MYELOID NEOPLASIA

Nilotinib is associated with a reduced incidence of BCR-ABL mutations vs imatinib in patients with newly diagnosed chronic myeloid leukemia in chronic phase

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Key Points

- Frontline nilotinib led to fewer, less diverse BCR-ABL mutations than imatinib in patients with chronic myeloid leukemia in chronic phase.
- Rates of progression to accelerated phase/blast crisis were lower with nilotinib than imatinib in patients with emergent BCR-ABL mutations.

In patients with chronic myeloid leukemia, BCR-ABL mutations contribute to resistance to tyrosine kinase inhibitor therapy. We examined the occurrence of treatment-emergent mutations and their impact on response in patients from the ENESTnd phase 3 trial. At the 3-year data cutoff, mutations were detected in approximately twice as many patients (21) on imatinib 400 mg once daily as on nilotinib (11 patients each on nilotinib 300 mg twice daily and nilotinib 400 mg twice daily). The majority of mutations occurred in patients with intermediate or high Sokal scores. Most mutations (14 [66.7%]) emerging during imatinib treatment were imatinib-resistant and nilotinib-sensitive. Incidence of the T315I mutation was low (found in 3, 2, and 3 patients on nilotinib 300 mg twice daily, nilotinib 400 mg twice daily, and imatinib, respectively) and mostly occurred in patients with high Sokal scores. Of the patients with emergent mutations, 1 of 11, 2 of 11, and 7 of 21 patients on nilotinib 300 mg twice daily, nilotinib 400 mg twice daily, and imatinib, respectively, progressed to accelerated phase/blast crisis (AP/BC) on treatment. Overall, nilotinib led to fewer treatment-emergent BCR-ABL mutations than imatinib and reduced rates of progression to AP/BC in patients with these mutations. (Clinicaltrials.gov NCT00471497). (*Blood.* 2013;121(18):3703-3708)

Introduction

Chronic myeloid leukemia (CML) is a hematopoietic malignancy associated with the translocation t(9;22)(q34;q11), resulting in the Philadelphia chromosome (Ph) and the presence of the constitutively activated tyrosine kinase BCR-ABL.[1] The development of imatinib (Glivec, Gleevec; Novartis Pharmaceuticals, East Hanover, NJ), a selective BCR-ABL tyrosine kinase inhibitor (TKI), established BCR-ABL inhibition as the standard of care for the treatment of CML. Despite the efficacy of imatinib in patients with Ph-positive (Ph+) CML in chronic phase (CP), approximately 15% of patients display resistance to imatinib or relapse after an initial response to therapy and thus have less favorable long-term outcomes.[2,3]

Nilotinib (Tasigna; Novartis Pharmaceuticals) was rationally designed to have enhanced selectivity and potency toward BCR-ABL compared with imatinib.[4] Nilotinib is recommended by the European LeukemiaNet (ELN)[5] and the National Comprehensive Cancer Network (NCCN)[6] for the treatment of adult patients with newly diagnosed Ph+ CML-CP and patients with imatinib-resistant or imatinib-intolerant Ph+ CML in CP or accelerated phase (AP) and has been approved in multiple countries for use in these indications.[7,8]

The randomized phase 3 study Evaluating Nilotinib Efficacy and Safety in Clinical Trials—Newly Diagnosed Patients (ENESTnd) compared nilotinib (300 mg twice daily and 400 mg twice daily) with imatinib in patients with newly diagnosed Ph+ CML-CP.[9-11] In ENESTnd, significantly fewer patients with CML-CP treated with either dose of nilotinib progressed to AP/blast crisis (BC),[9-11] and significantly more patients achieved complete cytogenetic response (CCyR),[9,10] major molecular response (MMR),[9-11] MR^4 (defined as *BCR-ABL* transcript levels ≤0.01% on the International Scale [IS]),[10,11] and $MR^{4.5}$ (defined as *BCR-ABL* transcript levels ≤0.0032% IS)[10,11] compared with patients treated with imatinib.

Mutations in the kinase domain of BCR-ABL are a common mechanism of resistance to TKI therapy, and these mutations have been detected in 40% to 60% of imatinib-resistant patients.[12-18] More than 90 distinct mutations in BCR-ABL have been identified, each

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Table 1. Polymorphisms identified at baseline

Variant	Nucleotide change	Codon	All patients (N = 846), n (%)	Nilotinib 300 mg twice daily (n = 282), n	Nilotinib 400 mg twice daily (n = 281), n	Imatinib 400 mg once daily (n = 283), n
T83T	ACT→ACG	c.252T→G	1 (0.1)	0	1	0
N96S	AAT→AGT	c.290A→G	1 (0.1)	0	1	0
T117T	ACG→ACA	c.344G→A	1 (0.1)	0	0	1
T204T	ACG→ACA	c.615G→A	1 (0.1)	1	0	0
T240T*	ACG→ACA	c.723G→A	2 (0.2)	1	1	0
Y253Y	TAC→TAT	c.762C→T	1 (0.1)	0	0	1
E499E*	GAA→GAG	c.1500A→G	53 (6.3)	21	17	15

All sequence variants were verified to be present in nontranslocated ABL; therefore, they are polymorphisms.
*T240T and E499E are previously reported polymorphisms.

conferring variable degrees of resistance to imatinib therapy.[17] Nilotinib has in vitro inhibitory activity against all tested imatinib-resistant mutants of BCR-ABL, except the T315I mutant.[4] Previous data have demonstrated that imatinib-resistant patients with CML-CP or CML-AP treated with nilotinib had high and durable rates of response for all mutations except T315I, Y253H, E255K/V, and F359C/V.[18-20] Here, we prospectively examined the occurrence of mutations emerging on treatment with nilotinib and imatinib in the ENESTnd study and their impact on response in patients with newly diagnosed Ph+ CML-CP.

Materials and methods

Patients, study design, and treatment

The ENESTnd study design has been previously described.[9-11] A total of 846 patients with CML-CP were randomized at a 1:1:1 ratio to receive nilotinib 300 mg twice daily, nilotinib 400 mg twice daily, or imatinib 400 mg once daily. Randomization was stratified according to the Sokal risk score[21] at the time of diagnosis. Eligibility criteria were previously reported[9] and are standard criteria for frontline CML-CP studies. This study was approved by the ethics committee or review board at each institution and was conducted in accordance with the Declaration of Helsinki. Prior to the study, all patients provided voluntary written informed consent.

Assessment of BCR-ABL mutational status

Mutational analysis of the BCR-ABL kinase domain by long-range reverse transcriptase polymerase chain reaction (PCR) amplification and direct sequencing (sensitivity, detection in 10% to 20% of Ph+ cells) was performed for all patients at baseline. If patients with baseline mutations (or polymorphisms) were identified, subsequent mutational analyses were planned every 3 months to monitor any changes in relation to drug response. For patients who did not have any baseline mutations or polymorphisms, triggers for subsequent mutational analyses were the end of treatment (including discontinuation) and lack of response or loss of response (failure to achieve MMR at 1 year, confirmed loss of MMR, or rise in *BCR-ABL* transcript level by fivefold or more from the lowest value achieved on study). Confirmed loss of MMR was defined as $BCR\text{-}ABL^{IS} > 0.1\%$, in association with a fivefold or more rise in *BCR-ABL* transcript levels from the lowest value achieved up to that time point, confirmed by a duplicate analysis of the same sample and by another PCR sample ≥4 weeks later. For patients with a mutation identified during therapy, follow-up mutational analyses were performed in subsequent samples until no mutation was detected, discontinuation of study, or death. For a patient with clinical relapse but a negative mutational result at the time of the relapse, continued mutational analyses may have been performed every 3 months as needed. *ABL* polymorphisms identified at baseline were confirmed by amplifying and sequencing the kinase domain region of both nontranslocated *ABL* alleles in the same samples.[22]

Analyses

All comparisons of the incidence of emerging mutations and outcomes among treatment arms were assessed using descriptive statistics. Due to the small number of patients in each treatment arm of ENESTnd who developed mutations, analyses were not powered to enable statistical comparisons between these subgroups. Retrospective analyses were performed on the full ENESTnd data set (N = 846), with a data cutoff of July 27, 2011 (3 years of follow-up[11]). Patients with emerging BCR-ABL mutations were identified, and types of mutations on treatment were assessed. Overall response status in patients with emerging mutations was summarized. The numbers of patients with progression to AP/BC, confirmed loss of MMR, or confirmed loss of CCyR (2 cytogenetic assessments ≥4 weeks apart with Ph+ >0%, regardless of the number of metaphases; or progression to AP/BC; or CML-related death) with and without emerging mutations were determined.

Results

Frequency of mutations on treatment

At baseline, no BCR-ABL mutations were detected for any patient. ABL polymorphisms were equally distributed among the 3 treatment arms (23 for nilotinib 300 mg twice daily, 20 for nilotinib 400 mg twice daily, and 17 for imatinib) (Table 1). With a minimum follow-up of 3 years, the number of patients who had at least 1 postbaseline mutational analysis was similar across the 3 treatment arms (228, 215, and 237 patients in the nilotinib 300 mg twice daily, nilotinib 400 mg twice daily, and imatinib arms, respectively; Table 2). However, emerging mutations were detected in approximately twice as many patients in the imatinib arm (21 patients) than in either nilotinib arm (11 patients each in the nilotinib 300 mg twice daily and nilotinib 400 mg twice daily arms; Table 2). In total, 16 different mutations affecting 13 amino acid residues within the BCR-ABL kinase domain were identified. The number of patients with multiple mutations detected was low and was similar in the nilotinib and imatinib arms: 3, 2, and 3 patients in the nilotinib 300 mg twice daily, nilotinib 400 mg twice daily, and imatinib arms, respectively.

The majority (66.7%) of patients with emerging mutations on imatinib had mutations previously identified to be resistant to imatinib but sensitive to nilotinib,[18] whereas 2 patients (20%) and no patients (0%) treated with nilotinib 300 mg twice daily and nilotinib 400 mg twice daily, respectively, had such mutations. In contrast, patients treated with nilotinib 300 mg or 400 mg twice daily more frequently had emerging mutations previously identified to be imatinib-resistant and less sensitive to nilotinib.[18] These mutations (Y253H, E255K/V, and F359C/V) were detected in 6 (54.5%) of 11, 9 (81.8%) of 11, and 4 (19.0%) of 21 patients with mutations treated with nilotinib 300 mg twice daily, nilotinib 400 mg twice daily, and imatinib, respectively. The incidence of T315I mutations was low

Table 2. Mutations identified during treatment

	Nilotinib 300 mg twice daily (n = 282), n	Nilotinib 400 mg twice daily (n = 281), n	Imatinib 400 mg once daily (n = 283), n
Patients with ≥1 postbaseline mutational analysis*	228	215	237
Patients with mutations	11	11	21
New mutations by Sokal score			
Low	1	2	1
Intermediate	5	3	8
High	5	6	12
Mutation category			
T315I	3	2	3
Less sensitive to nilotinib†	6	9	4
Other mutations‡	2§	0	14
Multiple mutations	3	2	3
Mutations‖			
M244V	0	0	4
G250E	1	0	2
Q252H	0	1	0
Y253H	4	4	2
E255K	1	3	0
E255V	0	1	0
D276G	0	0	2
T315I	3	2	3
M351T	0	0	2
E355G	0	0	1
F359C	0	0	1
F359I	0	0	2
F359V	4	2	1
H396R	0	0	1
E450G	0	0	1
E459K	1	0	2

*Triggers for postbaseline mutational analysis included mutations or polymorphisms at baseline, lack of response or loss of response on treatment, and end of treatment.

†Mutations less sensitive to nilotinib are E255K/V, F359C/V, and Y253H.

‡Includes imatinib-resistant, nilotinib-sensitive mutations (ie, all mutations except E255K/V, F359C/V, Y253H, and T315I).

§Of the 2 nilotinib-treated patients with other mutations, one had an E459K mutation and the other had a G250E mutation.

‖Individual mutation totals include patients with multiple mutations.

and was similar in the nilotinib and imatinib arms: 3, 2, and 3 patients in the nilotinib 300 mg twice daily, nilotinib 400 mg twice daily, and imatinib arms, respectively. Regardless of treatment, nearly all (39 [90.7%] of 43) emergent mutations were identified in patients with intermediate or high Sokal risk scores. Additionally, of the patients with a T315I mutation, 7 (87.5%) of 8 had high Sokal risk scores and 1 (12.5%) of 8 had an intermediate Sokal risk score at study entry.

Overall response status and disposition of patients with emergent mutations

Regardless of treatment, the majority of patients with emergent mutations exhibited suboptimal responses or treatment failure based on 2009 ELN CML management recommendations (defined for patients treated with imatinib 400 mg once daily) (Table 3).[5] Overall, 36.4% (4 of 11) and 54.5% (6 of 11) of patients with newly detectable mutations treated with nilotinib 300 mg twice daily and nilotinib 400 mg twice daily, respectively, failed treatment; 54.5% (6 of 11) and 18.2% (2 of 11) of patients in the nilotinib 300 mg twice daily and 400 mg twice daily arms, respectively, demonstrated suboptimal response to treatment. The remaining patient in the nilotinib 300 mg twice daily arm progressed after discontinuation of treatment. In the nilotinib 400 mg twice daily arm, 2 patients with mutations had confirmed loss of MMR, and 1 patient had an unconfirmed loss of MMR that was later regained as their worst responses. Of the 21 imatinib-treated patients with any emergent mutations, most (76.2% [16 of 21]) failed treatment, and 5 of 21 (23.8%) had suboptimal responses to therapy. All 8 patients with a T315I mutation had suboptimal response or treatment failure, although it should be noted that postbaseline mutational analyses were triggered by lack or loss of response.

At the 3-year data cutoff, 38 (88.4%) of the 43 patients with an emergent mutation had discontinued treatment, primarily because of suboptimal response or treatment failure. There were 5 patients (11.6%) who remained on treatment at the date of the data cutoff: 1 in the nilotinib 300 mg twice daily arm (F359V mutation), 2 in the nilotinib 400 mg twice daily arm (E255K and Y253H mutations), and 2 in the imatinib arm (F359C and T315I mutations). The F359V mutation was detected in the patient in the nilotinib 300 mg twice daily arm upon mutational analysis due to lack of MMR at 12 months; however, retrospective mutational analysis revealed that the mutation existed during the first 3 months of therapy. The mutation was not detected after 14 months, and the patient subsequently achieved both CCyR and MMR. The patient in the nilotinib 400 mg twice daily arm with the E255K mutation achieved CCyR but was not in MMR when the mutation was detected (about 33 months after start of study). The patient with the Y253H mutation in the nilotinib 400 mg twice daily arm achieved both CCyR and MMR but was not in MMR when the mutation was detected (about 33 months after start of study). The patient in the imatinib arm with the F359C mutation (first detected about 11 months after start of study) achieved both CCyR and MMR after mutation detection. The patient with the T315I mutation in the imatinib arm (first detected about 11 months after start of study) achieved CCyR but not MMR before the detection of mutation.

Progression to AP/BC and loss of response in patients with emergent mutations

Although most patients with emergent mutations in ENESTnd had suboptimal response or treatment failure, relatively fewer nilotinib-treated patients with emergent mutations than imatinib-treated patients with emergent mutations progressed to AP/BC or lost response on

Table 3. Response status in patients with emergent mutations

	Nilotinib 300 mg twice daily (n = 282), n	Nilotinib 400 mg twice daily (n = 281), n	Imatinib 400 mg once daily (n = 283), n
Patients with mutations	11	11	21
Response status			
Treatment failure*	4	6	16
Suboptimal response*	6	2	5
Confirmed loss of MMR*†	0	2	0
Other*‡	1	1	0

*Patients were counted only once under the worst-case response category.

†Loss of MMR was not considered a criterion for suboptimal response.

‡The patient in the nilotinib 300 mg twice daily arm progressed after discontinuation of treatment, and the patient in the nilotinib 400 mg twice daily arm had an unconfirmed loss of MMR but regained MMR after that.

Table 4. Mutational status in patients who progressed to AP/BC or lost response on treatment

Event type	Nilotinib 300 mg twice daily (n = 282), n	Nilotinib 400 mg twice daily (n = 281), n	Imatinib 400 mg once daily (n = 283), n
Progression to AP/BC on treatment*	2	3	12
Patients with mutations at time of progression	1	2	7
Type of mutation at time of progression	E459K	Y253H/T315I, E255V	M244V, Y253H, Y253H/F359I, M351T, F359I, E459K (2)
Patients who achieved MMR at any time	207	199	153
Patients with confirmed loss of MMR†	9	11	14
Patients with mutations at loss of MMR	0	4‡	3§
Type of mutation at loss of MMR		Y253H, Y253H/T315I, E255V, E255K	M244V, T315I, H396R/M351T
Patients who achieved CCyR at any time	245	238	218
Patients with confirmed loss of CCyR‖	2	3	5
Patients with mutations at loss of CCyR	0	2‡	3‡
Type of mutation at loss of CCyR		Y253H/T315I, E255V	Y253H/F359I, M351T, E459K

*Progression to AP/BC or death due to CML while on core study treatment. Estimated 3-y rates of progression-free survival in ENESTnd were previously reported (99.3%, 98.7%, and 95.2% for nilotinib 300 mg twice daily, nilotinib 400 mg twice daily, and imatinib, respectively; $P = .0059$ and .0185 for nilotinib 300 mg twice daily and nilotinib 400 mg twice daily, respectively, vs imatinib).[11]

†Confirmed loss of MMR was defined as $BCR\text{-}ABL^{IS}$ >0.1%, with a fivefold or more rise in *BCR-ABL* ratio from the lowest value achieved up to that time point, confirmed by a duplicate analysis of the same sample and by another PCR sample analyzed ≥4 weeks later.

‡Patients later progressed to AP/BC on treatment.

§Patients later discontinued due to suboptimal response.

‖Confirmed loss of CCyR was defined as 2 cytogenetic assessments ≥4 weeks apart with Ph+ >0%, regardless of the number of metaphases analyzed.

treatment (Table 4). In the nilotinib 300 mg twice daily arm, 1 (9.1%) of the 11 patients with a treatment-emergent mutation (E459K) progressed to AP/BC on treatment, without achieving CCyR or MMR, and no patient with an emergent mutation lost CCyR or MMR on treatment. In the nilotinib 400 mg twice daily arm, 2 (18.1%) of the 11 patients with a treatment-emergent mutation (1 patient each with Y253H/T315I and E255V) demonstrated losses of CCyR and MMR and subsequent progression to AP/BC on treatment. An additional 2 patients (18.1%; 1 patient each with Y253H and E255K) with treatment-emergent mutations on nilotinib 400 mg twice daily experienced a loss of MMR but no loss of CCyR or progression to AP/BC on treatment. In the imatinib arm, 7 (33.3%) of the 21 patients with treatment-emergent mutations progressed to AP/BC on treatment (1 patient each with M244V, Y253H, Y253H/F359I, M351T, and F359I, and 2 patients with E459K). Three of these patients also experienced a loss of CCyR (1 patient each with Y253H/F359I, M351T, and E459K) on treatment. An additional 3 patients (14.3%) with treatment-emergent mutations on imatinib (1 patient each with M244V, T315I, and H396R/M351T) experienced a loss of MMR but no loss of CCyR or progression to AP/BC on treatment.

Mutational status in patients with progression to AP/BC or loss of response

Analysis of all patients in ENESTnd demonstrated that some patients who progressed to AP/BC or lost response did not have treatment-emergent BCR-ABL mutations (Table 4). With 3 years of follow-up, 17 patients had progressed to AP/BC on treatment: 2 (<1%), 3 (1.1%), and 12 (4.2%) patients in the nilotinib 300 mg twice daily, nilotinib 400 mg twice daily, and imatinib arms, respectively. Of these, 1 (50.0%), 2 (66.7%), and 7 (58.3%) patients in the nilotinib 300 mg twice daily, nilotinib 400 mg daily, and imatinib arms, respectively, developed mutations on treatment.

A total of 34 patients in ENESTnd had a confirmed loss of MMR: 9 (4.3%) of 207, 11 (5.5%) of 199, and 14 (9.2%) of 153 patients treated with nilotinib 300 mg twice daily, nilotinib 400 mg twice daily, and imatinib, respectively. None of the 9 patients in the nilotinib 300 mg twice daily arm who lost MMR developed mutations during treatment. Of the 11 patients in the nilotinib 400 mg twice daily arm who lost MMR, 4 patients (36.4%) developed mutations during treatment (Y253H, E255K, E255V, and Y253H/T315I). Of the 14 patients who lost MMR in the imatinib arm, 3 patients (21.4%) developed mutations during treatment (M244V, T315I, and H396R/M351T). Half (17 [50.0%] of 34) the patients with emergent mutations who lost MMR subsequently regained the response.

Very few patients in ENESTnd had a confirmed loss of CCyR: 2 (<1%) of 245, 3 (1.3%) of 238, and 5 (2.3%) of 218 patients in the nilotinib 300 mg twice daily, nilotinib 400 mg twice daily, and imatinib arms, respectively. The 2 patients who lost CCyR in the nilotinib 300 mg twice daily arm did not have detectable mutations. Of the 3 patients who lost CCyR in the nilotinib 400 mg twice daily arm, 2 patients developed mutations during treatment (E255V and Y253H/T315I), lost MMR, and later progressed to AP/BC on treatment. Of the 5 patients who lost CCyR in the imatinib arm, 3 developed mutations during treatment (M351T, Y253H/F359I, and E459K) and later progressed to AP/BC on treatment.

Discussion

There have been more than 90 different BCR-ABL mutations identified in patients with imatinib resistance[17]; of these, only 15 amino acid substitutions account for 80% to 90% of all reported imatinib-resistant mutations, and 7 mutated codons (G250, Y253, E255, T315, M351, F359, and H396) account for 60% to 70%.[23] The mutation screening rate in ENESTnd may have been higher than that typically observed in clinical practice. In this study, fairly conservative triggers for mutational analysis (ie, lack of MMR at 1 year, confirmed loss of MMR, rise in *BCR-ABL* transcript level by fivefold or more from the lowest value achieved on study, and end of treatment/treatment discontinuation) were used to ensure that all patients with a higher probability of having an emergent mutation were identified. Among the 3 study arms, 16 different mutations were identified (Table 2), all of which have been previously reported in imatinib-resistant patients and were of relevant size (the proportion of mutant BCR-ABL/Ph+ cells to the total number of BCR-ABL/Ph+ cells) for the given sensitivity of the sequencing analyses.

With a minimum follow-up of 3 years, approximately twice as many emergent mutations were detected in patients in the imatinib arm as in either nilotinib arm. The rate of emergent mutations with frontline imatinib in this study is consistent with previously reported data.[13,24-27] The incidence of emerging mutations was comparable in both nilotinib arms, demonstrating that treatment with the lower dose of nilotinib (300 mg twice daily) does not increase the likelihood of developing emergent mutations.

In 2 patients treated with nilotinib 300 mg twice daily, the G250E and E459K BCR-ABL mutations became detectable. Although the emergence of these 2 mutations may have contributed to nilotinib resistance in these patients, on the basis of previous clinical data, these mutations are unlikely to be solely responsible for this resistance. The emergence of G250E and E459K mutations during nilotinib treatment has been previously observed in a phase 2 study of nilotinib in imatinib-resistant and -intolerant patients, in which all patients were treated with nilotinib 400 mg twice daily.[18] Preclinical studies have shown conflicting data on the degree of resistance of the G250E mutation to nilotinib, with variable results in biochemical and cell-based assays,[4,17,28,29] and clinical efficacy of nilotinib against the G250E mutant has been demonstrated in the aforementioned phase 2 study, with several patients achieving CCyR and MMR.[18]

Although preclinical studies with cells bearing the E459K BCR-ABL mutation have not been reported, modeling studies predict that this mutation would have a destabilizing effect on the inactive (DFG-out) conformation of the ABL tyrosine kinase domain to which both imatinib and nilotinib bind.[30,31] However, Kim et al reported a case of a patient with CML in BC bearing V299L and E459K compound mutations who responded to nilotinib,[30] suggesting that any loss in sensitivity conferred by the E459K mutation toward nilotinib is not insurmountable. Rather than reflecting a difference between the 2 nilotinib doses in suppressing the emergence of the G250E and E459K mutations, the absence of these mutations in the nilotinib 400 mg arm of ENESTnd may be due to the small number of patients who developed mutations on study. The incidence of the T315I mutation was similar with nilotinib and imatinib, demonstrating that there may not be a selective pressure for this highly resistant mutation in patients treated with nilotinib, a more potent and selective TKI, than imatinib. Overall, these results suggest that nilotinib is less likely than imatinib to lead to treatment-emergent mutations in patients with newly diagnosed CML.

Consistent with previous data from patients treated with imatinib,[24] the detection of emerging mutations in this study was more frequent in patients with intermediate or high Sokal score at study entry, irrespective of treatment arm. Moreover, the majority of patients with mutations treated with either drug in this study had suboptimal response or treatment failure. Previous data have demonstrated that patients with less-than-optimal responses[5] to therapy have poorer long-term outcomes and increased risk of progression compared with optimally responding patients.[32] Thus, the fact that mutations were more frequent in patients with higher prognostic risk in this study and that these patients were less likely to have optimal responses to therapy may support the hypothesis that the probability of developing a mutation is linked to a predisposition for developing resistance because of genomic instability.[33]

Many patients who lost responses or progressed to AP/BC in this study had a BCR-ABL mutation; however, mutations did not account for all cases of progression to advanced disease or loss of response. This observation suggests that alternative resistance mechanisms may be operative in some patients. Furthermore, it should be noted that our results cannot confirm whether treatment-emergent mutations were in fact driver mutations (and therefore responsible for the responses observed). Other factors not assessed in this analysis, such as interruption of therapy, may also have had an impact on the development of mutations and the responses observed. Nevertheless, our observations suggest that treatment with a more selective and potent TKI such as nilotinib suppresses the outgrowth of mutant clones and may ultimately increase the number of patients who have optimal responses to therapy and lead to fewer patients progressing to advanced disease.

In the overall ENESTnd population, fewer patients treated with either dose of nilotinib had suboptimal response or treatment failure compared with patients treated with imatinib at both 12- and 18-month milestones.[34] Patients treated with either dose of nilotinib in ENESTnd were significantly more likely to achieve CCyR than patients treated with imatinib,[10] and rates of MMR, MR^4, and $MR^{4.5}$ by 3 years were significantly higher in both nilotinib arms compared with the imatinib arm.[11] Nilotinib treatment was also associated with significantly reduced rates of progression to AP/BC and fewer CML-related deaths.[11] The decreased emergence of mutations seen with nilotinib relative to imatinib in this study may be a contributing factor to the improved outcomes seen with nilotinib. A study by Soverini et al[35] demonstrated that patients treated with frontline nilotinib therapy who achieved and maintained MMR rarely had BCR-ABL mutations, even when evaluated by high-sensitivity methods. However, because high-sensitivity screening was not performed in this study, it cannot be excluded that BCR-ABL kinase mutations existed below the level of detection in patients who achieved deeper responses with nilotinib.

In conclusion, this study demonstrates that the deeper molecular responses and more selective BCR-ABL inhibition achieved with nilotinib reduce the emergence of BCR-ABL mutations and protect patients from progression to advanced disease. These results further support the use of nilotinib in patients with newly diagnosed Ph+ CML-CP.

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Authorship

Contribution: A. Hochhaus, G.S., A. Hoenekopp, Y.S., H.M.K., and T.P.H. designed and performed research; A. Hochhaus, R.A.L., C.D.S., H.M.K., and T.P.H. provided study materials or patients; A. Hochhaus, R.A.L., G.E., G.R., C.D.S., M.K., A. Hoenekopp, Y.S., and H.M.K. collected and assembled data; A. Hochhaus, G.S., R.A.L., D.-W.K., M.E.K., A. Hoenekopp, X.F., Y.S., H.M.K., and T.P.H. analyzed and interpreted data; all authors drafted and approved the manuscript.

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References

1. Druker BJ. Translation of the Philadelphia chromosome into therapy for CML. *Blood.* 2008; 112(13):4808-4817.
2. Hochhaus A, O'Brien SG, Guilhot F, et al; IRIS Investigators. Six-year follow-up of patients receiving imatinib for the first-line treatment of chronic myeloid leukemia. *Leukemia.* 2009;23(6):1054-1061.
3. Deininger M, O'Brien SG, Guilhot F, et al. International randomized study of interferon vs STI571 (IRIS) 8-year follow up: sustained survival and low risk for progression or events in patients with newly diagnosed chronic myeloid leukemia in chronic phase (CML-CP) treated with imatinib. [abstract]. *Blood.* 2009;114:462[abstract 1126].
4. Weisberg E, Manley P, Mestan J, Cowan-Jacob S, Ray A, Griffin JD. AMN107 (nilotinib): a novel and selective inhibitor of BCR-ABL. *Br J Cancer.* 2006;94(12):1765-1769.
5. Baccarani M, Cortes J, Pane F, et al; European LeukemiaNet. Chronic myeloid leukemia: an update of concepts and management recommendations of European LeukemiaNet. *J Clin Oncol.* 2009;27(35):6041-6051.
6. National Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology: Chronic Myelogenous Leukemia. 2012;Version 3. 2013. Available at: http://www.nccn.org [Accessed February 19, 2013].
7. Tasigna [package insert]. East Hanover, NJ: Novartis Pharmaceuticals Corporation; 2012.
8. European Medicines Agency. Tasigna: summary of product characteristics [online]. Available at: http://www.emea.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000798/WC500034394.pdf [Accessed February 5, 2013].
9. Saglio G, Kim DW, Issaragrisil S, et al; ENESTnd Investigators. Nilotinib versus imatinib for newly diagnosed chronic myeloid leukemia. *N Engl J Med.* 2010;362(24):2251-2259.
10. Kantarjian HM, Hochhaus A, Saglio G, et al. Nilotinib versus imatinib for the treatment of patients with newly diagnosed chronic phase, Philadelphia chromosome-positive, chronic myeloid leukaemia: 24-month minimum follow-up of the phase 3 randomised ENESTnd trial. *Lancet Oncol.* 2011;12(9):841-851.
11. Larson RA, Hochhaus A, Hughes TP, et al. Nilotinib vs imatinib in patients with newly diagnosed Philadelphia chromosome-positive chronic myeloid leukemia in chronic phase: ENESTnd 3-year follow-up. *Leukemia.* 2012; 26(10):2197-2203.
12. Gorre ME, Mohammed M, Ellwood K, Hsu N, Paquette R, Rao PN, Sawyers CL. Clinical resistance to STI-571 cancer therapy caused by BCR-ABL gene mutation or amplification. *Science.* 2001;293(5531):876-880.
13. Jabbour E, Kantarjian H, Jones D, et al. Frequency and clinical significance of BCR-ABL mutations in patients with chronic myeloid leukemia treated with imatinib mesylate. *Leukemia.* 2006;20(10):1767-1773.
14. Hochhaus A, Kreil S, Corbin AS, et al. Molecular and chromosomal mechanisms of resistance to imatinib (STI571) therapy. *Leukemia.* 2002; 16(11):2190-2196.
15. Lahaye T, Riehm B, Berger U, et al. Response and resistance in 300 patients with BCR-ABL-positive leukemias treated with imatinib in a single center: a 4.5-year follow-up. *Cancer.* 2005;103(8): 1659-1669.
16. Soverini S, Gnani A, De Bendittis C, et al. BCR-ABL kinase domain mutations in imatinib and in second-generation tyrosine kinase inhibitor eras: a review of seven years of mutation analysis by the GIMEMA CML working party. *Haematologica.* 2011;96(s2):204-205[abstract 0486].
17. Soverini S, Hochhaus A, Nicolini FE, et al. BCR-ABL kinase domain mutation analysis in chronic myeloid leukemia patients treated with tyrosine kinase inhibitors: recommendations from an expert panel on behalf of European LeukemiaNet. *Blood.* 2011;118(5):1208-1215.
18. Hughes T, Saglio G, Branford S, et al. Impact of baseline BCR-ABL mutations on response to nilotinib in patients with chronic myeloid leukemia in chronic phase. *J Clin Oncol.* 2009;27(25): 4204-4210.
19. le Coutre P, Ottmann OG, Giles F, et al. Nilotinib (formerly AMN107), a highly selective BCR-ABL tyrosine kinase inhibitor, is active in patients with imatinib-resistant or -intolerant accelerated-phase chronic myelogenous leukemia. *Blood.* 2008; 111(4):1834-1839.
20. Kantarjian HM, Giles FJ, Bhalla KN, et al. Nilotinib is effective in patients with chronic myeloid leukemia in chronic phase after imatinib resistance or intolerance: 24-month follow-up results. *Blood.* 2011;117(4):1141-1145.
21. Sokal JE, Baccarani M, Russo D, Tura S. Staging and prognosis in chronic myelogenous leukemia. *Semin Hematol.* 1988;25(1):49-61.
22. Ernst T, Hoffmann J, Erben P, et al. ABL single nucleotide polymorphisms may masquerade as BCR-ABL mutations associated with resistance to tyrosine kinase inhibitors in patients with chronic myeloid leukemia. *Haematologica.* 2008;93(9): 1389-1393.
23. Jones D, Kamel-Reid S, Bahler D, et al. Laboratory practice guidelines for detecting and reporting BCR-ABL drug resistance mutations in chronic myelogenous leukemia and acute lymphoblastic leukemia: a report of the Association for Molecular Pathology. *J Mol Diagn.* 2009;11(1):4-11.
24. Khorashad JS, de Lavallade H, Apperley JF, et al. Finding of kinase domain mutations in patients with chronic phase chronic myeloid leukemia responding to imatinib may identify those at high risk of disease progression. *J Clin Oncol.* 2008; 26(29):4806-4813.
25. Soverini S, Colarossi S, Gnani A, et al. GIMEMA Working Party on Chronic Myeloid Leukemia. Contribution of ABL kinase domain mutations to imatinib resistance in different subsets of Philadelphia-positive patients: by the GIMEMA Working Party on Chronic Myeloid Leukemia. *Clin Cancer Res.* 2006;12(24):7374-7379.
26. Sherbenou DW, Wong MJ, Humayun A, et al. Mutations of the BCR-ABL-kinase domain occur in a minority of patients with stable complete cytogenetic response to imatinib. *Leukemia.* 2007;21(3):489-493.
27. Khorashad JS, Anand M, Marin D, et al. The presence of a BCR-ABL mutant allele in CML does not always explain clinical resistance to imatinib. *Leukemia.* 2006;20(4):658-663.
28. Weisberg E, Manley PW, Breitenstein W, et al. Characterization of AMN107, a selective inhibitor of native and mutant Bcr-Abl. *Cancer Cell.* 2005; 7(2):129-141.
29. Redaelli S, Piazza R, Rostagno R, et al. Activity of bosutinib, dasatinib, and nilotinib against 18 imatinib-resistant BCR/ABL mutants. *J Clin Oncol.* 2009;27(3):469-471.
30. Kim D, Kim DW, Cho BS, et al. Structural modeling of V299L and E459K Bcr-Abl mutation, and sequential therapy of tyrosine kinase inhibitors for the compound mutations. *Leuk Res.* 2009;33(9):1260-1265.
31. Vajpai N, Strauss A, Fendrich G, Cowan-Jacob SW, Manley PW, Grzesiek S, Jahnke W. Solution conformations and dynamics of ABL kinase-inhibitor complexes determined by NMR substantiate the different binding modes of imatinib/nilotinib and dasatinib. *J Biol Chem.* 2008;283(26):18292-18302.
32. Marin D, Milojkovic D, Olavarria E, et al. European LeukemiaNet criteria for failure or suboptimal response reliably identify patients with CML in early chronic phase treated with imatinib whose eventual outcome is poor. *Blood.* 2008;112(12): 4437-4444.
33. Jiang X, Saw KM, Eaves A, Eaves C. Instability of BCR-ABL gene in primary and cultured chronic myeloid leukemia stem cells *J Natl Cancer Inst.* 2007;99(9):680-693.
34. Saglio G, Hochhaus A, Guilhot F, et al. Nilotinib is associated with fewer treatment failures and suboptimal responses vs imatinib in patients with newly diagnosed chronic myeloid leukemia in chronic phase (CML-CP): results from ENESTnd. *Haematologica.* 2011;96(s2):58-59[abstract 0145].
35. Soverini S, Gnani A, De Benedittis C, et al. Low-level Bcr-Abl mutations are very rare in chronic myeloid leukemia patients who are in major molecular response on first-line nilotinib. *Leuk Res.* 2011;35(11):1527-1529.