A 220-Gene Targeted Next-Generation Sequencing Panel for the Detection of Variants in Diffuse Large B-Cell Lymphoma, Follicular Lymphoma, and Mantle Cell Lymphoma: Application to a Cohort of 85 Formalin-Fixed Paraffin-Embedded Diffuse Large B-Cell Lymphoma Biopsies

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Introduction

A 220-gene panel was designed using Nimblegen target capture probes to sequence exonic regions of genes reported to carry somatic mutations in DLBCL, FL, MCL, and CLL. Gene selection for the panel was based on published reports and public databases including Catalog of Somatic Mutations in Cancer (COSMIC).2

For the cases included in this panel, the following considerations were made: role in B-cell lymphomagenesis, reported role in B-cell lymphomagenesis, reported role in progression in the target diseases, frequency in each disease type, targets of novel therapies, genomic localization within a site of genomic imbalance in the target diseases, and member of either BCR signaling, WNT, or PI3K/AKT pathways.

Materials and Methods

- Nimblegen hybrid-capture design encompassing 4606 targets (exonic regions only)
- Application of their FlexPrep FF and formalin-fixed, paraffin-embedded (FFPE) material
- Starting material sequenced >100 ng FF, >1000 ng FFPE
- Libraries prepared using Kapa Hyper or HyperPlus kit
- Sequencing performed on an Illumina sequencing platform (MiSeq or HiSeq2000)

Alignment and Somatic Variant Calling

CLC Bio Medical Genomics Workbench Human ref genome/hs37d5/GRC38/22

Results

Initial Filtering

cdbbp 1000 Genomic, Homopolymer Map <1%:
- Non- synonymous and noncoding
- Homopolymer regions
- SIFT and PolyPhen prediction T and B

Final Filtering

- CNVkit or Seg/SegSeq
- Gapped regions
- Binned reads was done using a set of 8

Results-Variant Detection

Application of Panel to a Set of 85 FFPE DLBCL Specimens

- All FFPE DLBCL: 97% tumor content and mapped
- 26 samples were excluded due to DNA quality
- 1 sample was subjected to NGS for Focus Lymphoma Pathology

Clinico-Pathogenomic Associations with Detected Variants

Specimens were scored positive or negative for TP53 mutation, 1p deletion, or either one or the other and piling using log-rank analysis

Results-Copy Number Analysis

Copy number for each specimen was determined using either aCGH or CNVkit from NGS results.

Copy number analysis, on-target and off-target sequencing reads were partitioned into bins. Normalization of target reads was done using a set of 8 binned controls to determine copy number.

Results-Average Coverage of Regions Reported to Harbor Resistance Mutations in Brustolin Treated Patients

Coverage

Gene Target Region/Reported Resistance Mutation Average Coverage

BTK Exon 15/C461Y 3415

PLCG2 Exon 1/334LH 5696

EAAT2 Exon 1/80610W 4202

ITGAV Exon 1/9079V 4252

ITGAV Exon 2/742D 5916

ITGAV Exon 2/348 5150

Conclusions

- Using a hybrid capture panel, we are able to validate target performance metrics using a set of 62 FFPE and 32 FF samples. For almost all of the FF samples, less than 2% of the targets did not reach a coverage of at least 60X in 90% of the regions. For FFPE, less than 5% of the targets did not reach this same metric.

- Pilot showed correlation between variants associated with GCB and non-GCB subtypes, thus validating the clinical robustness of the panel.

- Panel can be used to assess copy number changes using on-target reads and CNVkit algorithm.

- Somatic and/or copy number relevant resistance mutations achieve an average coverage high enough to detect low level variants.

References


www.cancergenetics.com