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Next-generation sequencing improves *BCR-ABL1* mutation detection in Philadelphia chromosome-positive acute lymphoblastic leukaemia

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Summary

BCR-ABL1 kinase domain mutation testing in tyrosine kinase inhibitor (TKI)-resistant Philadelphia chromosome-positive (Ph+) acute lymphoblastic leukaemia (ALL) patients is routinely performed by Sanger sequencing (SS). Recently, next-generation sequencing (NGS)-based approaches have been developed that afford greater sensitivity and straightforward discrimination between compound and polyclonal mutations. We performed a study to compare the results of SS and NGS in a consecutive cohort of 171 Ph+ ALL patients. At diagnosis, 0/44 and 3/44 patients were positive for mutations by SS and NGS respectively. Out of 47 patients with haematologic resistance, 45 had mutations according to both methods, but in 25 patients NGS revealed additional mutations undetectable by SS. Out of 80 patients in complete haematologic response but with *BCR-ABL1* ≥0·1%, 28 (35%) and 52 (65%) were positive by SS and NGS respectively. Moreover, in 12 patients positive by SS, NGS detected additional mutations. NGS resolved clonal complexity in 34 patients with multiple mutations at the same or different codons and identified 35 compound mutations. Our study demonstrates that, in Ph+ ALL on TKI therapy, NGS enables more accurate assessment of mutation status both in patients who fail therapy and in patients with minimal residual disease above 0·1%.

Keywords: acute lymphoblastic leukemia, BCR-ABL1, mutations, tyrosine kinase inhibitors, NGS.

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Introduction

Incorporation of BCR-ABL1 tyrosine kinase inhibitors (TKIs) in the first-line treatment of adult Philadelphia chromosome-positive (Ph+) acute lymphoblastic leukaemia (ALL) has led to an increase in complete haematological response (CHR) rates up to 95–100%, thus enabling more patients to undergo allogeneic stem cell transplantation (SCT) (recently reviewed in Soverini *et al.*, 2019[1]). However, the stability of response to TKIs is frequently undermined by the development of resistance, which is of particular concern in the elderly and unfit population, which is not eligible for transplant or intensive chemotherapy regimens. Moreover, the persistence of detectable minimal residual disease (MRD) is a well-established negative prognostic factor.[2–4] Disease persistence or recurrence have been associated with point mutations in the BCR-ABL1 kinase domain (KD).[5–9] Detection of some imatinib-resistant mutations predicts for the efficacy or the inefficacy of the other TKIs.[1] The T315I mutation, in particular, can only be addressed, as yet, by the third-generation TKI ponatinib.[10] Accurate mutation profiling is always necessary in case of relapse, but it is of value also at other key time points for decision-making, like end of induction or consolidation, and if a TKI is not tolerated and must be changed.[11,12]

The traditional approach to mutation testing is based on Sanger sequencing (SS) of the entire KD of the *BCR-ABL1* transcript.[13–15] In recent years, next-generation sequencing (NGS) has been proposed as an alternative because of the greater sensitivity and the clonal nature of the analysis — enabling the detection of small mutant subclones as well as the straightforward identification of compound mutations (CMs).[16–19] The latter are when two distinct mutations are acquired by the same *BCR-ABL1* molecule, as opposed to polyclonality.[20] Like individual mutations, different CMs seem to exhibit different TKI sensitivity profiles.[21–23] A few small, retrospective studies in Ph+ ALL have suggested that NGS paints a more accurate picture of mutation status,[16] and may pick up emerging TKI-resistant mutations earlier than SS in approximately half of the patients who will later experience haematological relapse.[24]

Here we report the results of a study aimed to compare *BCR-ABL1* KD mutation screening by NGS and by SS in a

consecutive series of 171 Ph+ ALL patients who were analysed either at diagnosis or during TKI treatment.

Materials and methods

Patients

Between May 2015 and May 2019, a total of 171 adult Ph+ ALL patients were enrolled in the study. Forty-four patients were analysed at the time of diagnosis, prior to any therapy; 127 patients were studied during treatment, either because of refractoriness/relapse ($n = 47$) or because of molecularly detectable MRD positivity after induction or consolidation therapy ($n = 80$). MRD-positive patients were eligible if they had a *BCR-ABL1/ABL1* transcript ratio ≥0·1% by real-time quantitative polymerase chain reaction (qPCR), because at transcript levels below 0·1% library preparation and sequencing are not always successful and, even when successful, might yield unreliable or unreproducible results.[25] Patient disposition is shown in Table I. Patients were treated in first line or in subsequent lines with different regimens according to local protocols, but all regimens incorporated a TKI (imatinib, dasatinib or ponatinib) at standard, recommended dose. This study was noninterventional: first, second- and subsequent-line treatment was not guided by NGS results. For each patient, a 5-ml bone marrow (BM) sample was shipped to the central reference laboratory in Bologna from one of 25 participating Haematology Centres in Italy. Each sample was analysed in parallel by SS and by NGS, as described below. This study (ref. 113/2014/U/Tess) was approved by the Ethical Committees of the S. Orsola Hospital in Bologna (promoter and sponsor Institution) and of all the other centres. Patients signed written informed consent for participation.

Table I. Patient disposition.

Total no. of patients	171
Median age, yrs (range)	57 (18–78)
Gender (M:F)	85:86
At diagnosis, prior to any treatment	44
Lack or loss of CHR	47
In CHR, but MRD-positive (*BCR-ABL1* > 0·1%)	80
Last TKI incorporated in the treatment regimen:	
Imatinib	42
Dasatinib	68
Ponatinib	17
e1a2 (p190)	113
b2a2 or b3a2 (p210)	58

yrs, years; M, male; F, female; CHR, complete haematological remission; MRD, minimal residual disease; TKI, tyrosine kinase inhibitor.

BCR-ABL1 KD mutation screening by SS

SS-based analysis was performed on an ABI PRISM 3730 (Applied Biosystems, Foster City, CA, USA) as previously described.[26] The lower detection limit of NGS was 20%.

BCR-ABL1 KD mutation screening by NGS

NGS-based screening of the *BCR-ABL1* KD was performed on a Roche (Basel, Switzerland) GS Junior instrument until April 2017, and on an Illumina (San Diego, CA, USA) MiSeq instrument from May 2017 on, according to a custom deep-sequencing protocol of a panel of six 400-bp amplicons generated by nested reverse transcription-polymerase chain reaction (RT-PCR), that has been reported in detail elsewhere.[16] Briefly, amplicon design was performed so that the key mutation hotspots, P-loop, T315, F317, and A-loop, were always interrogated twice by independent amplicons. RT and the first step of PCR amplification of the breakpoint region and of the KD were done similarly to the SS-based protocol. Adapters and indexes were introduced in the sequencing amplicons using fusion primers during the second step of PCR amplification (for sequencing on the Roche GS Junior) or via end-repairing, A-tailing and ligation after the second step of PCR amplification (for sequencing on the Illumina MiSeq). Setup and evaluation of accuracy and reproducibility of the NGS-based method have been described.[16,27] Read alignment to the *ABL1* reference sequence (GenBank accession number NM_005157.5), variant calling at nucleotide positions corresponding to amino acids 235 through 498 (that correspond to the KD of ABL1[25]) annotation and filtration were done using the Amplicon Suite software (SmartSeq s.r.l., Novara, Italy), which was implemented to maximise the reliability of variant calls based on an algorithm integrating specific acceptability criteria and estimation of error rates at each nucleotide position calculated using a retrospective dataset of patients and donors. Acceptability criteria have been reported.[27] The lower detection limit of NGS was 3%; variants below 3%, if detected, were filtered out. *Cis* or *trans* configuration of mutation pairs, indicating CMs or polyclonality, respectively, was determined correcting for the likelihood of PCR recombination as described by Deininger *et al.*[28] The '35INS' insertion/truncation mutants[29] were excluded from the analyses.

Definitions

In this study, variants were defined as 'high-level' when identified in ≥20% of *BCR-ABL1* transcripts (hence detectable both by NGS and by SS) and as 'low-level' when identified in 3–20% of *BCR-ABL1* transcripts (hence detectable by NGS but not by SS).

Results

Newly diagnosed Ph+ ALL

Of the 44 patients who were analysed at the time of diagnosis, none was positive for mutations by SS and three (7%) were positive for mutations by NGS. One patient had a V289A (variant transcript frequency, 3·4%); one patient had a D276G (4%) and a F359V (3·5%); one patient had an E255K (3·3%). The first patient was enrolled in a clinical trial of ponatinib first line (NCT01641107). The second and the third patient were enrolled in a clinical trial of dasatinib first line as induction treatment, followed by blinatumomab (NCT02744768). Thus, all three patients happened to receive a TKI active against the mutation(s) that had been detected by NGS, and as expected, they achieved a CHR and a molecular response (follow-up: 18, 22 and 29 months).

TKI-resistant Ph+ ALL

Out of 127 patients with relapsed/refractory disease or MRD persistence, 74 (58%) and 98 (77%) were positive for mutations by SS and by NGS respectively (Fig 1A). All the mutations identified by SS were also identified by NGS. The advantage of NGS over SS consisted not only in the detection of mutations in 24 (19% of the total) patients who were negative by SS, but also in the identification of additional mutations in 37 (29% of the total) patients who were positive by SS (Fig 1A). All but three (R362K, L370R, Y413C) of the mutations identified by NGS but not by SS could be recognised as poorly sensitive either to the TKI the patient was receiving at the time of testing, or to the previous TKI(s).

In the setting of haematologic resistance (Fig 1B), 45/47 patients had mutations according to SS and no additional patient was found to have mutations by NGS. In 25 (56%) cases, however, NGS revealed a more complex mutation status, with one or more mutations that were undetectable by SS. In the setting of MRD positivity (*BCR-ABL1* transcript levels >0·1%; Fig 1C), 28/80 (35%) patients were positive by SS and 52/80 (65%) by NGS. Moreover, 12 patients positive by SS had additional mutations detectable by NGS only. Whenever the selective pressure was maintained, all the MRD-positive patients who were negative by SS but had mutations known to confer resistance to the ongoing TKI according to NGS subsequently relapsed.

Table II lists the relative frequency of mutations as assessed by SS (total: 107) and by NGS (total: 189) respectively. T315I, that is resistant to imatinib and to all the available second-generation TKIs, was by far the most frequent mutation: it was identified in 45·9% of patients by SS and in 58·2% of patients by NGS. The other mutations most recurrently detected by both methods were E255K (resistant to imatinib, nilotinib and bosutinib), Y253H (resistant to imatinib and nilotinib), F317L (resistant to imatinib and dasatinib) and E255V (resistant to imatinib, nilotinib, and bosutinib). No association was observed between transcript type and mutation prevalence.

Of the 74 patients positive by SS, 29 (39%) had more than one mutation (up to three). Of the 98 patients positive by NGS, 58 (59%) had more than one mutation (up to 11). Five of these 58 patients had multiple mutations at the same codon which only NGS could disentangle. Two patients had an E255K and an E255V resulting from two different nucleotide changes at the same codon (GAG > AAG and GAG > GTG respectively) (Fig 2A). One patient had three F317 variants in separate subclones (F317L resulting from a TTC > CTC nucleotide substitution, F317I resulting from a TTC > ATC substitution and F317C resulting from a TTC > TGC substitution; Fig 2B). One patient had an identical V299L resulting from two distinct nucleotide substitutions in independent subclones (GTG > TTG and GTG > CTG) and harboured four separate additional subclones, three with the same F317L resulting from three distinct nucleotide changes (TTC > CTC, TTC > TTG and TTC > TTA) and one with the F317I (TTC > ATC). Lastly, one patient had two variants of the F317L mutation in two separate subclones (arising from TTC > CTC and TTC > TTG nucleotide changes respectively) and an F317I (TTC > ATC) in a third subclone.

Moreover, 57/58 patients had multiple mutations at different codons. Analysis of *cis* or *trans* configuration led to identify a total of 35 CMs in 29 patients (30% of all the patients positive for mutations by NGS). Twenty-two out of 29 patients with CMs had been referred because of no or loss of CHR, 7/29 because of MRD increase. CMs were exclusively detected in patients who had received ≥2 TKIs; at the time of analysis, 16 patients were on dasatinib therapy and 13 on ponatinib. Table III details the type and relative frequency of the CMs identified, and the TKI the patients were receiving at the time of testing. In all the cases, each mutation pair included either a T315I or an F317L coupled with one of the most recurrent imatinib-resistant mutations. T315I/E255K and T315I/E255V were by far the most frequent pairs (10 and 8 patients respectively), and were detected in association with either dasatinib or ponatinib haematologic or molecular resistance. While no single mutation was found in association with ponatinib resistance, CMs were detected in 13/17 cases (besides T315I/E255V and T315I/E255K, we also found T315I/F359V, T315I/G250E, T315I/Y253F, T315I/Q252H). Detailed mutation results for each of the 127 patients are reported in Table S1.

Discussion

BCR-ABL1 KD mutation screening is essential in relapsed/refractory Ph+ ALL[11,12] since mutation profile is the most important piece of information for the selection of the most appropriate therapeutic alternative in this critical category of patients. For several years, SS has been the gold standard for testing, but nowadays NGS is replacing SS in many

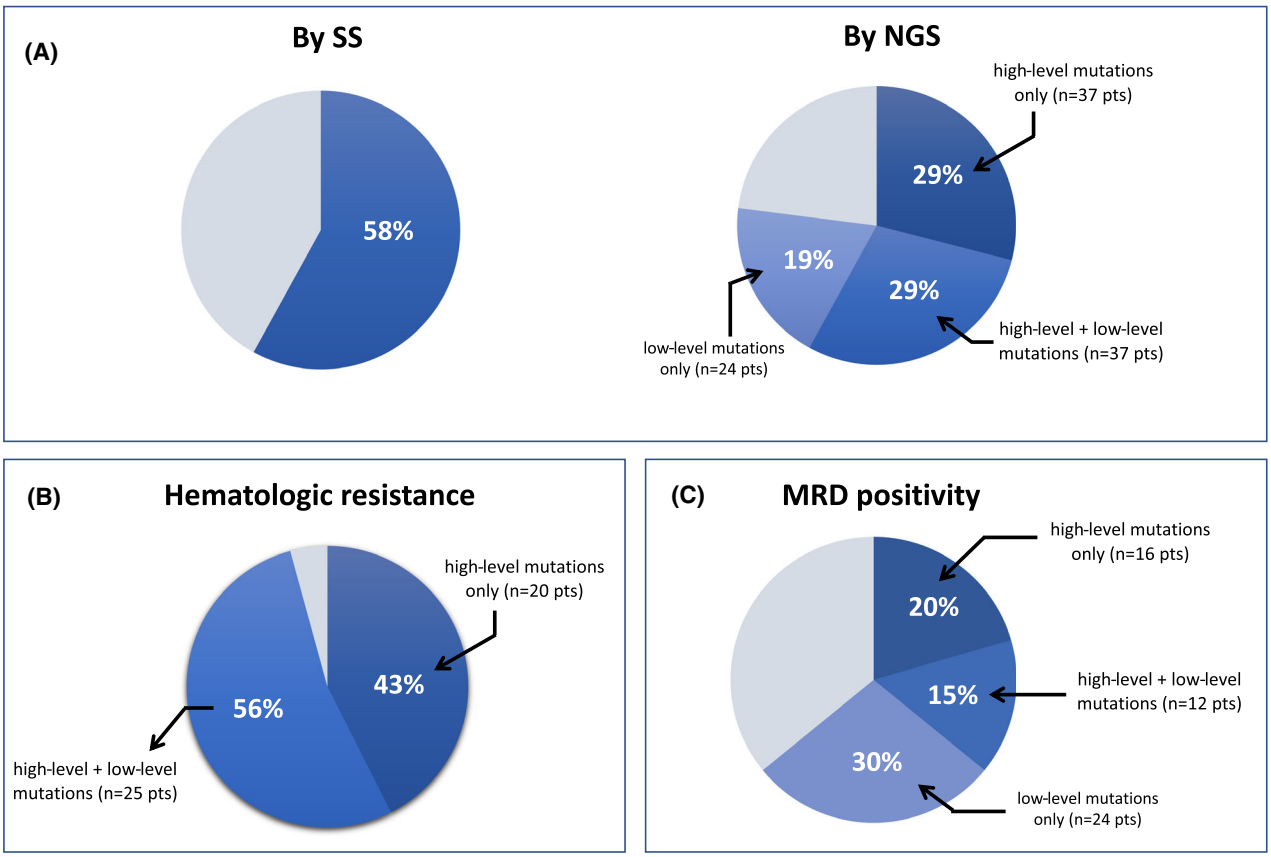

Fig 1. (A) Percentage of TKI-resistant patients positive for mutations by Sanger sequencing (SS) and by next-generation sequencing (NGS). Among patients positive for mutations by NGS, 37 (29%) had high-level mutations only (≥20%; detectable by SS, too); another 37 (29%) had both one or more high-level mutations and one or more low-level mutations (≤20%; detectable by NGS only); 24 (19%) had only low-level mutations. (B) Breakdown of high- and low-level mutations detected in the setting of haematologic resistance (n = 47 patients). Eleven patients were studied because of the lack of complete haematological remission (CHR) to a first- (n = 2), second- (n = 3) or third-line (n = 6) TKI. All patients had mutations according to both SS and NGS, but in 5/11 patients NGS detected additional mutations. The remaining 36 patients were studied because of loss of CHR. Thirty-four had mutations according to both SS and NGS, but in 20/34 patients NGS detected additional mutations, for a total of 25 patients with a more complex mutation status as revealed by NGS. (C) Breakdown of high- and low-level mutations detected in the setting of MRD persistence (*BCR-ABL1* ≥ 0·1%; n = 80 patients). [Colour figure can be viewed at wileyonlinelibrary.com]

diagnostic laboratories. This is the first study designed to evaluate the informativity and feasibility of NGS — as compared to SS — for routine *BCR-ABL1* KD mutation testing in a cohort of Ph+ ALL patients.

To this purpose, we compared NGS and SS results in 127 consecutive Ph+ ALL patients with relapsed/refractory disease or MRD positivity at the end of induction or during consolidation therapy (for technical reasons related to the feasibility of library preparation, patients had to display *BCR-ABL1* transcript levels >0·1%). Our data show that in about half of the patients on TKI therapy in whom there was an indication for *BCR-ABL1* KD mutation testing, SS provided inaccurate information about mutation status (Fig 1A).

In the setting of haematological resistance, the key advantage of NGS over SS was to highlight a more complex mutation status in more than half of the patients — who were found to harbour low-level mutations additional to those detectable by SS. As the number of options for treating relapsed and refractory Ph+ ALL patients have increased, it is vital to rely on the most accurate mutation information to select the potentially best therapy for each individual case, in an attempt to maximise long-term outcomes. In the setting of MRD persistence, NGS was found to detect mutations in nearly twice as many patients as compared to SS: 52/80 as against 28/80. This means that NGS could pick up emerging TKI-resistant mutations in 24 (30%) cases. This would enable choosing the best TKI as an earlier therapeutic intervention and possibly prevent or delay haematological relapse — that is associated with inferior outcomes and impacts on SCT effectiveness. Indeed, in our study, all patients who remained on the same TKI subsequently relapsed. Among the mutations that can go undetected by SS but may be picked by NGS, some individual or CMs will have an influence on the choice of the TKI. The T315I mutation confers insensitivity to imatinib and all second-generation TKIs but can be overcome by ponatinib. Monoclonal

Table II. Relative frequency of mutations as assessed by Sanger sequencing (SS) *versus* next-generation sequencing (NGS).

Mutation	No. of patients with the mutation as assessed by SS (%*)	No. of patients with the mutation as assessed by NGS (%*)
T315I	34 (45·9%)	57 (58·2%)
E255K	16 (21·6%)	24 (24·5%)
Y253H	11 (14·9%)	15 (15·3%)
F317L	9 (12·2%)	19 (19·4%)
E255V	8 (10·8%)	12 (12·2%)
V299L	5 (6·8%)	12 (12·2%)
F359V	5 (6·8%)	8 (8·3%)
G250E	4 (5·4%)	6 (6·1%)
F317I	4 (5·4%)	5 (5·1%)
Q252H	3 (4·1%)	5 (5·1%)
E459K	2 (2·7%)	4 (4·1%)
D276G	2 (2·7%)	3 (3%)
F317C	1 (1·3%)	1 (1%)
M351T	1 (1·3%)	2 (2%)
Y253F	1 (1·3%)	4 (4·1%)
L248V	1 (1·3%)	2 (2%)
M244V	ND	1 (1%)
E279K	ND	1 (1%)
E355G	ND	1 (1%)
I418V	ND	1 (1%)
R362K	ND	1 (1%)
Y413C	ND	1 (1%)
L370R	ND	1 (1%)

*The sum of the percentages is greater than 100 because several patients had more than one mutation.

antibodies like blinatumomab or inotuzumab can also be considered because of their different mode of action.[30] Thus, (timely) detection of this mutation is of utmost importance. In our study, SS missed a T315I mutation in 24/127 (19%) patients. Similarly, detection of an F317L mutation in a patient on imatinib should discourage the choice of dasatinib as second-line therapy. Moreover, both *in silico* molecular modelling and *in vitro* studies have recently suggested that some T315I-inclusive CMs confer resistance not only to imatinib and all second-generation TKIs, but also to ponatinib.[21-23] In our study, almost 40% of patients had two (or more) mutations by SS, and the percentage increased to almost 60% when NGS was used. In all these cases, determining the *cis* or *trans* configuration of mutation pairs is important. This piece of information can indirectly be inferred from SS traces only when the percentage of both mutations is greater than 50%; in all the remaining cases, a cumbersome procedure of cloning would be needed — which is not feasible on a routine basis. NGS, in contrast, provides a straightforward method to assess the clonal configuration of T315I-inclusive mutation pairs and with its greater sensitivity may pick ponatinib-resistant CMs even when present at a relatively small abundance. This would prevent the unnecessary exposure to a fairly toxic drug that would most likely be ineffective. Twelve T315I-inclusive CMs have been predicted to have little or no sensitivity to ponatinib based on the IC_{50} values calculated in cell line models,[22,23] though *in vivo* data are scarce.[22,31] In our study, 12/12 patients who did not achieve or who lost CHR on ponatinib indeed harboured one ($n = 8$) or two ($n = 4$) CMs. In particular, T315I/E255V and T315I/E255K were the most frequent CMs found in association with ponatinib resistance.

We also investigated whether the greater sensitivity of NGS (lower detection limit, 3% as compared to 15–20% in SS[19,27]) might enable the detection of mutations in newly diagnosed patients. Detection of *BCR-ABL1* KD mutations in pretherapy samples had occasionally been reported.[7,8,24,32,33] NGS enabled us to identify mutations in only 3/44 patients. It can thus be concluded that prior to any treatment, mutations if present occur in a very small proportion of transcripts — lower, in most of the cases, than the NGS detection limit. This is in line with the results of two recent studies that took advantage of approaches capable to further enhance sensitivity of several orders of magnitude. Using an ultra-accurate and sensitive approach of duplex sequencing, Short *et al.*[34] found pretreatment mutations with a median variant allelic frequency of 0·008% in 49/63 (78%) of the patients analysed. A strategy of allele-specific oligonucleotide droplet digital PCR enabled Cayuela *et al.*[35] to identify the T315I mutation at levels ranging from 0·00051% to 0·0013% in 24% of 63 tested patients. Interestingly, both studies revealed that only in few instances these 'very low-level' mutations correlated with poor response to regimes of intensive chemotherapy, TKIs, and allogeneic SCT. Taken together, our findings and the two studies mentioned above suggest that pretreatment mutation testing is likely to be of very limited clinical value. SS and NGS seem not to have enough sensitivity, whereas more sensitive approaches may pick up very rare mutations that are unlikely to impact on outcome, at least when intensive treatment regimens are used.

Timely decision-making is essential in Ph+ ALL: centralisation of NGS testing in a limited number of reference laboratories is thus mandatory. This will also facilitate standardisation and organisation of periodical quality control rounds that are critical requirements to ensure accuracy and reproducibility of results. Ideally, laboratories should already be involved in MRD monitoring of Ph+ ALL patients, so that mutation testing may be activated timely whenever necessary on the same RNA or cDNA sample used for MRD assessment. Sequencing of samples in batches of reasonable size will also be needed for cost-effectiveness. This would not be a problem for medium- to large-scale laboratories, where NGS is nowadays routinely used to analyse larger and larger numbers of leukaemia and solid tumour samples (thus equipment and expertise are already available) given that *BCR-ABL1* libraries can be pooled and sequenced together with other gene libraries. Moreover, a good inter-laboratory

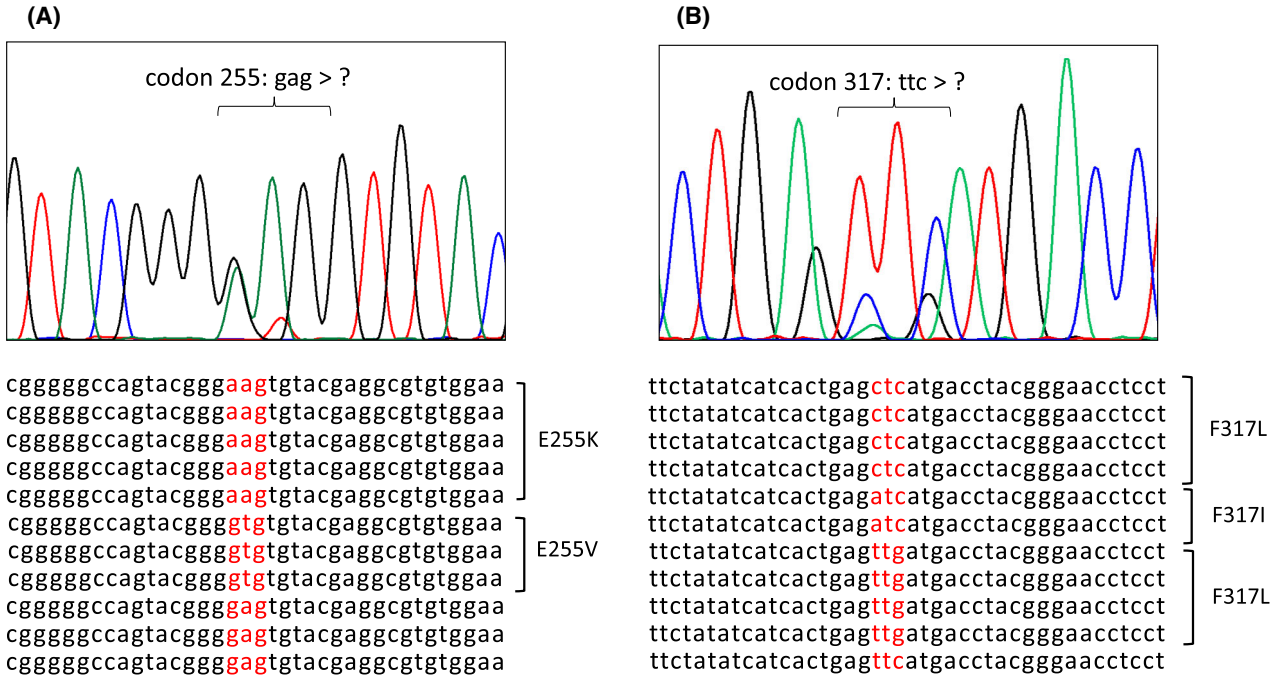

Fig 2. Representative examples of patients with multiple mutations at the same codon. (A) UPN #11 had two nucleotide substitutions at adjacent positions in codon 255. Mutations were detectable by Sanger sequencing (SS), but the resulting amino acid substitutions (E255K, E255V) could only be determined after the alignment of next-generation sequencing (NGS) clonal reads. (B) UPN #122 had two overlapping nucleotide substitutions at the first position of codon 317 and an additional one at the third position. NGS reads showed that the three nucleotide substitutions were acquired by three distinct clones and resulted in two F317L mutations and one F317I mutation. [Colour figure can be viewed at wileyonlinelibrary.com]

Table III. Type and relative frequency of the compound mutations identified in our study.

Compound mutation	No. of patients	TKI at the time of testing
T315I/E255K	10	ponatinib (n = 6); dasatinib (n = 4)
T315I/E255V	8	ponatinib (n = 7); dasatinib (n = 1)
T315I/F359V	3	ponatinib (n = 2); dasatinib (n = 1)
T315I/Y253H	3	dasatinib
F317L/E255K	3	dasatinib
F317L/F359V	2	dasatinib
F317L/Y253H	1	dasatinib
T315I/Q252H	1	ponatinib
T315I/G250E	1	ponatinib
F317L/G250E	1	dasatinib
T315I/Y253F	1	ponatinib
T315I/M351T	1	dasatinib

The therapy at the time of compound mutation detection is specified. Six patients (five on ponatinib, one on dasatinib) were found to harbour two different compound mutations. TKI, tyrosine kinase inhibitor.

reproducibility of the NGS methodology, both in general and for *BCR-ABL1* mutation testing, has been shown by several studies.[27,36–39] It is important to underline that the use of NGS in Ph+ ALL should not be restrained by either technical or financial issues. The cost of the drugs is much higher than the cost of testing (estimated to be around €100 per sample[27]). Besides imatinib, dasatinib and ponatinib, other treatment modalities have recently become available or might become available in the near future. Asciminib (a TKI targeting the myristoyl-binding pocket rather than the ATP-binding site) has been evaluated in a phase 1 trial enrolling both chronic myeloid leukaemia (CML) and Ph+ ALL patients. In CML cell lines and patients, asciminib has been reported to be active against the T315I as well as many other individual mutations.[40] Interestingly, combination with asciminib seems to restore the efficacy of ponatinib (even at very low doses) against many CMs.[40] Even more importantly, in multi-TKI-resistant patients where remission induction prior to transplant is the clear path, immune-based therapies like monoclonal antibodies and CAR-T are a valuable option, since their efficacy is not influenced by mutation status. Thus, accurate assessment of mutation status by NGS will have a double utility. On one hand, it will enable better selection among different BCR-ABL1-targeted TKIs. On the other hand, it will help identify a subset of high-risk patients with a 'mutator' phenotype, where a switch to another TKI that will most likely be ineffective should be avoided now that better alternatives are available.

We acknowledge that there have been no studies evaluating and comparing the clinical impact of using NGS rather than SS, like no studies had ever tested the clinical impact of

SS-based mutation testing on patient outcome. Such studies are almost impossible to design and to perform in such a rare condition as Ph+ ALL. Yet, the biological rationale supporting mutation testing is clear and strong. For this reason, the use of NGS for *BCR-ABL1* KD mutation screening has already been endorsed by the European LeukemiaNet panel of experts who compiled the 2020 recommendations for the treatment of CML.[41]

Author contributions

Simona Soverini, Margherita Martelli, Luana Bavaro, Caterina De Benedittis, Michele Baccarani, Giovanni Martinelli and Michele Cavo designed and performed the study, analysed the data and wrote the manuscript. The remaining authors enrolled patients and provided clinical information. All authors reviewed critically the manuscript and gave final approval for submission.

Conflicts of interests

SS has received speaker fees from Incyte Biosciences (Wilmington, DE, USA); SG has received speaker fees from Pfizer (New York, NY, USA), Novartis (Basel, Switzerland) and Incyte Biosciences; MBo has received honoraria from Novartis, Incyte Biosciences and Pfizer; EA has received speaker fees from Novartis, Bristol-Myers Squibb (New York, NY, USA), Incyte Biosciences and Pfizer; FA has received honoraria for advisory boards from Incyte Biosciences; MA has received honoraria for advisory boards from Incyte Biosciences, Novartis and Pfizer; FSt has received honoraria from Bristol-Myers Squibb, Incyte Biosciences, Novartis and Pfizer; MBa has been a consultant for Incyte Biosciences, Takeda (Tokyo, Japan) and Novartis; GM has been a consultant and has received speaker fees from Incyte Biosciences and Pfizer. The remaining authors declare that they have no competing interests.

Funding

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table SI. Detailed mutation status of each of the 127 TKI-resistant patients analysed in parallel by Sanger sequencing (SS) and by next-generation sequencing (NGS).

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