RESEARCH ARTICLE

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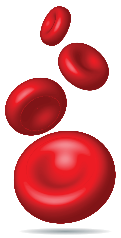
Rotation of nilotinib and imatinib for first-line treatment of chronic phase chronic myeloid leukemia



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The introduction of second-generation tyrosine-kinase inhibitors (TKIs) has generated a lively debate on the choice of first-line TKI in chronic phase, chronic myeloid leukemia (CML). Despite the TKIs have different efficacy and toxicity profiles, the planned use of two TKIs has never been investigated. We report on a phase 2 study that was designed to evaluate efficacy and safety of a treatment alternating nilotinib and imatinib, in newly diagnosed BCR-ABL1 positive, chronic phase, CML patients. One hundred twenty-three patients were enrolled. Median age was 56 years. The probabilities of achieving a complete cytogenetic response, a major molecular response, and a deep molecular response (MR 4.0) by 2 years were 93%, 87%, and 61%, respectively. The 5-year overall survival and progression-free survival were 89%. Response rates and survival are in the range of those reported with nilotinib alone. Moreover, we observed a relatively low rate of cardiovascular adverse events (5%). These data show that the different efficacy and toxicity profiles of TKIs could be favorably exploited by alternating their use.

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Introduction

Fifteen years after the introduction of imatinib, at least four other tyrosine kinase inhibitors (TKIs) have become available for the treatment of Philadelphia chromosome-positive (Ph+), BCR-ABL1+, chronic myeloid leukemia (CML) [1–4]. All these TKIs belong to the same class and share the same target, namely the proteins that are coded by the BCR-ABL1 fusion gene. However, there are several differences among these TKIs, concerning: the pharmacokinetic profile, the inhibitory efficacy on wild-type or mutated BCR-ABL1 [2,4–7], and, the inhibition of TKs other than BCR-ABL1 (so-called off–target inhibition) [2,4]. All these differences, influencing the efficacy and safety, suggest that it would have been interesting to test TKIs in combination, similarly to what occurs in many leukemias and cancers, where the sequential or concomitant administration of effective drugs is common. This was not yet done in CML, where a tradition of single-agent therapy, initiated with spleen radiation and continued with busulfan, hydroxyurea, and interferon-α, was maintained with TKIs. The investigation of new treatment policies that maintain a high

Additional Supporting Information may be found in the online version of this article.

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therapeutic efficacy, improve the safety profile, and, possibly, have lower costs, is of utmost importance in CML. In this context, the high efficacy of nilotinib, and the cardiovascular safety and the lower cost of imatinib (even more with the upcoming generic formulation) represent key characteristics that might be interestingly combined. On this basis, the GIMEMA CML WP designed and conducted a multicenter, prospective, phase 2 study of a combination of nilotinib and imatinib, given sequentially for 3-month periods for a minimum of two years. We report here the final evaluation of this trial, focused on response rates, safety profile, and outcome.

Patients and Methods

Study protocol. One hundred and twenty-three adult (≥18 years old) patients with newly diagnosed (≤6 months), chronic phase (CP), Ph+, BCR-ABL1+, CML were enrolled between February and August 2009, at 38 GIMEMA Clinical Centers, in a phase 2, single-arm study where treatment was initiated with nilotinib, 400 mg twice daily (BID) for 3 months, then was continued with imatinib, 400 mg once daily (OD) for other 3 months. Thereafter, the two drugs were given in a rotation of 3-month periods, for a total of 24 months (core phase of the study). Three days of drug washout were planned at the end of each 3-month period. During the core phase, the patients were required to stay on the 2-drugs rotating regime, unless safety or efficacy issues have occurred. After the first 2 years, physicians were free to carry on with the 2-drugs rotating regime, or to select between imatinib and nilotinib; patients were then followed for at least 3 more years (Supporting Information Fig. 1). The study (Clinical Trials gov. NCT00769327) was approved by the Ethics Committees or the Institutional Review Boards of all participating centers, and was conducted according to Good Clinical Practice and the Declaration of Helsinki. All patients gave written informed consent. Exclusion criteria were a performance status ≥ 2, uncontrolled serious medical conditions, and prior treatment with TKIs (except for imatinib ≤ 30 days). The primary objective of the study was the evaluation of the 12-month complete cytogenetic response rate. Secondary objectives included the cytogenetic and molecular responses rates during the first 24 months of the study, the analysis of failures, adverse events, and survival.

Definition of risk score and CML phase. The baseline risk score was calculated according to Sokal [8]. CP, accelerated phase (AP), and blast phase (BP) were defined according to ELN[2]. Treatment failures were retrospectively evaluated according to 2013 ELN recommendations [2].

Cytogenetic response. The cytogenetic response (CyR) was assessed by chromosome banding analysis (CBA) of at least 20 marrow cell metaphases, at 3, 6, 9, 12, 18, and 24 months, and defined according to ELN [9]. Fluorescence in situ hybridization (FISH) on peripheral blood could be used to define the Complete CyR (CCyR: ≤1% of BCR-ABL1 positive nuclei over at least 200 nuclei analyzed) [2].

Molecular response. Molecular response (MR) was assessed by RT-PCR of peripheral blood cells, at one Center (Bologna) for 2 years, then at GIMEMA Labnet laboratories, once they had been standardized, and had received their conversion factor, allowing the expression of the results according to the International Scale (IS) [10]. Early Molecular Response (EMR) was defined as BCR-ABL1 transcripts level ≤ 10% at 3 months. Major Molecular Response (MMR) and MR 4.0 were defined as BCR-ABL1 transcripts ≤0.1%, and ≤0.01%, respectively, in samples with more than 10,000 ABL1 copies [11]. Molecular tests were performed every 3 months until a MMR was achieved and confirmed, then every 6 months. Mutational screening of BCR-ABL1 kinase domain point mutations was performed in case of progression, using conventional Sanger Sequencing, as reported elsewhere [12].

Adverse events. We analyzed the adverse events (AEs) occurred during the first year of study to correlate their frequency, recurrence, and severity with the TKI that was taken when the AE occurred. If an AE persisted for more than 15 days after the planned change of treatment, the AE was associated with both drugs. Among AEs, particular attention was given to arterial thrombotic/sclerotic events (ATEs), which were defined as peripheral arterial obstructive disease (PAOD), acute coronary syndrome (acute myocardial infarction [MI]; instable angina), chronic ischemic heart disease (stable angina), significant carotid stenosis and ischemic stroke.

Statistical analysis. The rate of cytogenetic and molecular response is reported both "at" a time point, calculated dividing the number of patients with that response at that time point by the number of all enrolled patients (not evaluable patients, for any reason, were considered as non-responders), and "by" a time point, calculated by the Kaplan and Meier method as the cumulative incidence or probability of having achieved that response within that time period. It is acknowledged that the latter calculation overestimates the response, but this value allows a comparison, though indirect, with many other studies, where the response rates were reported only, or mainly, "by" a time point.

Overall survival (OS) and progression-free survival (PFS) were calculated from the first day of treatment to death by any cause (OS), including death after allogeneic stem cell transplantation (SCT), and to progression or death, whichever came first (PFS), by the method of Kaplan and Meier [13].

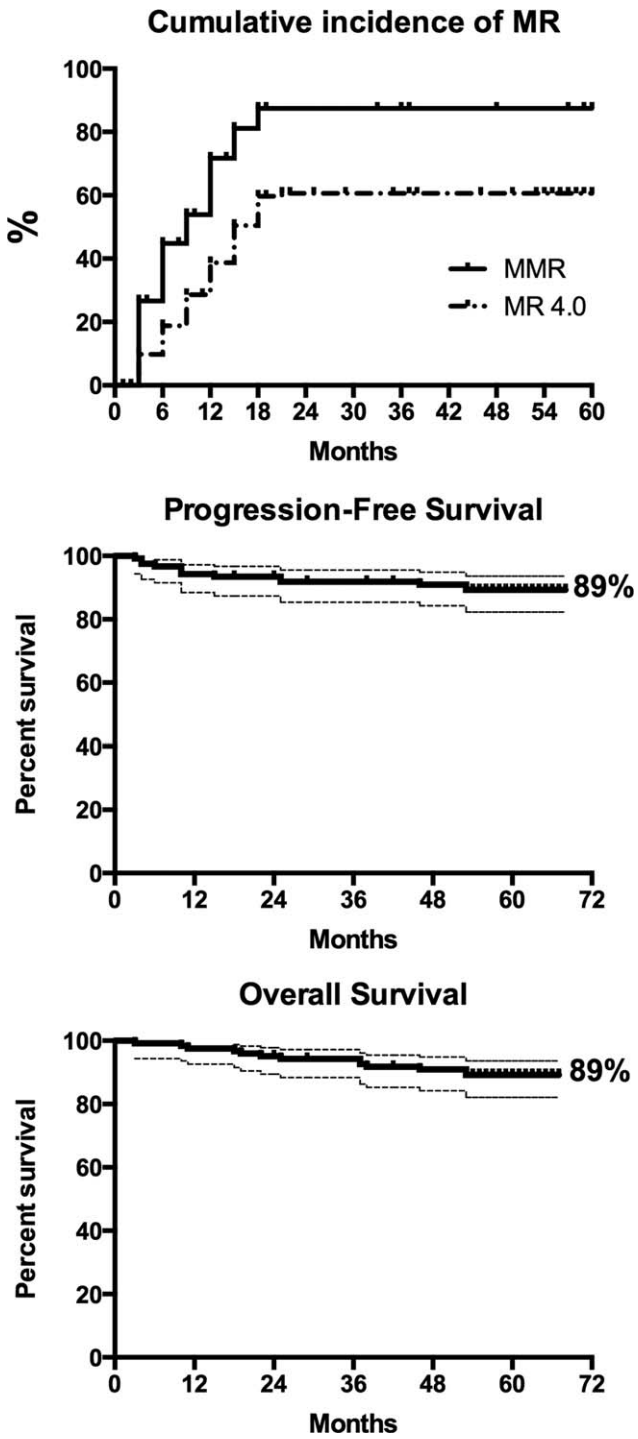

Figure 1. Cumulative incidence of major molecular response and of MR 4.0 (A), and five-year survival (B, C). Major molecular response (MMR): BCR-ABL1 ≤ 0.1%; ABL copies ≥ 10,000. Deep molecular response (MR 4.0): BCR-ABL1 ≤ 0.01%; ABL copies ≥ 10,000. Events considered for overall-survival: deaths for any cause; for progression-free survival: progression to accelerated/blast phase, and deaths for any cause.

Results

Patients

Baseline characteristics of patients are shown in Supporting Information Table I. Males were 52%. Noteworthy is the median age of 56 years, corresponding to the age that was found in population-based studies in Italy and in Europe[14]. The proportion of Sokal

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TABLE I. Cytogenetic and Molecular Response During the Core Phase of the Study (24 Months)

		3 mo %	6 mo %	9 mo %	12 mo %	18 mo %	24 mo %
CCyR	at[a]	70	79	NA	75	65	63
CCyR	by[b]	70	85	NA	91	91	93
EMR	at[a]	91	NA	NA	NA	NA	NA
MMR	by[b]	27	45	54	72	87	87
MR 4.0	at[a]	10	13	15	23	43	44
MR 4.0	by[b]	10	19	29	39	60	61

[a] The rates "at" were calculated by dividing the number of patients with that response at that time point by the number of all enrolled patients ($n = 123$); not evaluable patients (for any reason) were considered as non-responders.
[b] The rates "by" express the cumulative probability of achieving that response over that time period.
CCyR, no Ph+ metaphases out of at least 20 marrow cell metaphases or FISH ≤ 1% BCR-ABL positive nuclei over at least 200 nuclei analyzed; EMR, BCR-ABL1 ≤ 10% at 3 months; MMR, Major Molecular Response (BCR-ABL1 ≤ 0.1%); ABL copies ≥ 10,000; MR 4.0, BCR-ABL1 ≤ 0.01%; ABL copies ≥ 10,000; NA, not applicable.

TABLE II. Patients' Disposition

	End of core phase (24 months) *n* (%)	End of study (60 months) *n* (%)
On study	**103 (84)**	**85 (69)**
On rotation schedule	82 (67)	14 (11)
On nilotinib alone	9 (7)	44 (36)
On imatinib alone	12 (10)	27 (22)
Off-study[a]	**20 (16)**	**38 (31)**
Dead	7 (6)	13 (11)
On dasatinib	8 (7)	16 (13)
On other treatments	4 (3)	4 (3)
Alive after ASCT	0	1
Off treatment	0	1
Lost to follow-up	1	3 (2)

[a] Off-study: includes the patients who discontinued both study drugs, for any reason.
ASCT, Allogeneic Stem Cell Transplantation.

high-risk patients (22%) was as expected[14]. The median follow-up of living patients was 60 months (range 54 to 67 months).

Cytogenetic and molecular response

The rates of cytogenetic and molecular response are shown in Table I. At the end of the first period of nilotinib treatment (3 months), 91% of patients had achieved the EMR. In 2008 the prognostic impact of EMR was not yet recognized, thus, according to protocol, treatment was not changed in the 11 (9%) patients without EMR. Notably, 2 of them subsequently progressed to BP, and only 3 achieved a MMR. The complete cytogenetic response rate at 12 months was 75%. At the end of the core phase (24 months), 63% of patients were in CCyR, 54% in MMR, and 44% in MR 4.0 (for all calculations, non evaluable patients were considered as non-responders). The cumulative incidences by 24 months of CCyR, MMR, and MR4.0 were 93%, 87%, and 61%, respectively. Thereafter, these rates did not change significantly, with a plateau from the third year on (Fig. 1). The MMR rate by 24 months was significantly lower for Sokal high-risk patients compared to intermediate and low risk ones (96%, 90%, and 69%, respectively; $P = 0.005$); moreover, high-risk and intermediate risk patients had lower MR 4.0 rates compared to low risk patients (55% vs. 73%, respectively; $P = 0.016$).

Patient disposition and outcome

Patient disposition at the end of the core phase (24 months) and at the end of study (60 months) is shown in Table II. One hundred and three patients (84%) were still on study at month 24, with 83/123 (67%) still on the 2-drug rotation regime. Eighty-five patients (69%) were on study at month 60, but only 14/123 (11%) were on the rotation regime, while 44/123 (36%) had chosen to continue with nilotinib alone, 300 mg BID, and 27/123 (22%) with imatinib alone. Dasatinib was the most frequent second-line drug (14% of patients). The 5-year PFS and OS was 89% (95% CI: 82-94%) (Fig. 1). Overall, 6 patients were submitted to allogeneic SCT, 4 of them after progression to BP (all died after SCT, with active disease), and 2 in CP, after failure of second-line treatment (one died of SCT, one is alive and in remission).

Seven patients (6%) progressed to BP, of whom 4, suddenly, with a lymphoid phenotype, and 3, through an AP, with a myeloid phenotype (Supporting Information Table II). Five of seven progressions occurred during the first year. Notably, 5/7 had previously achieved the EMR. All four patients with a lymphoid phenotype had a mutation vs. none of the 3 patients with a myeloid phenotype.

Overall, 13/123 patients (11%) died, of whom 7 after progression to BP and with active disease, one after SCT in CP, 2 of other malignancies (prostate cancer, 68 years old, after 20 months of therapy; bladder cancer, 77 years old, after 4 months of therapy), one of a massive pulmonary embolism (deep vein thrombosis secondary to pelvic fracture), one of a cerebral hemorrhage with thrombocytopenia ($27x10^9$/L) during the 3rd month of the first nilotinib period, and one of ischemic stroke at 81 years of age, 54 months after diagnosis, while on dasatinib treatment (off-study for failure at 24 months) (Supporting Information Table III).

Adverse events

Non-hematologic adverse events (AEs) and laboratory abnormalities are listed in Table III, Supporting Information Table IV, and Supporting Information Figs. 2 and 3. During the first year, 256 AEs were observed: 42% associated with nilotinib (first and/or third quarter), 31% with imatinib (second and/or fourth quarter), and 27% with both drugs. Moreover, 64% of the AEs were limited to one quarter, mainly the first, as expected.

The most common AEs were periorbital edema (31.7% of the patients, all grade 1/2), skin rash (30.7% all grades, 1.6% grade 3), muscle pain/cramps (26% all grades; 2.4% grade 3), and fatigue (21.1% all grades; 0.8% grade 3). Periorbital edema was more frequently associated with imatinib, although during nilotinib treatment it did not completely resolve, or persisted for > 15 days, in 9.8% of the patients. Skin rash was more common with nilotinib, but, similarly, during imatinib treatment it did not completely resolve, or persisted for > 15 days, in 8.1% of patients.

ATEs were reported in 6 patients (5%) (Supporting Information Table V). Three patients (age 70, male; age 78, female; age 89, female) developed a myocardial infarction, which was managed with percutaneous trans-luminal angioplasty (two patients), or medical treatment (one patient). All these 3 patients permanently discontinued nilotinib. A patient (age 75, female) was diagnosed of unstable angina, which was managed with medical treatment, and resumed the alternating

TABLE III. Adverse Events Observed During the First Year of Study, According to Treatment (Nilotinib Only, Imatinib Only, Both Nilotinib and Imatinib)

	Ascribed to nilotinib		Ascribed to imatinib		Ascribed to both TKIs		Total	
	All Grades %	Grades 3/4%	All grades %	Grades 3/4 %	All grades %	Grades 3/4 %	All grades %	Grades 3/4 %
Periorbital edema	2.4	0	19.5	0	9.8	0	31.7	0
Skin rash	21.1	1.6	1.6	0	8.1	0	30.9	1.6
Muscle pain/cramps	5.7	0	8.9	0	11.4	2.4	26.0	2.4
Fatigue	9.8	0	2.4	0.8	8.9	0	21.1	0.8
Pruritus	13	1.6	0.8	0	4.1	0.8	17.9	2.4
Abdominal pain/diarrhea	5.7	1.6	6.5	0	3.3	0	15.4	1.6
Fluid retention[a]	2.4	0	9.8	1.6	1.6	0	13.8	1.6
Bone pain/Joint pain	9.8	0.8	0.8	0.8	1.6	0.8	12.2	2.4
Conjunctivitis/Dry eye	4.1	0	4.9	0.8	2.4	0	11.4	0.8
Gastric pain	6.5	2.4	1.6	0	0.8	0.8	8.9	3.3
Nausea/Vomiting	2.4	0	2.4	0	2.4	0	7.3	0
Headache	4.9	0	0.8	0	0.8	0	6.5	0
Alopecia	2.4	0	2.4	0	0.8	0	5.6	0

[a] Other than periorbital edema.

Percentage of patients with AEs. We reported here the AEs (all grades) with a cumulative incidence $\geq$ 5% by 12 months, considering all 123 enrolled patients. We analyzed whether an AE was observed in the same patient during more treatment periods. Therefore, AEs were divided in three groups: AEs ascribed to nilotinib only, to imatinib only, or to both drugs. If a pre-existing AE persisted for more than 15 days after the planned change of treatment, the AE was associated with both drugs.

regime. Another patient (age 70, male) complained of worsening claudication, which was controlled with medical treatment, but prompted the discontinuation of nilotinib. One asymptomatic patient (age 59, male) developed a carotid stenosis, of moderate grade but progressively worsening, and discontinued nilotinib.

Discussion

The results of the treatment of newly diagnosed, CP, CML with a standard dose of imatinib (400 mg OD), together with second- and third-line treatment with second generation TKIs, in case of failure, non-optimal response, intolerance or toxicity, are already excellent [1–4], and few space is left for improvement. The combination of TKIs with interferon-α or other agents has been recently discussed [15,16], but currently, with the exception of interferon-α, the efforts to improve the outcome focus on a larger use of second generation TKIs in first-line, as well as on an early switch from imatinib to second generation TKIs [17–25]. It was reported that these policies resulted in a faster achievement of more and deeper molecular responses, in a marginal improvement of PFS, but not of OS. The obstacles to an earlier and larger use of nilotinib are cost [26] and toxicity, particularly the concern of vascular complications [27–34]. On the contrary, the cardiovascular safety of imatinib, together with its lower cost, particularly with the upcoming generic formulation, may consolidate its use as first-line treatment for the majority of CML patients. So far, no attempts were made to investigate the use of TKIs in combination, as it is the case in many leukemias and cancers, where the sequential or concomitant administration of effective drugs is common, and beneficial.

When nilotinib became available for the second-line treatment of CML, we considered that a combination of nilotinib and imatinib could have been more effective than imatinib alone, and maybe as effective as nilotinib alone, but less toxic and less expensive. The concomitant administration of two TKIs may raise pharmacodynamics and pharmacokinetics issues impacting on safety, efficacy, dosing, and schedule. The sequential administration of two TKIs could avoid these issues, and therefore may be preferable. We selected the 3-month rotating schedule considering pharmacokinetics aspects (some days are required to reach a steady-state plasma drug concentration), and taking care of designing a schedule that would have been easy to comply with, and that coincided with the routine molecular monitoring.

The standard, approved dose of nilotinib first-line is now 300 mg BID. However, when this study was designed, in 2008, the tested dose of nilotinib in second-line was 400 mg BID, and the results of the ENESTnd study were not yet available [35]; therefore, the dose of 400 mg BID was selected.

In our trial, the cumulative incidence of CCyR by 12 months was 91%, and the cumulative incidences of MMR and MR 4.0 by 24 months were 87% and 61%, respectively. Though any comparison among different studies is biased, the MMR (Supporting Information Table VI) and the MR 4.0 rates achieved in this trial were at the high-end of those reported so far with single-agent TKI [35–49]. Risk distribution can affect the response: here, the proportion of high Sokal risk patients was 22%, vs. 16% to 29% in other studies. After the 24-month core phase, all patients were allowed to move from the 2-drug rotation regime to single drug treatment based on a physician's choice. All patients were then followed until a minimum of 5 years, a follow-up that is equal to, or longer than, that of the majority of the studies of second-generation TKIs in first-line. At 5 years, 69% of the patients were on treatment with the study drugs, although only a minority (11%) was still on the rotation regime. Several reasons, including the selection of the best tolerated drug between imatinib and nilotinib, the level of molecular response achieved, the success of the ENESTnd study [35,37], and costs, may have influenced the decision to continue nilotinib or imatinib alone (36 and 22% of the patients, respectively).

The 5-year PFS and OS were 89%, an outcome that is in the range of what reported in prior trials (Supporting Information Table VI) with TKIs in first-line. Moreover, these results are particularly significant if we consider the impact of age on survival [50]. Indeed, in our study patients' median age (56 years) was almost 10 years higher than that of the ENESTnd study (46 and 47 years in imatinib and nilotinib arms, respectively) [35] and the DASISION study (49 and 46 years in imatinib and dasatinib arms, respectively) [40], and, importantly, it was close to that reported in population based Registries in Italy and Europe [14]. Age is also associated to an increased incidence of arterial thrombotic events; here, despite the significantly higher median age compared to the ENESTnd study, the rate of arterial thrombotic events was 5%, similar to that reported in the nilotinib 300 mg BID arm (7.5%), and lower than that reported in the nilotinib 400 mg BID arm of that study (13.4%) [39].

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Noteworthy, five of seven progressions occurred within the first year, including 2 patients that had not previously achieved an EMR (BCR-ABL1 $\leq$ 10% at 3 months). It is possible that a prolongation of the first period of nilotinib could have been useful, particularly in the patients without EMR. Overall, 12 (10%) patients developed a secondary resistance (loss of a previous achieved level of response; details in Supporting Information Table VII); however, since mutations were identified in 2 patients only, and conferred resistance to both nilotinib and imatinib, it is likely that the rotation of the drugs did not play a major role in these events.

In summary, this study showed long-term outcomes and molecular responses fully comparable to those reported in previously published trials with first-line TKIs in CP CML. The safety data, particularly concerning cardiovascular adverse events, suggest that the alternating regime could be safer than nilotinib alone. Moreover, even if a detailed cost analysis was not planned in this study, it is conceivable that this policy may result in lower costs compared to nilotinib alone. We conclude that in newly diagnosed, CP, CML patients the initial treatment with this 2-TKI rotation regime may be an alternative to single-TKI therapy.

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