Brazilian multicenter study to characterize analytical performance and expert's concordance, using target-directed sequencing of FFPE samples

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INTRODUCTION

- Precision oncology relies on genomic data that is rapidly generated and reliably analyzed and interpreted. In Brazil oncologists often send tumor samples abroad for testing, leading to longer wait for results, potentially impacting patient outcomes.
- The Tarsila Project is an interlaboratory study to strengthen in-house testing and benchmark the performance of seven local reference institutions. Results from the project showcased the high quality of Next-generation sequencing (NGS) results as a diagnostic method Formalin-Fixed Paraffin-Embedded (FFPE) samples in Brazil's main molecular oncology centers.
- The concordance of samples sequenced with the Ion Torrent™ Oncomine™ Focus Assay (Thermo Fisher Scientific - TFS), a 52-gene panel widely adopted for solid tumor testing, was compared to estimate repeatability and reproducibility, as well as the clinical experience of participants to review and report variants.

Table 1: Oncomine Focus Assay (OFA) 52 genes list categorized by somatic type.

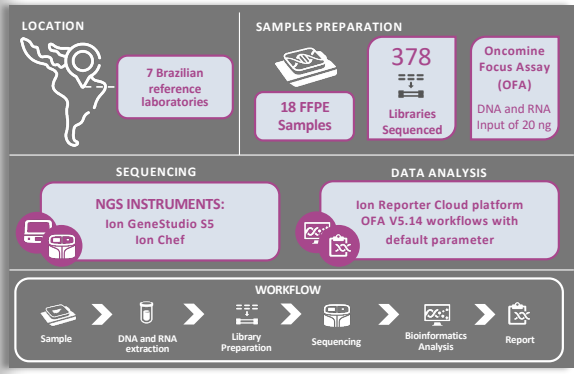
Hotspot genes	Copy number variants	Fusion drivers
AKT1, ALK, AR, BRAF, CDK4, CTNNB1, DDR2, EGFR, ERBB2, ERBB3, ERBB4, ESR1, FGFR2, FGFR3, GNA11, GNAQ, HRAS, IDH1, IDH2, JAK1, JAK2, JAK3, KIT, KRAS, MAP2K1, MAP2K2, MET, MTOR, NRAS, PDGFRA, PIK3CA, RAF1, RET,	AKT1, ALK, AR, BRAF, CCND1, CDK4, CDK6, EGFR, ERBB2, FGFR1, FGFR2, FGFR3, FGFR4, KIT, KRAS, MET, MYC, MYCN, PDGFRA, PIK3CA	ABL1, AKT3, ALK, AXL, BRAF, EGFR, ERBB2, ERG, ETV1, ETV4, ETV5, FGFR1 FGFR2, FGFR3, MET, NTRK1, NTRK2, NTRK3, PDGFRA, PPARG, RAF1, RET, ROS1

MATERIALS AND METHODS

FFPE lung tumor samples were provided by three institutions, centralized histopathology was performed, and only samples with >30% tumor content were included. Participants received nine blinded samples (three commercial controls and six clinical samples), performed DNA and RNA isolation (according to each lab's validated protocol), library construction, sequencing, analysis and reporting. Each clinical sample was sent to three different labs. Libraries were prepared in triplicate in each lab. A total of 378 libraries (189 paired DNA/RNA) were included.

Labs analyzed and reported results according to internally validated protocols and provided raw data, filtered data, and clinical reports. An independent analysis was performed by TFS with Oncomine Focus workflow v5.14 on Ion Reporter.

Figure 1: Tarsila Project Infographic: Methodology and workflow.

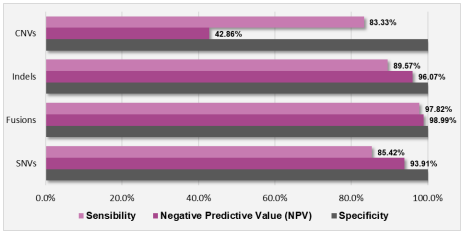


RESULTS

- Overall, 82% of libraries met all DNA/RNA QC metrics. Results from controls and previously characterized clinical samples showed an accuracy for Single Nucleotide Variant (SNV) and indels above 96% (n variants = 873 SNVs, 227 indels).

Figure 2: Reproducibility and repeatability statistical analysis with a 95% confidence interval.

Figure 3: Samples sensibility, NPV and specificity statistical analysis.



- For gene fusions and Copy Number Variations (CNVs) an accuracy of 99.6% and 85.1% was detected (n = 330 fusions, 19 CNVs), respectively. As no false positives were called on the negative controls, specificity was 100% for all variant types.
- Inter-laboratory repeatability was above 90%, even considering samples that did not pass QC.
- Most of the discordances and failures to detect variants originated from a single run that delivered poor results, presumably due to issues during library construction.

CONCLUSIONS

The high concordance rate of the clinical reports emitted by the participants shows that NGS data is reliably generated and interpreted in Brazil. Oncology patients may benefit from having their samples analyzed faster locally, rather than sending them abroad. We obtained important agreement levels between the results of the labs, evidencing a high accuracy in the detection and reporting of genetic variants.

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