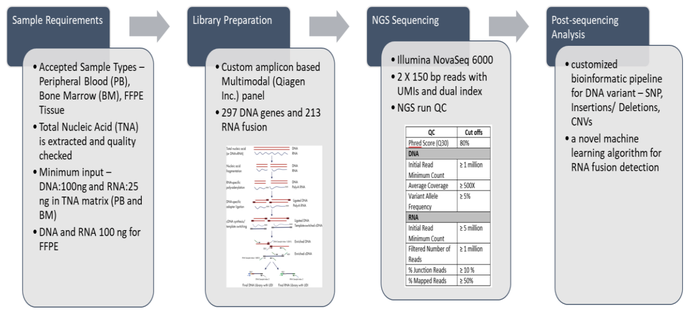
AACR 2023 Abstract #219

Single-tube NGS profiling allows identification of molecular signature in ALL patients

Lina Zelinger · Samuel Koo · Charmeine Ko · Francys Alarcon · Kenneth B. Thomas · Segun C. Jung · Fernando J. Lopez Diaz · Fei Ye · Shashikant Kulkarni
NeoGenomics Laboratories, Aliso Viejo, CA; Houston, TX; Carlsbad, CA

Background: Acute lymphoblastic leukemia (ALL) can be classified to subtypes by molecular signatures correlating to prognosis and treatment regimen, making accurate molecular profiling of interest for both clinical and research communities.
NGS technology allow for identification of a wide array of molecular events (e.g. structural variants and single nucleotide variants (SNVs)), and integrate these with global profiles of gene expression.
Most tests screen either DNA or RNA to provide a piece of a complex molecular puzzle, resulting in higher cost, processing time and amounts of input material. We report a single tube NGS assay that uses total nucleic acid as input for simultaneous screening of DNA and RNA (allowing us to profile gene expression, fusions and multiple variants), establishing a work frame for comprehensive molecular profiling in a single test.

Methods: 243 clinical ALL samples (206 bone marrow and 37 peripheral blood) were used in this study, split in to two cohorts, 102 for initial investigation and 141 for reproducibility testing. Libraries were done using a custom Qiagen Multimodal NGS panel of 302 DNA and 229 RNA targets. Amplicon libraries were sequenced with UDI on Illumina NovaSeq 6000. Data were analyzed by in-house bioinformatic pipeline. Expression values were normalized to GUSB. Previous in-house studies were used to establish cut-offs for upregulation of 31 genes associated with ALL. Variant enrichment for genes was done by normalizing the number of variants to the size of the gene and genes with elevated variant loads were identified. Pathway enrichment analysis was done using gProfiler2.



Conclusions: We demonstrate the use of a single tube multimodal NGS assay for comprehensive genomic profiling. Our analysis shows that this powerful and cost-effective tool can help identify molecular signatures, guiding diagnostic and therapeutic management for ALL patients.

Gene Expression Analysis Highlights Molecular Signatures in ALL Patients

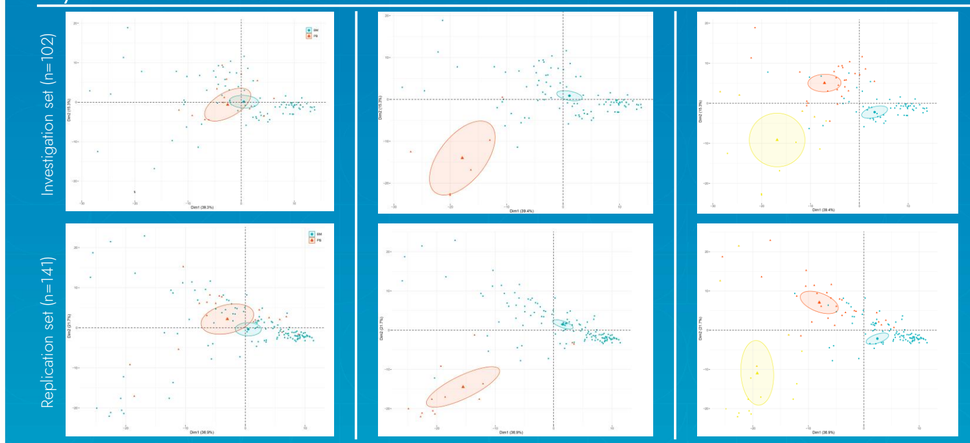
PCA demonstrates three clusters based on number of over expressed genes

- 229 RNA targets are covered by our ALL NGS panel allowing us to explore the expression profiles of these genes, to identify molecular signatures and biomarkers.
- PCA plots were generated based on the normalized (to *GUSB*) expression of all genes, and then colored coded by number of over-expressed biomarkers in each sample. Three clusters are seen based on number of OE genes, low (0-1), medium (8-11), and high (>17 genes). Each cluster, was investigated for molecular hallmarks.
- Over expressed genes suggest specific interaction and pathway activation within each cluster providing a more detailed stratification of ALL patients and allowing for a more accurate molecular diagnosis and monitoring.
- In 40% of both set of patients no significant over-expressed biomarker was observed, suggesting that further analysis might identify additional biomarkers.

ABL1	IL3	PBX1	ZNF384
ABL2	JAK2	PDGFRA	
BCR	KMT2A	PDGFRB	
CRLF2	MEF2D	PTK2B	
CSF1R	MLLT10	RUNX1	
EPOR	NTRK1	TAL1	
ETV6	NTRK2	TCF3	
FGFR1	NTRK3	TLX1	
FLT3	NUP98	TLX3	
IKZF1	PAX5	TYK2	

Literature studies were used to identify 31 genes as a clinically important expression biomarkers for heme conditions

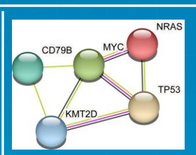
Key features of the two ALL cohorts



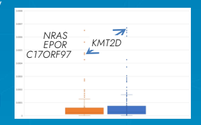
A. Tissue of origin does not impact clustering pattern and show that both sample type can be analyzed together. BM – Bone Marrow; PB – Peripheral Blood

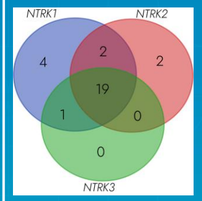
B. Samples of patient with over expression of *EPOR* in the high variability cluster suggesting a common molecular landscape

C. High variability cluster showed an elevated kinase activity, as well as *PDGFRA/B* and *TLX1/3* (yellow), and medium cluster shows upregulation of *RUNX1* and *PAX5* (red)

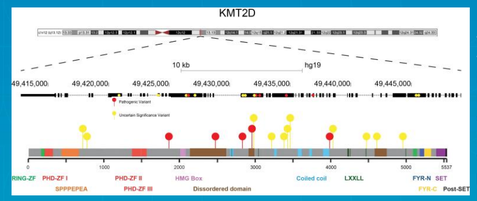


Genes enriched for variants suggest that these are key players in the disease development landscape. 7 genes showed enrichment for variants in both of our cohorts *(NRAS, TP53, CD79B, C17ORF97, DDX41, KMT2D and MYC)*. KMT2D was especially interesting as it interacts with established oncogenes as MYC and TP53 but its role in adult ALL is not very well defined.





28 patients (11.5%) showed an upregulation of kinases activity, with 67.9% of these display upregulation of all three NRTK genes tested.



16 *KMT2D* variants were identified in 50 out of the 243 (11.8%) samples participating in the study.

Some of the VOUS are located in the same or close proximity to functional domains and exons suggesting that they can have functional impact.

lina.zelinger@neogenomics.com

NEO GENOMICS