CHARACTERIZATION OF BCR-ABL1 KINASE DOMAIN DELETIONS AND INSERTIONS IN CML PATIENTS AFTER TREATMENT WITH TYROSINE KINASE INHIBITORS

Authors: GUSTAVO TREVIZANI STELZER, SIMONE TEIXEIRA BONECKER DOS SANTOS, ILANA ZALCBERG RENAULT, LUCIANA MAYUMI GUTIYAMA

Institution: Laboratório de Biologia Molecular, Centro de Transplante de Medula Óssea - Instituto Nacional de Câncer José Alencar Gomes da Silva

INTRODUCTION

Although chronic myelogenous leukemia (CML) is one of the most successful therapeutic cases in oncology, resistance to treatment with tyrosine kinase inhibitors (TKI) remains a relevant clinical problem, 20-30% of patients will fail theTKI treatment. BCR-ABL1 kinase domain (KD) point mutations represent the most relevant mechanism of resistance described, it corresponds to 35% of first line treatment failure. In addition, a high occurrence of deletions and insertions in the KD of non-responders was observed (Fig. 1). The most frequent events observed are the deletion of whole exon 7 (Del 7, approximately 30%); and the insertion of 35 bp between exons 8 and 9 (INS35, approximately 40%). However, different to point mutations, either the occurrence during the TKI treatment or the role of these variants in the patients' response to TKIs are not a consensus in the literature yet.

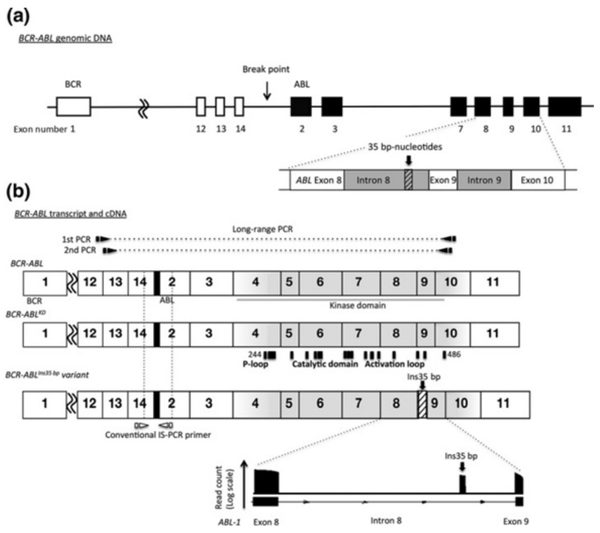


Figure 1: INS 35 pb genomic scheme in BCR-ABL gene. (a) Genomic DNA. (b) Transcript and cDNA negative and positive for INS35. (Yuda et al., 2017)

METHODS AND RESULTS

Table 1: Characteristics of the patients analyzed in the study. Response was defined according to European Leukemia Net recommendations (Baccarani et al., 2013)

Patient samples	293
Age at diagnosis (median in years)	43 (2-83)
Male	173 (59%)
Treatment (median in months)	78 (12-234)
Fase	
Chronic	176
Accelerate	11
Blast crisis	2
NI	104
Inhibitor	
Imatinib	170
Dasatinib	61
Nilotinib	61
NI	1
Response	
Optimal Responders	53
Late Responders	40
Primary Resistants	136
Secondary Resistants	64

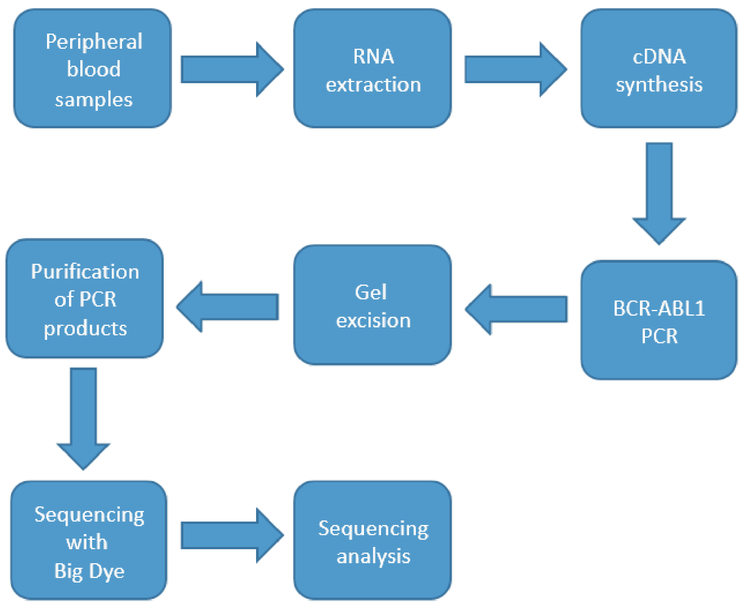


Figure 2: Flowchart of the methodology used in the study.

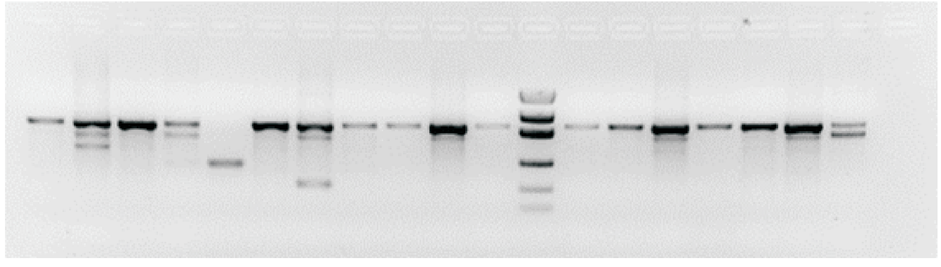
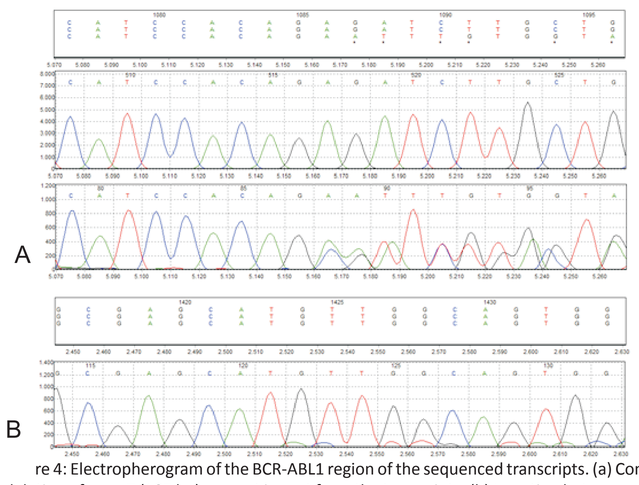
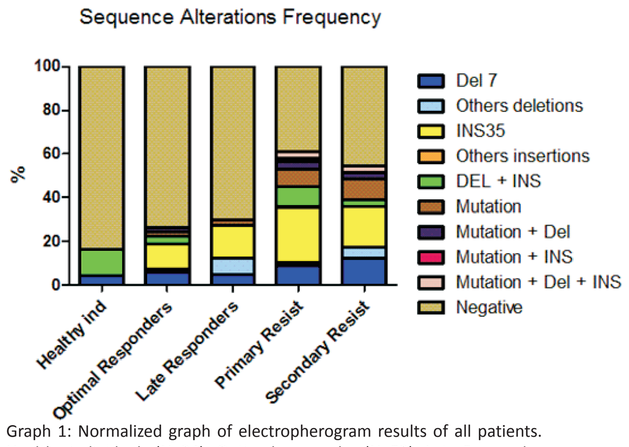


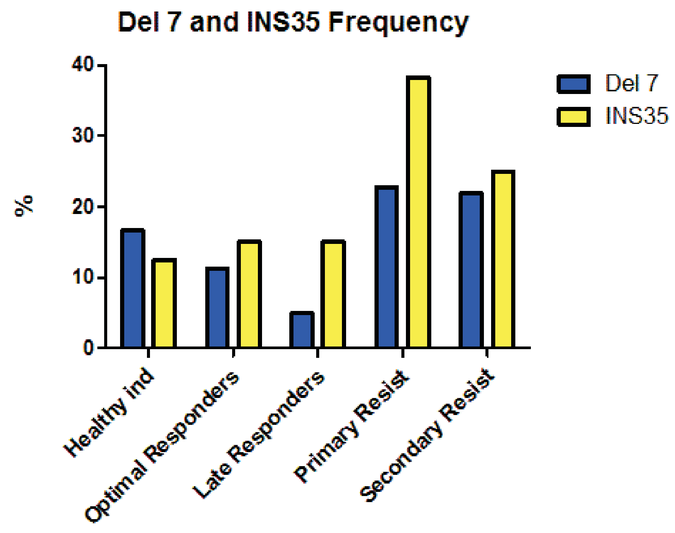
Figure 3: PCR products of kinase domain amplification on a 2% agarose gel stained with ethidium bromide under UV light. ABL KD WT pointed by arrow. Deleted ABL KD pointed by bracket.



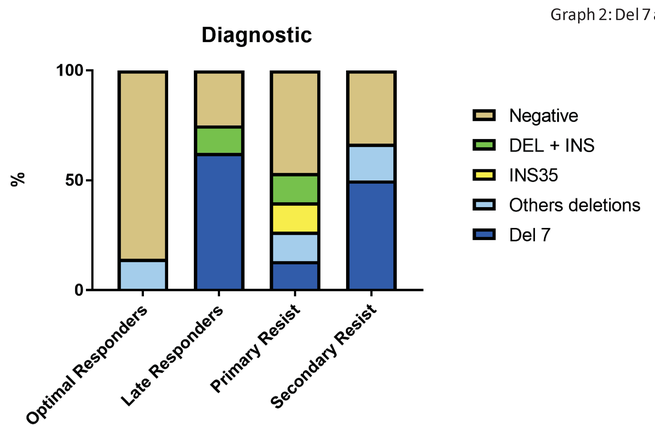
re 4: Electropherogram of the BCR-ABL1 region of the sequenced transcripts. (a) Complete deletion of exon 7 (185 bp) present in part from the transcripts; (b) Insertion between exons 8 and 9 (35 bp).



Graph 1: Normalized graph of electropherogram results of all patients. Healthy individuals (N=24); Optimal responder (N=53); Late Responder (N=40); Primary Resistant (N=136); Secondary Resistant (N=64). $p < 0.05$ for INS35 (*)



Graph 2: Del 7 and INS35 Frequency.



Graph 3: Normalized graph of sequenced patients at diagnosis. Optimal responders (N=7); Late responders (N=8); Primary resistant (N=15); Secondary resistant (N=6).

CONCLUSION

❖Del 7 and INS35 are variants observed also in healthy individuals, which lead us to infer that these alterations are alternative splicing isoforms.

❖Del 7 and INS35 are the most frequent variants among CML patients with TKI resistance with over 12 months of follow up. The occurence of INS35 in primary resistant patients is statistically different either from healthy individuals, optimal and late responders.

❖At diagnosis, Del 7 occurence statistically differs among the response groups. It is most represented in Late responders and Secondary resistant patients. We still need to validate this data in a larger cohort but, despite of the number of samples investigated, this data strongly suggests a role of exon 7 deletion in TKI effectiveness.

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

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