusions Neutropenia Thrombocytopenia Cardiovascular events Anemia Pulmonary hypertension	37 29 22 15 13 5	259 (519)	DASISION[6]
Bosutinib (400 mg/ daily)	Diarrhea Nausea Thrombocytopenia Increased ALT Increased AST Hematological Musculoskeletal Infections	70.1 35.1 35.1 30.6 22.8 45.5 29.5 44.4	268 (536)	BFORE[7]

ALT, alanyl aminotransferase; AST, aspartate aminotrasferase; TKI, tyrosine kinase inhibitor.

achievable in about 50% of patients with complete molecular remission or DMR lasting 2 years or more.

This first observation was confirmed in many other studies (Table 5).

Therefore, from 2010 onward, the main objective of CML therapy was no longer survival, which was considered a goal guaranteed by the achievement of MMR, but the TFR, based on the expectation that the proportion of patients achieving a stable DMR could be significantly increased by time through the extensive use of the more potent second-generation TKIs.

In ENEST freedom[21,22] patients with 1-year DMR after NIL maintained the TFR in about 50% of the cases at 12 years. Similarly, in the EUROSKI trial, 50% of the patients who discontinued any TKI after stable DMR for 1 year maintained the TFR.[23] Similarly, a recent study from the MD Anderson Center reports a TFR rate of 80% if the duration of DMR at the MR4.5 is 6 years.[24] Finally, in the DASFREE trial, 46% of the patients in sustained DMR for 1 year maintained the TFR after DAS discontinuation.[25] Similar results have been reproduced also in the routine practice. The Spanish group reported a nationwide series of 236 CML patients who discontinued TKI outside clinical trials. TKI

Table 4. Comparison of ELN 2020 and NCCN 2020 guidelines.

Levels of response	NCCN 2020	ELN 2020
	BCR::ABL1/ABL	
Optimal response		
Baseline	–	–
3 months	<10%	⩽10%
6 months	<10%	⩽1%
12 months	⩽1%	⩽0.1%
>12 months	⩽0.1%	⩽0.1%
Warning		
Baseline	–	High-risk ACA
3 months	>10%	>10%
6 months	–	>1–10%
12 months	>1–10%	>0.1–1%
>12 months	–	>0.1–1%, loss of ⩽0.1%
Failure		
Baseline	–	–
3 months	–	>10% confirmed
6 months	>10%	>10%
12 months	>10%	>1%
>12 months	>1%	>1%, resistance mutations, high-risk ACA

ACA, additional cytogenetical abnormalities; ELN, European Leukemia Net; NCCN, National Comprehensive Cancer Network.

therapy was resumed due to MMR loss in 52 patients (22%). In this study, the TFR rate at 4 years was 64% and no patient had disease progression.[26]

Another real-life Italian study was conducted on 293 patients who discontinued TKI in DMR (72% were on IM at the time of discontinuation). Eighty-eight percent of patients discontinued as per clinical practice, and the reasons for stopping treatment were as follows: toxicity (20%), pregnancy (6%), and a shared decision between treating physician and patient (62%). After a median follow-up of 34 months (range, 12–161) TFR at 12 months was 68% for IM, 73% for second-generation TKI.[27] The German group recently reported the practice among 33 German Centers (797 patients). The mean time from TKI initiation to discontinuation was 7.2 years; the mean duration of MR4 before TFR was 3.5 years. After entering TFR, 53.2% of patients remained in MR4 or better.[28] Finally, the Chinese group retrospectively analyzed 190 patients who stopped TKI. With a median follow-up after stopping TKI treatment of 17 months, the estimated TFR were 76.9%, 68.8%, and 65.5% at 6, 12, and 24 months.[29]

Alternative strategies with respect to TKI discontinuation

While IM induces a deep MR over a longer median time as compared to second-generation TKIs, these last, in turn, increase the percentage of possible candidates to discontinuation in a median shorter time but also the percentage of patients who had to discontinue the therapy because of toxicity or intolerance.

At that time, it was around 2010, a different strategy was first investigated by the Italian group of CML study. It consisted of an intermittent (1 month ON and 1 month OFF) IM schedule for those patients in stable complete cytogenetic response (CcyR). This treatment was first explored in the phase II INTERIM study, and it was demonstrated to be feasible and safe.[30] After 6 years of follow-up, 16/76 patients (21%) have lost CCyR and MMR, and 16 patients (21%) have lost MMR only. Neither progression to BP nor CML-related deaths were recorded. All the patients who had lost the CCyR regained the CCyR after resuming IM continuously and 60% are on intermittent treatment in CCyR and MMR or MR4.0.[31] In fact, CCyR and MMR have been reported as equal in terms of survival by NCCN.[19] Furthermore, grade I–II side effects disappeared in more than 50% of the patients on intermittent treatment.

A second study (DESTINY) investigating a de-escalation and then the stop of IM/NIL/DAS was proposed by Clark *et al.* to get the TFR. Adult patients with chronic myeloid leukemia in first CP, who had received TKI therapy for 3 years or more, with at least a sustained MMR

Table 5. Clinical trials investigating TFR in Ph+-CML patients.

References	Study acronym	Inclusion criteria	Pts (n°)	TKI	Line of therapy	Long-term (⩾2 years) TFR
Imagawa J, *Lancet Hematol* 2015	DADI	DMR ⩾ 1 year	88	DAS	First	49% (6 months)
Etienne G, *JCO* 2017	STIM	DMR ⩾ 2 years	100	IM	First	38%
Campiotti L, *Eur J Cancer* 2017	Meta-analysis	Undetectable	509	IM	First	59% (6 months)
Rea D, *Blood* 2017	STOP 2G-TKI	DMR ⩾ 2 years	60	DAS/NIL	First/second	54%
Hochhaus A, *Leukemia* 2017	ENEST-freddom	DMR ⩾ 2 years	190	NIL	First	52% (12 months)
Ross DM, *Leukemia* 2018	TWISTER	DMR ⩾ 2 years	40	IM	First	45%
Lee SE, *Leukemia* 2018	KID	DMR ⩾ 2 years	90	IM	First	59%
Ross DM, *J Cancer Res Clin Oncol* 2018	ENEST-freedom	DMR = 1 years	190	NIL	First	49%
Mahon FX, *Ann Int Med* 2018	ENEStop	DMR = 1 year	126	NIL	Second	53%
Okada M, *Clin Lymph Myeloma Leuk* 2018	DADI	DMR = 1 year	63	DAS	Second	44%
Saussele S, *Lancet Oncol* 2018	EUROSKI	DMR = 1 year	758	Any	First	50%
Shah NP, *Leuk Lymph* 2020	DASFREE	DMR = 1 year	84	DAS	First/second	46%
Kimura S, *Lancet Hematol* 2020	DADI	DMR ⩾ 2 years	68	DAS	First	55% (6 months)

DMR, deep molecular response; DAS, dasatinib; IM, imatinib; NIL, nilotinib; Pts, patients; TFR, treatment free remission; TKI, tyrosine kinase inhibitor.

in the 12 months de-escalated to half the standard dose for 12 months, then stopped for a further 24 months. Forty-nine patients in the MMR group and 125 in the MR4 group have been enrolled. Recurrence-free survival was 72% and 36% in the MR4 and MMR groups, respectively. All recurrences regained MMR within 5 months of treatment resumption and no disease progression was seen.[32] Another critical issue was covered in the study by Iurlo *et al.* This retrospective, multicentric analysis focuses on 248 CML patients who discontinued TKI after a low-dose treatment more frequently because of comorbidities. In all, 245 of these patients were in DMR at the time of TKI discontinuation, and 69% of them maintained this response after a median follow-up of 24.9 months. The factors that influenced the probability of maintaining TFR were as follows: the absence of TKIs' resistance, DMR duration before discontinuation longer than 6.8 years, and e14a2 fusion transcript. The authors concluded that a low dose of TKI before treatment discontinuation does not seem to hamper the probability of achieving a DMR and, thus, the probability of maintaining the TFR.[33]

Ten years later, a summary of the TFR strategy pursued from the studies aimed at speeding and increasing the DMRs can be done.

These studies clearly showed that the rate of patients who could be candidates for treatment discontinuation was no more than 50–60% and,

moreover, that the TFR rate was ever 50%. In other words, it was evident that the benefit of the TFR strategy could be restricted to no more than 25–30% of the entire CML population. This is an important benefit, but it regards a small proportion of CML patients, similar to what happened in the past for the allogeneic Hematopoietic Stem Cells Transplantation (allo-SCT) or IFNα.

Toward the identification of the best candidates for TFR

The difficulty in the identification of the patients eligible for TFR led to the inadequacy of Real Time quantitative PCR (RT-qPCR) to assess correctly and precisely the stable DMR as one of the reasons.

In the last years, many efforts have been made to overcome the intrinsic technical limits of RT-qPCR, both improving the workflow and looking at further new tools.[34] Among them, one of the most promising technologies is the digital PCR (dPCR). Indeed, the dPCR was developed to allow the detection of small amounts of target nucleic acids. The rationale of this emerging technology is the performance of an absolute quantification of the target thanks to the random distribution of molecules in a high number of partitions (chip wells or water-in-oil droplets) which serve as micro-reactions.[35,36] Today, the most common dPCR platforms present up to 20,000 micro-reactions.

Recently, several groups have faced dPCR for *BCR::ABL1* transcript absolute quantification in CML patients[37–42] and the accuracy and sensitivity of this approach have been demonstrated by all the reported data. These agree in confirming the accuracy and sensitivity of dPCR in *BCR::ABL1* transcript absolute quantification. Moreover, a better selection of CML patients eligible for TKIs discontinuation has been described in several publications comparing RT-qPCR to dPCR,[39,43–46] as well as the ability of dPCR to define the stability of the deep response during TFR.[47] In addition, some groups tested dPCR *BCR::ABL1* quantification also in pediatric CML cases both approaching cDNA and genomic DNA. dPCR resulted in more sensitivity than qPCR in pediatric patient settings, too.[48,49] The dPCR quantification of genomic *BCR::ABL1* has been successfully explored also in adult CML cases aiming to detect leukemic stem cells negative for *BCR::ABL1* transcript and to establish a personalized approach to CML patient management.[50–52] Last but not least, the application of dPCR resulted able to performing a precise, sensitive, and accurate quantification of *BCR::ABL1* transcripts shuttled by circulating extracellular vesicles,[53,54] similar to what was observed in another type of leukemia.[55–57]

Considering these encouraging results, it is not surprising that new commercial assays have become available for *BCR::ABL1* transcript detection by dPCR[58] and that coordinated multicentric studies are optimizing and standardizing CML MRD monitoring by dPCR.[59,60] These data are constantly confirmed by new evidence and altogether stress the utility of moving to dPCR for minimal residual disease (MRD) monitoring in CML patients, in particular in subjects presenting low levels of *BCR::ABL1* transcript and potentially eligible for stopping TKI therapy. In fact, the biased performance of the *BCR::ABL1* molecular detection and quantification may impact the selection of CML patients in therapy-stopping trials[45,61] or in the routine practice TKI discontinuation.

TKI dose reduction and TKI de-escalation as new scenarios to find the minimum effective dose to maintain MMR/DMR

IM needs a longer time and even if NIL or DAS are more potent and quicker, they are more toxic than IM. Last but not least, even if more potent second generation were registered for the first-line CML treatment to improve the rate of MMR and the depth of MR, the sensitivity and the accuracy of minimal residual disease monitoring did not improve in the same way because the new dPCR did not replace the conventional RT-qPCR.

Facing the evidence of a minimal increase in efficacy, in terms of stable DMR, and, on the other hand, of a high increase in off-target drug toxicity (e.g. cardiovascular toxicity, pleural effusion, diarrhea), the dose optimization of second-generation TKIs in the first line has established more recently as the dominant therapeutic strategy. Lower dose DAS (50 mg daily) was tested as front-line therapy in newly diagnosed CML-CP.[62] After a minimum follow-up of 12 months, 81 patients were evaluable. Two patients came off

the study in less than 3 months. On the 81 patients enrolled, the cumulative rates for a CCyR by 6 and 12 months were 77% and 95%, respectively. The cumulative rates for MMR, MR4.0, and MR4.5 by 12 months were 81%, 55%, and 49%, respectively. Five patients (6%) developed pleural effusions; four of these patients (80%) required a dose reduction. At a median follow-up of 24 months, none of the patients had disease transformation to an AP or BP. The 2-year event-free and OS rates were 100%.

Jabbour *et al.* explored DAS 50 mg *versus* 100 mg on a cohort of 233 patients with newly diagnosed CML-CP (low dose = 83 patients; standard dose = 150 patients). By propensity score analysis, 77 patients in each cohort without significant baseline differences were identified. After a median follow-up of 60 months, the 3-year MMR rates were 92% and 84% for low-dose and standard-dose DAS, respectively ($p = 0.23$). DAS 50 mg/day induced a higher cumulative incidence of MR4.0 (77% *versus* 66%; $p = 0.04$) and MR4.5 (77% *versus* 62%; $p = 0.02$) at 3 years. The rate of any grade pleural effusion was 5% with DAS 50 mg/day compared to 21% with 100 mg/day.[63]

At the 2017 American Society of haematology (ASH) meeting, Rea *et al.* reported on the NILORED trial that explored the effect of a reduction of NIL to a single-day dose, after the achievement of MMR. In all, 67 patients were presented as they completed 1 year of NIL single-day dose reduction. NIL was reduced to 450–300 mg daily. Kaplan–Meier analysis of survival without unconfirmed MMR loss was 97% at 12 months and analysis by molecular response category showed that none of the patients who were at least in MR4.0 at baseline lost MMR.[64]

Moreover, Brummendorf *et al.* looked at the impact of TKI dose reduction among the patients included in the BYOND study. This study explored the efficacy and toxicity of BOS after failure of previous TKI, and the authors focused on those patients who underwent a BOS dose reduction due to toxicity/tolerability. Dose reduction up to 200 mg/daily did not change the rate of achievement or maintenance of MMR.[65]

In 2021, Rousselot *et al.* explored the issue of DAS dose adjustment based on therapeutic drug monitoring (TDM). Eligible patients ($n = 287$) started DAS at 100 mg per day followed by DAS TDM. Patients considered overdosed were randomized ($n = 80$) between a dose reduction strategy (TDM arm) and standard of care (control arm). A major reduction in the cumulative incidence of pleural effusion was observed in the TDM arm (4% *versus* 15%; 11% *versus* 35%, and 12% *versus* 39% at 1, 2, and 3 years, respectively; $p = 0.0094$). Molecular responses were superimposable in all arms.[66,67]

As previously reported, the phase II DESTINY study showed that initial de-escalation before discontinuation might improve the success of TFR protocols, as the recurrence-free survival was 72% and 36% in the MR4.0 and MMR groups, respectively. All recurrences regained MMR within 5 months of treatment resumption.[32]

Furthermore, the policy of intermittent TKI treatment explored in the INTERIM trial (FIXED arm: 1 month ON and 1 month OFF) was randomly compared with a progressive intermittent TKI treatment (PROGRESSIVE arm: 1 month ON and 1 month OFF for the first year, 1 month ON and 2 months OFF for the second year, and 1 month ON and 3 months OFF for the third year) (OPTkIMA trial) in elderly patients with CML in stable MMR or MR4. The probability of maintaining the MMR of the 166 patients who completed the first year of OPTkIMA was 81%.[65] After the second year of treatment, the rate of MMR loss was 6% in the FIXED arm *versus* 20% in the PROGRESSIVE arm ($p = 0.006$) but, interestingly, in the third year, the percentage of patients who resumed continuous TKI treatment for MR3 loss was quite comparable in the two arms (2% *versus* 9%; $p = 0.06$) (unpublished data).

At the 2021 ASH meeting, Breccia *et al.* presented the preliminary results of the DANTE study, exploring a dose optimization strategy for TFR. Briefly, patients with sustained MR4.0 after at least 3 years of NIL $\geqslant$400 mg/day were addressed to a consolidation phase, testing the dose of NIL 300 mg/day for 48 weeks. This phase was followed by NIL interruption if MR4.0 was maintained or NIL continuation at the same dose in case of MMR or resumption of standard dose if MMR loss. In all, 47 patients completed the consolidation phase, and 76.9% of them maintained the DMR.[68]

Finally, Martin Roland *et al.* recently published an interesting real-life study on 80 CML patients, of whom 71 received a lower dose of TKI and 25 were discontinued (9 of whom without previous dose reduction). 15.4% of the patients treated with low-dose TKI had a molecular recurrence, and the mean molecular recurrence-free survival was 24.6 months. Following TKI discontinuation and with a median follow-up of 29.2 months, the MMR was maintained in all but four patients, leading to an estimated TFR of 38.9 months. The authors concluded that low-dose treatment and/or TKI discontinuation is feasible and safe in patients who may suffer AEs that may increase the probability of low treatment adherence or impair the QoL.

The limits of strategies to reach the TFR

All these data entail that the choice of TKI must be done not only based on the goal of DMR to pursue the TFR strategy but also taking into account the incidence and severity of different TKI side effects, in particular the off-target effects in the long term.

This means that a critical selection at baseline of TKI should be considered case by case and that the choice of TKI should be evaluated on the patient's age, disease risk, and degree of response to therapy, and, thus, based on the relationship between costs and benefits. The Gruppo Italiano Malattie EMatologiche dell'Adulto (GIMEMA; Italian group for Hematologic Diseases of the Adult) CML Working Party (WP) has developed a project aimed at selecting the treatment policies that may increase the probability of TFR, considering four variables: the need for TFR, the TKIs, the characteristics of leukemia, and the patient. A Delphi-like method was used to reach a consensus among the representatives of 50 centers of the CML WP. A consensus was reached on the assessment of disease risk [EUTOS Long-Term Survival (ELTS) score], on the definition of the most appropriate age boundaries for the choice of first-line treatment, on the choice of the TKI for first-line treatment, and on the definition of the responses that do not require a change of the TKI (*BCR::ABL1* $\leqslant$10% at 3 months, $\leqslant$1% at 6 months, $\leqslant$0.1% at 12 months, $\leqslant$0.01% at 24 months), and of the responses that require a change of the TKI, when the goal is TFR (*BCR::ABL1* $>$10% at 3 and 6 months, $>$1% at 12 months, and $>$0.1% at 24 months). A consensus was reached for the choice of second-generation TKIs, in all young patients (18–40 years) and intermediate- and high-risk adult patients (41–65 years), and for the choice of IM, in low-risk elderly patients (66–80 years) and all very elderly patients ($>$80 years).[69]

All these observations and considerations suggest that the TFR may be reasonable and a valid objective but, at present, it is clear that this objective cannot be neither pursued nor achieved by most patients and that the profile of the optimal candidate is not yet well defined.

Indeed, although Sokal's low risk, depth/duration of DMR, and duration of TKI therapy longer than 4–6 years are recognized as the main factors correlating with TFR, the rate of patients who could be candidates for treatment discontinuation was no more than 25–30% of the entire CML population and that the great majority of CML patients are still destined to receive the TKI daily therapy forever.[69]

For these patients, INTERIM, DESTINY and, more recently, the OPTKIMA studies opened the perspective of de-scalation of chronic therapy for maintaining the MMR, reducing toxicity, and possibly improving the QoL. Therefore, a strategy based on the principle of the minimum effective dose, totally different from that of the maximum tolerated dose for reaching the TFR, has been re-evaluated and taken into consideration not only following these studies but also from the numerous studies exploring lower TKIs doses, as above reported.

Another important observation is that the PFS and OS of patients with MMR/DMR in continuous treatment or DMR in TFR are absolutely quite similar and no significant differences can be observed. In other words, different strategies can be applied, and they lead to the same PFS and OS. It is a matter of understanding if and how much that specific strategy is justified for that category of patients.

TKI and pregnancies

Taking into account that the life expectancy of CML patients is now superimposable to that of sex and age-matched healthy people, family

planning is increasingly important for many patients with childbearing potential. In particular, the issue of pregnancy should be addressed both for those patients who achieve the TFR and for those patients who plan a pregnancy or discover to be pregnant while on TKIs.

Planned and unplanned pregnancies in patients with CML can be managed with a strict collaboration between a hematologist, obstetrician-gynecologist, and neonatologist. It is important to properly educate the patients and their relatives on the pros and potential cons of pregnancy during TKI treatment. Abruzzese *et al.* identified key issues in therapy management during pregnancy in chronic myeloid leukemia patients, considering time from TKI therapy and tumor burden. Following these points is strongly suggested in clinical practice, to safely proceed with the pregnancy and delivery.[70,71]

Conclusions and future perspectives

Considering the reported evolution of the therapeutic management of adult CML patients in the last 20 years, we think that CML therapy is partially personalized, and this aspect should be improved. CML therapeutic strategy has traced the road for other personalized treatments in other hematologic malignancies and solid tumors, and now it should be based on different TKIs for different objectives in different categories of patients. TKI potency and duration of treatment should be balanced between efficacy and toxicity, and the TFR or chronic treatment strategy should be chosen according to the risk of disease, the patient's age, and/or the comorbidities of the patients. A balanced integration of the factors related to the drug (e.g. safety, tolerability, or costs), the patient (e.g. age, comorbidities, or compliance and lifestyle), and the center (e.g. drug availability, clinical experience, or drug monitorization preferences) was suggested also by Ciftciler and Haznerdaroglu[72] in 2021. The authors stressed the need for a comprehensive analysis of the different elements that may affect the efficacy of the TKI treatment for the selection of the best therapeutic strategy. This is not only in terms of drug molecule choice and the known side effects[73] but also the consideration of dosage optimization, the patients' social conditions, and the need for access to the hematologic center.

Nowadays, we remark also that any strategy has to be driven by a precise MRD assessment. In this regard, it is time to move from RT-qPCR to dPCR for detecting and monitoring the levels of *BCR::ABL1* transcript, particularly for TFR purposes. In fact, overcoming the limits of RT-qPCR is a necessary step forward to optimize TKI therapy and discontinuation. dPCR can lead to a better sensitivity and accuracy of MRD assessment and, consequently, to a better selection of patients eligible for TKI discontinuation strategies, as above reported. Altogether, these data stress the utility of moving to dPCR for MRD monitoring in CML patients, in particular in subjects presenting low levels of *BCR::ABL1* transcript and potentially eligible for stopping TKI therapy. In fact, the biased performance of the *BCR::ABL1* molecular detection and quantification may influence the selection of CML patients in therapy-stopping trials[45,61] or in the routine care TKI discontinuation, as above reported.

The eradication of disease remains an ambitious objective and for its achievement, new drugs or new combinations will be necessary as well as new tools for *BCR::ABL1* assessment. In this regard, the above-mentioned introduction of dPCR and the detection of *BCR::ABL1* transcript in new biological matrixes (e.g. extracellular vesicles) are expected to be widely explored.

Moreover, a fundamental step will be going back from the clinic to biology to further study the pathogenesis, disease progression, and most of all to screen the efficacy and toxicity of new drugs aiming to target the CML leukemic cells. In this scenario, the settling of new animal models is the most promising approach. Many efforts led to the availability of murine CML models suitable for *in vivo* testing of specific TKIs,[74–77] and allowing the investigation of key players in disease development.[78] Drosophila melanogaster has been also used as an alternative CML animal model.[79,80] Both Mus musculus and Drosophila melanogaster present pros and cons when cost- and time-effectiveness as well as human comparability are considered.

Recently, two stable zebrafish model for a CML-like disease has been generated. Xu *et al.* reported the generation of a transgenic zebrafish line using a synthetic human *BCR::ABL1* transcript introduced by a transgenic construct in zebrafish

embryos. After multiple activations of *BCR::ABL1* expression, the fish developed a myeloproliferative disease resembling human CML in 6–12 months, as expected considering the median human CML onset.[81] Differently, an Italian group introduced a new CML zebrafish model developed using a human *BCR::ABL1* transcript isolated from a CML patient. To model Ph+ CML, the human fusion transcript under the control of the Gal4/upstream activating sequence has been used to generate a transgenic line using the Tol2 transposition system and then crossed with the hsp70 (heat-inducible promoter) Gal4 line to recapitulate hematologic characteristics and molecular biology features of CML. This model presents an early development of CML-like disease in embryos with proliferating hematopoietic cells in the caudal hematopoietic tissue and seems a new tool for real-time observation of leukemic cells pathogenesis and analysis of *BCR::ABL1*-dependent signaling pathways.[82] Both the zebrafish models are expected to be excellent tools for rapid and effective high-throughput drug screening. In fact, Zebrafish has proven to be a versatile and reliable experimental *in vivo* tool to study human hematopoiesis and model hematological malignancies. Transgenic technologies enable the generation of specific leukemia types by the expression of human oncogenes under specific promoters.[83]

In conclusion, the most of patients affected with CML and receiving TKI can expect to have a 'normal' life expectancy and a QoL that is comparable to that of age- and sex-matched healthy people. There are numerous options available for first- and second-generation treatment selection authorized in the first line. The selection of the primary TKI will depend on whose CML patients are more likely to have disease progression or develop drug resistance. It is crucial to harmonize the best available knowledge, specific patient features, and medical expertise when choosing the optimal TKI for CML therapy. Key topics of novel investigations for a tailored strategy in the field of CML include gene expression profile, the CML-leukemic stem cell reserve, and next-generation genomics approach for the investigation of TKI and multi-drug resistance genes. Moreover, the availability of new tools, such as molecular techniques and animal models, will improve and support novel studies for a personalized approach in the field of CML.

Declarations

Ethics approval and consent to participate

This is a review paper of information freely available in the public domain (i.e. a review of work already published by others). Thus, it does not require ethics approval or informed consent.

Consent for publication

Not applicable.

Author contributions

Domenico Russo: Conceptualization; Writing – original draft.

Michele Malagola: Conceptualization; Writing – original draft.

Nicola Polverelli: Writing – original draft.

Mirko Farina: Writing – original draft.

Federica Re: Writing – original draft.

Simona Bernardi: Conceptualization; Writing – original draft.

Acknowledgements

None.

Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

Competing interests

The authors declare that there is no conflict of interest.

Availability of data and materials

All the reported data are referred to published results.

ORCID iD

Simona Bernardi https://orcid.org/0000-0002-3494-2624

References

1. Hochhaus A, Larson RA, Guilhot F, *et al.* Long-term outcomes of imatinib treatment for chronic myeloid leukemia. *N Engl J Med* 2017; 376: 917–927.
2. Hehlmann R, Lauseker M, Saußele S, *et al.* Assessment of imatinib as first-line treatment of

chronic myeloid leukemia: 10-year survival results of the randomized CML study IV and impact of non-CML determinants. *Leukemia* 2017; 31: 2398.

3. Kalmanti L, Saussele S, Lauseker M, *et al*. Safety and efficacy of imatinib in CML over a period of 10 years: data from the randomized CML-study IV. *Leukemia* 2015; 29: 1123–1132.
4. Guilhot F, Rigal-Huguet F, Guilhot J, *et al*. Long-term outcome of imatinib 400 mg compared to imatinib 600 mg or imatinib 400 mg daily in combination with cytarabine or pegylated interferon alpha 2a for chronic myeloid leukaemia: results from the French SPIRIT phase III randomised trial. *Leukemia* 2021; 35, 2332–2345.
5. Preudhomme C, Guilhot J, Nicolini FE, *et al*. Imatinib plus peginterferon alfa-2a in chronic myeloid leukemia. *N Engl J Med* 2010; 363: 2511–2521.
6. Simonsson B, Gedde-Dahl T, Markevärn B, *et al*. Combination of pegylated IFN-α2b with imatinib increases molecular response rates in patients with low- or intermediate-risk chronic myeloid leukemia. *Blood* 2011; 118: 3228–3235.
7. Hochhaus A, Saglio G, Hughes TP, *et al*. Long-term benefits and risks of frontline nilotinib vs imatinib for chronic myeloid leukemia in chronic phase: 5-year update of the randomized ENESTnd trial. *Leukemia* 2016; 30: 1044–1054.
8. Cortes JE, Saglio G, Kantarjian HM, *et al*. Final 5-year study results of DASISION: the dasatinib versus imatinib study in treatment-naïve chronic myeloid leukemia patients trial. *J Clin Oncol* 2016; 34: 2333–2340.
9. Cortes JE, Gambacorti-Passerini C, Deininger MW, *et al*. Bosutinib versus imatinib for newly diagnosed chronic myeloid leukemia: results from the randomized BFORE trial. *J Clin Oncol* 2018; 36: 231–237.
10. Kantarjian HM, Hughes TP, Larson RA, *et al*. Correction to: Long-term outcomes with frontline nilotinib versus imatinib in newly diagnosed chronic myeloid leukemia in chronic phase: ENESTnd 10-year analysis. *Leukemia* 2021; 35: 2142–2143. Erratum for: *Leukemia* 2021; 35: 440–453.
11. Baccarani M, Deininger MW, Rosti G, *et al*. European LeukemiaNet recommendations for the management of chronic myeloid leukemia: 2013. *Blood* 2013; 122: 872–884.
12. Hochhaus A, Baccarani M, Silver RT, *et al*. European LeukemiaNet 2020 recommendations for treating chronic myeloid leukemia. *Leukemia* 2020; 34: 966–984.
13. Baccarani M, Saglio 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* 2006; 108: 1809–1820.
14. Baccarani M, Cortes J, Pane F, *et al*. Chronic myeloid leukemia: an update of concepts and management recommendations of European LeukemiaNet. *J Clin Oncol* 2009; 27: 6041–6051.
15. O'Brien S, Berman E, Borghaei H, *et al*. Chronic myelogenous leukemia. *J Natl Compr Canc Netw* 2009; 7: 984–1023.
16. O'Brien S, Abboud CN, Akhtari M, *et al*. Chronic myelogenous leukemia. *J Natl Compr Cancer Netw* 2012; 10: 64–110.
17. Pallera A, Altman JK, Berman E, *et al*. NCCN Guidelines Insights: chronic myeloid leukemia, version 1.2017. *J Natl Compr Cancer Netw* 2016; 14: 1505–1512.
18. Deininger MW, Shah NP, Altman JK, *et al*. Chronic myeloid leukemia, version 2.2021, NCCN Clinical Practice Guidelines in Oncology. *J Natl Compr Cancer Netw* 2020; 18: 1385–1415.
19. Radich JP, Zelenetz AD, Chan WC, *et al*. NCCN task force report: molecular markers in leukemias and lymphomas. *J Natl Compr Cancer Netw* 2009; 7: S-1.
20. Mahon F-X, Réa D, Guilhot J, *et al*. Discontinuation of imatinib in patients with chronic myeloid leukaemia who have maintained complete molecular remission for at least 2 years: the prospective, multicentre Stop Imatinib (STIM) trial. *Lancet Oncol* 2010; 11: 1029–1035.
21. Hochhaus A, Masszi T, Giles FJ, *et al*. Treatment-free remission following frontline nilotinib in patients with chronic myeloid leukemia in chronic phase: results from the ENESTfreedom study. *Leukemia* 2017; 31: 1525–1531.
22. Ross DM, Masszi T, Gómez Casares MT, *et al*. Durable treatment-free remission in patients with chronic myeloid leukemia in chronic phase following frontline nilotinib: 96-week update of the ENESTfreedom study. *J Cancer Res Clin Oncol* 2018; 144: 945.
23. Saussele S, Richter J, Guilhot J, *et al*. Discontinuation of tyrosine kinase inhibitor therapy in chronic myeloid leukaemia (EURO-SKI): a prespecified interim analysis of a prospective, multicentre, non-randomised, trial. *Lancet Oncol* 2018; 19: 747–757.

24. Haddad FG, Sasaki K, Issa GC, *et al.* Treatment-free remission in patients with chronic myeloid leukemia following the discontinuation of tyrosine kinase inhibitors. *Am J Hematol* 2022; 97: 856–864.

25. Shah NP, García-Gutiérrez V, Jiménez-Velasco A, *et al.* Dasatinib discontinuation in patients with chronic-phase chronic myeloid leukemia and stable deep molecular response: the DASFREE study. *Leuk Lymphoma* 2020; 61: 650–659.

26. Hernández-Boluda JC, Pereira A, Pastor-Galán I, *et al.* Feasibility of treatment discontinuation in chronic myeloid leukemia in clinical practice: results from a nationwide series of 236 patients. *Blood Cancer J* 2018; 8: 91.

27. Fava C, Rege-Cambrin G, Dogliotti I, *et al.* Observational study of chronic myeloid leukemia italian patients who discontinued tyrosine kinase inhibitors in clinical practice. *Haematologica* 2019; 104: 1589–1596.

28. Dengler J, Tesch H, Jentsch-Ullrich K, *et al.* Treatment-free remission in real-world chronic myeloid leukemia patients: insights from German hematology practices. *Acta Haematol* 2022; 145: 603–610.

29. Chen Y, Zhao H, Guo J, *et al.* Successful treatment discontinuation in CML patients with full-dose and low-dose TKI: results from real-world practice. *Front Pharmacol* 2023; 14: 1101743.

30. Russo D, Martinelli G, Malagola M, *et al.* Effects and outcome of a policy of intermittent imatinib treatment in elderly patients with chronic myeloid leukemia. *Blood* 2013; 121: 5138–5144.

31. Russo D, Malagola M, Skert C, *et al.* Managing chronic myeloid leukaemia in the elderly with intermittent imatinib treatment. *Blood Cancer J* 2015; 5: e347.

32. Clark RE, Polydoros F, Apperley JF, *et al.* De-escalation of tyrosine kinase inhibitor therapy before complete treatment discontinuation in patients with chronic myeloid leukaemia (DESTINY): a non-randomised, phase 2 trial. *Lancet Haematol* 2019; 6: e375–e383.

33. Iurlo A, Cattaneo D, Consonni D, *et al.* Treatment discontinuation following low-dose TKIs in 248 chronic myeloid leukemia patients: updated results from a campus CML real-life study. *Front Pharmacol* 2023; 14: 1154377.

34. Izzo B, Gottardi EM, Errichiello S, *et al.* Monitoring chronic myeloid leukemia: how molecular tools may drive therapeutic approaches. *Front Oncol* 2019; 9: 833.

35. Soverini S, Bernardi S and Galimberti S. Molecular testing in cml between old and new methods: are we at a turning point? *J Clin Med* 2020; 9: 3865.

36. Bernardi S, Malagola M, Farina M, *et al.* Digital PCR as a new method for minimal residual disease monitoring and treatment free remission management in chronic myeloid leukemia patients: is it reliable? *Hemato* 2023; 4: 1–11.

37. Berdeja JG, Heinrich MC, Dakhil SR, *et al.* Rates of deep molecular response by digital and conventional PCR with frontline nilotinib in newly diagnosed chronic myeloid leukemia: a landmark analysis. *Leuk Lymphoma* 2019; 60: 2384–2393.

38. Goh HG, Lin M, Fukushima T, *et al.* Sensitive quantitation of minimal residual disease in chronic myeloid leukemia using nanofluidic digital polymerase chain reaction assay. *Leuk Lymphoma* 2011; 52: 896–904.

39. Wang W-J, Zheng C-F, Liu Z, *et al.* Droplet digital PCR for BCR/ABL(P210) detection of chronic myeloid leukemia: a high sensitive method of the minimal residual disease and disease progression. *Eur J Haematol* 2018; 101: 291–296.

40. Bernardi S, Ruggieri G, Malagola M, *et al.* Digital PCR (Dpcr) a step forward to detection and quantification of minimal residual disease (MRD) in Ph+/BCR-ABL1 chronic myeloid leukemia (CML). *J. Mol Biomark Diagn* 2017; 8: 3.

41. Kjaer L, Skov V, Andersen MT, *et al.* Variant-specific discrepancy when quantitating *BCR-ABL1* e13a2 and e14a2 transcripts using the Europe Against Cancer qPCR assay. *Eur J Haematol* 2019; 103: 26–34, doi:10.1111/ejh.13238.

42. Bernardi S, Bonifacio M, Iurlo A, *et al.* "Variant-specific discrepancy when quantitating *BCR-ABL1* e13a2 and e14a2 transcripts using the Europe Against Cancer qPCR assay." Is dPCR the key? *Eur J Haematol* 2019; 103: 272–273.

43. Mori S, Vagge E, Le Coutre P, *et al.* Age and dPCR can predict relapse in CML patients who discontinued imatinib: the ISAV study. *Am J Hematol* 2015; 90: 910–914.

44. Bernardi S, Malagola M, Zanaglio C, *et al.* Digital PCR improves the quantitation of DMR and the selection of CML candidates to TKIs discontinuation. *Cancer Med* 2019; 8: 2041–2055.

45. Zanaglio C, Bernardi S, Gandolfi L, *et al.* RT-qPCR versus digital PCR: how do they impact differently on clinical management of

chronic myeloid leukemia patients? *Case Rep Oncol* 2020; 13: 1263–1269.

46. Nicolini FE, Dulucq S, Boureau L, *et al.* Evaluation of residual disease and TKI duration are predictive factors for molecular recurrence after stopping imatinib first-line in chronic phase CML patients. *Clin Cancer Res* 2019; 25: 6606–6613.
47. Abruzzese E, Bocchia M, Trawinska MM, *et al.* Minimal residual disease detection at RNA and leukemic stem cell (LSC) levels: comparison of RT-qPCR, d-PCR and CD26+ stem cell measurements in chronic myeloid leukemia (CML) patients in deep molecular response (DMR). *Cancers* 2023; 15: 4112.
48. Krumbholz M, Karl M, Tauer JT, *et al.* Genomic *BCR-ABL1* breakpoints in pediatric chronic myeloid leukemia. *Genes Chromosomes Cancer* 2012; 51: 1045–1053.
49. Krumbholz M, Goerlitz K, Albert C, *et al.* Large amplicon droplet digital PCR for DNA-based monitoring of pediatric chronic myeloid leukaemia. *J Cell Mol Med* 2019; 23: 4955–4961.
50. Dello Sbarba P, Rovida E, Marzi I, *et al.* One more stem cell niche: how the sensitivity of chronic myeloid leukemia cells to imatinib mesylate is modulated within a 'hypoxic' environment. *Hypoxia* 2014; 2: 1–10.
51. Lund HL, Hughesman CB, McNeil K, *et al.* Initial diagnosis of chronic myelogenous leukemia based on quantification of M-BCR status using droplet digital PCR. *Anal Bioanal Chem* 2016; 408: 1079–1094.
52. Cumbo C, Impera L, Minervini CF, *et al.* Genomic *BCR-ABL1* breakpoint characterization by a multistrategy approach for 'personalized monitoring' of residual disease in chronic myeloid leukemia patients. *Oncotarget* 2018; 9: 10978–10986.
53. Bernardi S, Foroni C, Zanaglio C, *et al.* Feasibility of tumor-derived exosome enrichment in the onco-hematology leukemic model of chronic myeloid leukemia. *Int J Mol Med* 2019; 44; 2133–2144.
54. Bernardi S, Malagola M, Polverelli N, *et al.* Exosomes in chronic myeloid leukemia: are we reading a new reliable message? *Acta Haematol* 2020; 143: 509–510.
55. Bernardi S and Farina M. Exosomes and extracellular vesicles in myeloid neoplasia: the multiple and complex roles played by these 'magic bullets'. *Biology (Basel)* 2021; 10: 105.
56. Bernardi S, Zanaglio C, Farina M, *et al.* DsDNA from extracellular vesicles (EVs) in adult AML. *Ann Hematol* 2021; 105: 1355–1356.
57. Bernardi S, Farina M, Bosio K, *et al.* Feasibility of leukemia-derived exosome enrichment and co-isolated dsDNA sequencing in acute myeloid leukemia patients: a proof of concept for new leukemia biomarkers detection. *Cancers (Basel)* 2022; 14: 4504.
58. Chung HJ, Hur M, Yoon S, *et al.* Performance evaluation of the QXdX *BCR-ABL* %Is droplet digital PCR assay. *Ann Lab Med* 2020; 40: 72–75.
59. Fava C, Bernardi S, Gottardi EM, *et al.* Standardization of two Dpcr platforms for detection of *BCR/ABL1* – minimal residual disease (MRD) in Ph+ chronic myeloid leukemia (CML). *Blood* 2017; 130: 2867–2867.
60. Fava C, Bernardi S, Gottardi EM, *et al.* Alignment of Qx100/Qx200 droplet digital (Bio-Rad) and QuantStudio 3D (Thermofisher) digital PCR for quantification of *BCR-ABL1* in Ph+ chronic myeloid leukemia. *Diseases* 2021; 9: 35.
61. Spiess B, Rinaldetti S, Naumann N, *et al.* Diagnostic performance of the molecular *BCR-ABL1* monitoring system may impact on inclusion of CML patients in stopping trials. *PLoS One* 2019; 14: e0214305.
62. Naqvi K, Jabbour E, Skinner J, *et al.* Long-term follow-up of lower dose dasatinib 50 mg daily as frontline therapy in newly diagnosed chronic phase chronic myeloid leukemia. *Cancer* 2020; 126: 67.
63. Jabbour E, Sasaki K, Haddad FG, *et al.* Low-dose dasatinib 50 mg/day versus standard-dose dasatinib 100 mg/day as frontline therapy in chronic myeloid leukemia in chronic phase: a propensity score analysis. *Am J Hematol* 2022; 97: 1413–1418.
64. Rea D, Cayuela J-M, Dulucq S, *et al.* Molecular responses after switching from a standard-dose twice-daily nilotinib regimen to a reduced-dose once-daily schedule in patients with chronic myeloid leukemia: a real life observational study (NILO-RED). *Blood* 2017; 130: 318–318.
65. Brümmendorf TH, Gambacorti-Passerini C, Clark R, *et al.* Vascular, and hypertension safety of bosutinib vs imatinib for newly diagnosed chronic myeloid leukemia in the BFORE trial, https://library.ehaweb.org/eha/2019/24th/266213/tim.h.brmmendorf.cardiac.vascular.and.hypertension.safety.of.bosutinib.vs.html?f=menu=6*browseby=8*sortby=2*media=2*ce_

id=1550*ot_id=20973 (2019, accessed on Mar 20, 2023).

66. Rousselot P, Mollica L, Guilhot J, *et al*. Dasatinib dose optimisation based on therapeutic drug monitoring reduces pleural effusion rates in chronic myeloid leukaemia patients. *Br J Haematol* 2021; 194: 393–402.
67. Malagola M, Iurlo A, Abruzzese E, *et al*. Molecular response and quality of life in chronic myeloid leukemia patients treated with intermittent TKIs: first interim analysis of OPTkIMA study. *Cancer Med* 2021; 10: 1726–1737.
68. Breccia M, Abruzzese E, Stagno F, *et al*. First interim analysis of the Italian Dante study: de-escalation before treatment-free remission in patients with chronic myeloid leukemia treated with first-line nilotinib. *Blood* 2021; 138: 1474.
69. Baccarani M, Abruzzese E., Accurso V, *et al*. Managing chronic myeloid leukemia for treatment-free remission: a proposal from the GIMEMA CML WP. *Blood Adv* 2019; 3: 4280–4290.
70. Abruzzese E, Mauro M, Apperley J, *et al*. Tyrosine kinase inhibitors and pregnancy in chronic myeloid leukemia: opinion, evidence, and recommendations. *Ther Adv Hematol* 2020; 11: 2040620720966120.
71. Robertson HF and Apperley JF. Treatment of CML in pregnancy. *Hematology Am Soc Hematol Educ Program* 2022; 2022: 123–128.
72. Ciftciler R and Haznedaroglu IC. Tailored tyrosine kinase inhibitor (TKI) treatment of chronic myeloid leukemia (CML) based on current evidence. *Eur Rev Med Pharmacol Sci* 2021; 25: 7787–7798.
73. Lipton JH, Brümmendorf TH, Gambacorti-Passerini C, *et al*. Long-term safety review of tyrosine kinase inhibitors in chronic myeloid leukemia – what to look for when treatment-free remission is not an option. *Blood Rev* 2022; 56: 100968.
74. Wolff NC, Veach DR, Tong WP, *et al*. PD166326, a novel tyrosine kinase inhibitor, has greater antileukemic activity than imatinib mesylate in a murine model of chronic myeloid leukemia. *Blood* 2005; 105: 3995–4003.
75. Schubert C, Chatain N, Braunschweig T, *et al*. The SCLtTAxBCR-ABL transgenic mouse model closely reflects the differential effects of dasatinib on normal and malignant hematopoiesis in chronic phase-CML patients. *Oncotarget* 2017; 8: 34736–34749.
76. Cheng H, Hao S, Liu Y, *et al*. Leukemic marrow infiltration reveals a novel role for Egr3 as a potent inhibitor of normal hematopoietic stem cell proliferation. *Blood* 2015; 126: 1302–1313.
77. Peng C, Chen Y, Yang Z, *et al*. PTEN is a tumor suppressor in CML stem cells and BCR-ABL-induced leukemias in mice. *Blood* 2010; 115: 626–635.
78. Dash AB, Williams IR, Kutok JL, *et al*. A murine model of CML blast crisis induced by cooperation between BCR/ABL and NUP98/HOXA9. *Proc Natl Acad Sci U S A* 2002; 99: 7622–7627.
79. Al Outa A, Abubaker D, Bazarbachi A, *et al*. Validation of a drosophila model of wild-type and T315I mutated *BCR-ABL1* in chronic myeloid leukemia: an effective platform for treatment screening. *Haematologica* 2020; 105: 387–397.
80. Bernardoni R, Giordani G, Signorino E, *et al*. A new *BCR-ABL1* drosophila model as a powerful tool to elucidate the pathogenesis and progression of chronic myeloid leukemia. *Haematologica* 2019; 104: 717–728.
81. Xu M, Ye Y, Ye Z, *et al*. Human BCR/ABL1 induces chronic myeloid leukemia-like disease in zebrafish. *Haematologica* 2020; 105: 674–686.
82. Zizioli D, Bernardi S, Varinelli M, *et al*. Development of *BCR-ABL1* transgenic zebrafish model reproducing chronic myeloid leukemia (CML) like-disease and providing a new insight into CML mechanisms. *Cells* 2021; 10: 445.
83. Zizioli D, Mione M, Varinelli M, *et al*. Zebrafish disease models in hematology: Highlights on biological and translational impact. *Biochim Biophys Acta Mol Basis Dis* 2019; 1865: 620–633.