# Characterization of alternative splicing events in the Tyrosine Kinase Domain of BCR-ABL1 transcripts in CML patients during TKI

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## INTRODUCTION

Chronic Myeloid Leukemia (CML) is characterized by the presence of the Philadelphia chromossome (Figure 1) that encodes the BCR-ABL1 oncoprotein, a necessary condition for malignant transformation. Tyrosine kinase inhibitors (TKI), specific for BCR-ABL1, bind to the ATP binding site in the tyrosine kinase domain of ABL1, inhibiting the transduction of proliferation and cell death signals (Figure 2).

The use of TKI in patients with CML is the most successful case of a targeted therapy. However, resistance to TKI remains an unsolved clinical problem. The main mechanism associated with resistance is the presence of point mutations in the kinase domain. However, direct sequencing of the kinase domain of CML patients shows, in addition to mutations, insertions and deletions. Unlike point mutations, splicing variants don't have their role well described in literature, and it is not known the value of deletions and insertions in resistance to TKIs.

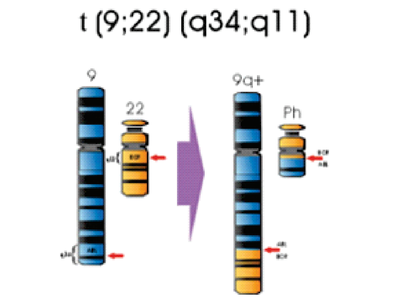



Figure 1: Translocation between chromossomes 9 and 22, producing the Piladelphia Chromossome (Ph).
Available at: http://www.unesp.br/prope/projtecn/Saude/saude48a.htm

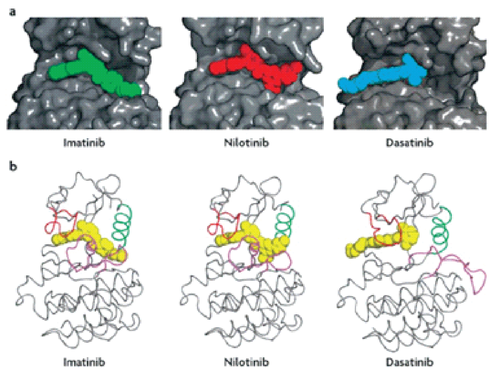



Figure 2: Tyrosine kinase protein complexed with different tyrosine kinase inhibitors. (a) Three-dimensional shaping of tyrosine kinase protein while binded with different TKIs. (b) Protein-bound scheme in condensed structure.
Available at: Nature Reviews Cancer, 2007, Nature Publishing Group.

## METHODOLOGY

Characterization of the occurrence of insertions and deletions in the tyrosine kinase domain of the BCR-ABL1 transcripts, and evaluation of it's possible role in the resistance to TKI. The study was conducted with responders and late responders to TKIs compared to patients failing treatment due to primary and secondary resistance. Response and resistance was defined with data provided by European Leukemia Net (Table 1). Samples were submitted to direct sequencing and the analysis was performed by *Mutation Surveyor V5.0.1 ® SoftGenetics* specific analysis program.

Table 1: Parameters used to evaluate the response of patients with CML using TKI in first line treatment.

| | Optimal | Warning | Failure |
|---|---|---|---|
| Baseline | NA | High risk<br>Or<br>CCA/Ph+, major route | NA |
| 3 mo | BCR-ABL1 ≤10%<br>and/or<br>Ph+ ≤35% | BCR-ABL1 >10%<br>and/or<br>Ph+ 36-95% | Non-CHR<br>and/or<br>Ph+ >95% |
| 6 mo | BCR-ABL1 <1%<br>and/or<br>Ph+ 0 | BCR-ABL1 1-10%<br>and/or<br>Ph+ 1-35% | BCR-ABL1 >10%<br>and/or<br>Ph+ >35% |
| 12 mo | BCR-ABL1 ≤0.1% | BCR-ABL1 >0.1-1% | BCR-ABL1 >1%<br>and/or<br>Ph+ >0 |

Source: Baccarani et al. 2013.

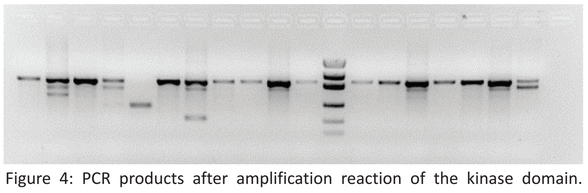


Figure 4: PCR products after amplification reaction of the kinase domain. Agarose gel 2% with ethidium bromide under UV light.

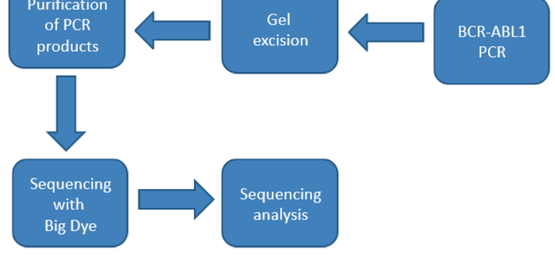



Figure 3: Flowchart of the methodology used in the study.

## RESULTS

| Patient samples | 44 |
|---|---|
| Age at diagnosis (median in years) | 45 (12-73) |
| Male | 30 (68,2%) |
| Treatment (median in months) | 73 (13-181) |
| Fase | |
| Chronic | 31 (70%) |
| Acelerate | 2 (4,5%) |
| NI | 11 (25%) |
| Inhibidor | |
| Imatinib | 31 (70%) |
| Nilotinib | 4 (9%) |
| Dasatinib | 9 (20,5%) |

Figure 5: Characteristics of the cohort of patients analyzed in the study.

Patients were grouped acoording to their response to TKI treatment: 12 of them were optimal responders, 8 were late responders, 16 had primary resistance and 8 acquired resistance during treatment (secondary resistance) (Graph 1). The most frequent insertion is that of 35 bp (ins35) (Figure 6C) and the most frequent deletion was the complete exon 7 (185 bp) (Figure 6A). 37.5% (6/16) of patients with primary resistance had ins35 and only one patient presented a point mutation (T315I), while in the group of patients in secondary resistance, 4 (50%) patients obtained this same insertion (Graph 2) . Regarding the deletions, two patients with primary resistance and one in secondary resistance had complete deletions of exon 7 (185 bp) (Graph 2). The presence of partial deletion of exons 7 (72 bp) (Figure 6 B) was observed in one optimal responder patient and in one late responder (Graph 2).

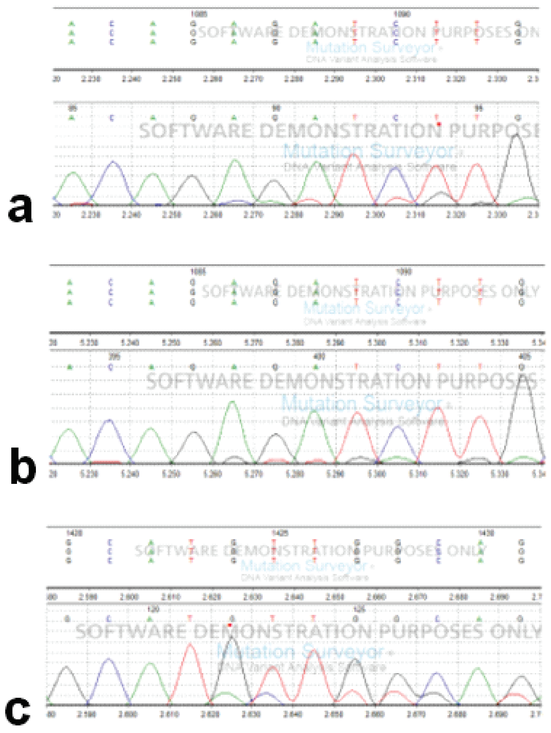
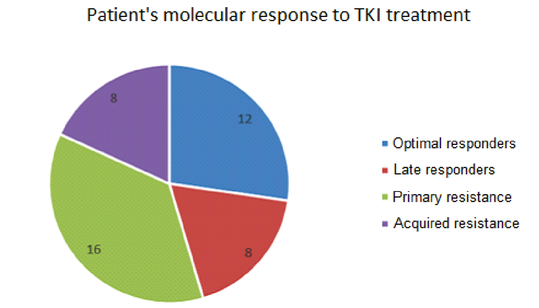
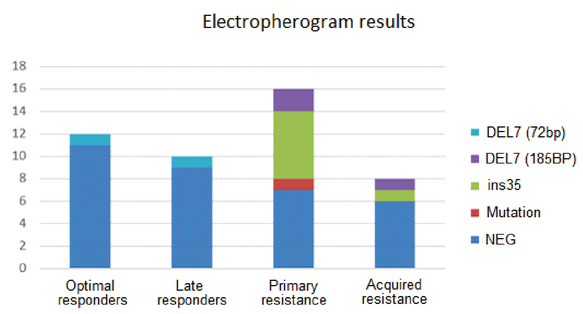



Figure 6: Electropherogram of the BCR-ABL1 region of the sequenced transcripts. (a) Complete deletion of exon 7 (185 bp) present in part from the transcripts; (b) partial deletion of exon 7 (72 bp) present in some of the transcripts; (c) Insertion between exons 8 and 9 (35 bp).




Graph 1: Distribution of the cohort of patients in relation to hematological response.




Graph 2: Graph of electropherograms results of sequenced patients.

## DISCUSSION

The presence of insertion was exclusively in patients with inhibitor resistance, suggesting a relation between this genetic alteration and different types of resistance, since, according to data in the literature (Lee et al., 2008), this insertion could alter the three-dimensional structure of the BCR-ABL1 chimeric protein, impairing the performance of the inhibitor. The presence of 185 bp deletions would theoretically affect the structure in the same manner as the insertion and, due to the number of bases, the decoding of the remaining sequence would be frameshifted. The 72 bp deletion would compromise only a portion of the protein, since this would be an in frame deletion.

## CONCLUSION

Through the characterization of deletion and insertion events of patients in different categories responses, a prevalence of frame-shift type insertion and deletion events can be observed in patients who fail treatment, whereas patients with optimal or late responses presented in frame deletions.

## PERSPECTIVES

Increase the N of the study to confirm if the proportion of insertion and deletion events remains in the different response groups and whether the data are statistically significant.

Analysis of healthy donor sequences in order to evaluate the frequency of the same insertions and deletions found in CML patients.

Development of ASO-PCR for these alterations in order to minimize the costs of the identification of these splicing variants.

## REFERENCES

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Lee, T.S., Ma, W., Zhang, X., Giles, F., Cortes, J., Kantarjian, H., Albitar, M. (2008). BCR-ABL alternative splicing as a common mechanism for imatinib resistance: evidence from molecular dynamics simulations. Mol. Cancer Ther. 7, 3834 – 3841.

Projeto Gráfico: Setor de Edição e Informação Técnico-Científica / INCA