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NGS assay for simultaneous screening of DNA and RNA provides a comprehensive solution guiding clinical management of ALL patients

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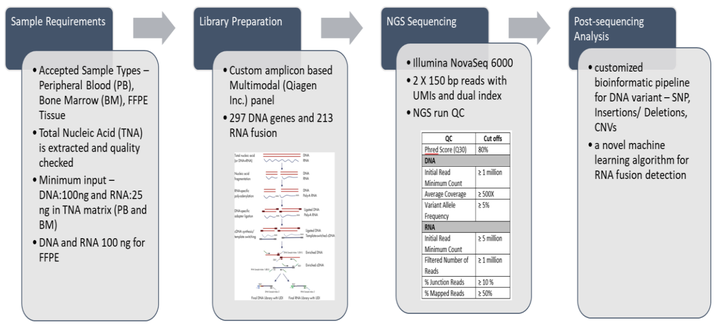
Introduction:

Accurate molecular characterization of acute lymphoblastic leukemia (ALL) subtypes is crucial for clinical management and guiding therapeutic intervention. Over 20 B-ALL subtypes are defined by molecular signatures based on genetic variants and gene expression profiles. Upregulation of CRLF2 expression has been reported as a key molecular indicator associated with poor prognosis in Ph-like ALL patients. Upregulation of CRLF2 can be caused by gene fusion (e.g. IGH::CRLF2 or CSF2RA::CRLF2) or mutations in CRLF2 and IKZF1. Most panel-based assays screen either DNA or RNA, requiring higher input material and multiple workflows leading to higher cost and longer processing time. We report a single tube NGS panel that uses total nucleic acid as input for simultaneous screening of DNA and RNA, providing a comprehensive genetic profile for diagnosis, and therapeutic guidance of Ph-like ALL patients.



Conclusions:

We demonstrate the use of a single tube multimodal NGS assay for comprehensive genomics profiling that simultaneously screens DNA and RNA for expression and variants. It is a powerful and cost-effective tool to help classify ALL samples for clinical management and guiding therapeutic intervention.

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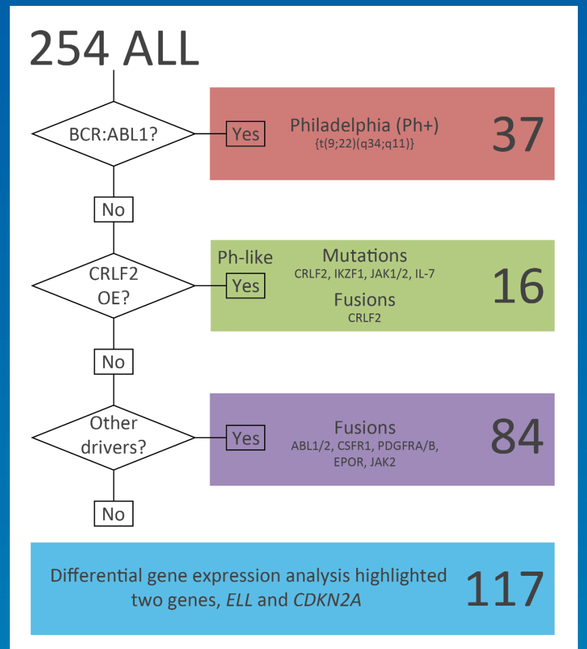
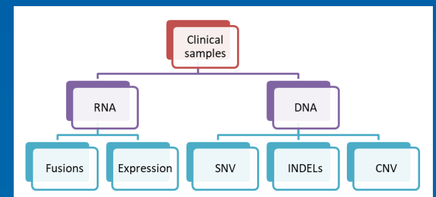
Comprehensive genomics profiling simultaneously screens DNA and RNA for expression and variants

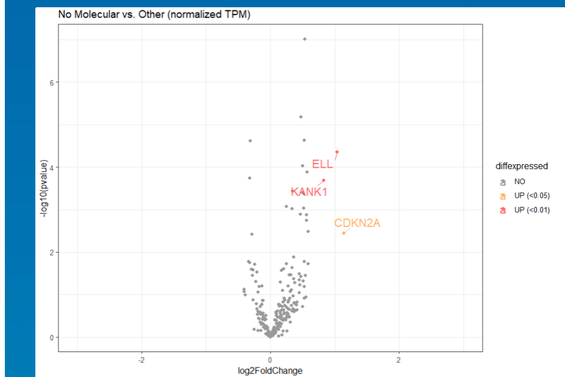


Key highlights

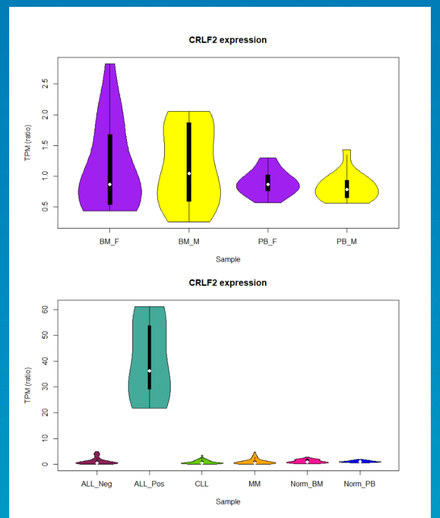
- *CRLF2* expression segregates Ph-like patients from other clinical diagnosis and healthy donors
- In addition to BCR::ABL, the most commonly affected gene in Ph+ patients was *IKZF1*
- *IKZF1* and *JAK1/2* were frequently altered in Ph-like cases
- The most common fusions identified in the non-Ph cases were *KMT2A* (3.5%, 7/201) and *RUNX1* (3/201, 1.5%)
- In 58% of cases (117/201) we were not able to identify a fusion molecular driver prompting us to investigate gene expression profiles in these patients



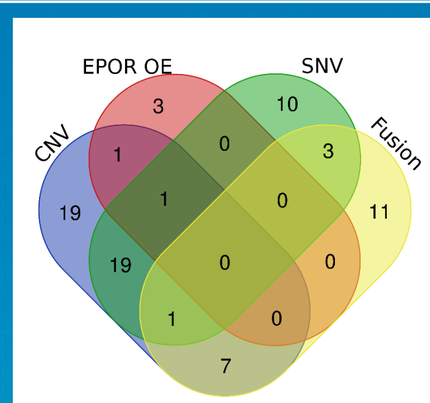
Molecular events investigated in each sample



In 58% (117/201) of samples we did not identify a known molecular driver. We then performed differential gene expression analysis to identify genes that are uniquely upregulated in the group with no molecular driver compared to the rest of the cohort (84). Our goal was to investigate gene expression profiles to gain insight in to what could be the driver in these cases. Three genes came up as significantly up regulated (p-value < 0.05) in this analysis, *ELL*, *KANK1* and *CDKN2A*. All three have established links to either hematological conditions or hereditary cancer. *ELL* is known to be a fusion partner of *KMT2A* in AML and recently reported to be observed in pediatric T-ALL, *KANK1* (potential tumor suppressor) has been shown to be fused with *PDGFRB* leading to myeloproliferative neoplasm, and *CDKN2A* has been reported to be mutated in several types of hereditary cancers and its expression has been suggested as a prognostic marker with poor outcome for ALL.

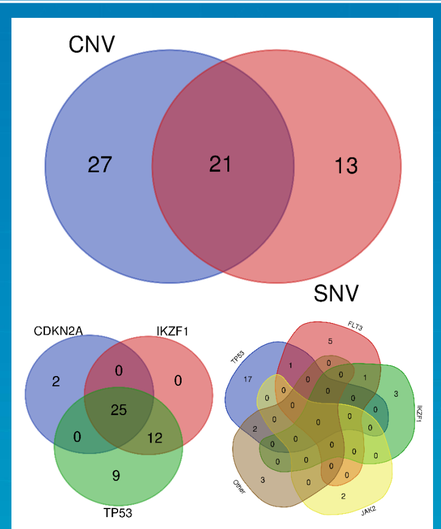


A. CRLF2 expression was tested across sample type, gender and clinical diagnosis to assure uniformity of assay performance in a pilot cohort.

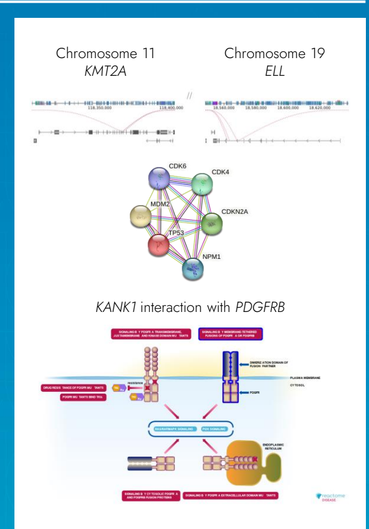


KMT2A::AFF1 was the most common fusion identified in these patients, which has been reported to be more frequent in women and linked to lower survival rates as well as higher median WBC count.

B. The most common molecular drivers in non-Ph patients. In 9 cases no data was available for expression or fusion detection, so these were excluded from the differential expression analysis.



C. Molecular findings in non Ph patients show that about a third of the patients show both CNV and SNV variation, but while CNV tend to overlap within samples SNV show a patient specific pattern.



KMT2A::ELL fusion illustration is from http://www.vizome.org/

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