Opportunities of Genome Imaging for Genetic Diagnosis in Acute Lymphoblastic Leukemia.

Acute lymphoblastic leukemia (ALL) is a prevalent hematopoietic malignancy that requires urgent medical intervention to prevent inferior outcome. ALL is characterized by recurrent structural rearrangements, whole chromosome copy number changes and microdeletions of prognostic value. Currently an extensive panel of tests is required, including karyotype, FISH, arrays or Multiple Ligation-dependent Probe Amplification (MLPA) and PCR-based diagnostic methods. Testing is thus costly and cascade testing is often performed resulting in lengthy turn-around-times (TAT). This does not conform to study protocols that require rapid results to stratify patients into different treatment arms and to identify those who could benefit from "targeted" therapies, such as the Ph-like ALLs. It is therefore interesting to examine new technologies, like genome imaging (Bionano Genomics). This technique utilizes ultra-long linear DNA molecules, enzymatically labeled at specific sequence motifs, to detect numerical and structural aberrations (>500bp) genome wide with a TAT of less than one week.

Objective: assess whether implementation of this technology could (partially) replace current analytical strategies.

Methods: 10 diagnostic T- and 10 diagnostic B-ALL cases were included. All samples were analyzed with the Rare Variant and the De Novo Assembly Pipelines. Filters were set to detect chromosomal aberrations sized >5 Mb. For submicroscopic deletions and gains, only regions encompassing clinically relevant loci were investigated.

Results:

- Bionano results were concordant with MLPA, a technique routinely used for rapid detection of key CNAs.
- All recurrent translocations identified by routine strategies were correctly identified by Bionano.

Bionano further identified:

- A t(5;11) *TCF7-SPI1* in a case with a failed karyotype (confirmed by another method).
- A t(12;22) *ZNF384-EP300* fusion in a case with a normal karyotype. This recurrent rare fusion was confirmed by FISH.

In general there was an excellent concordance of whole/partial chromosome gains and losses and for hyperdiploidy (observed in 3 B-ALLs in this series). Moreover, Bionano identified also hyperdiploidy in a case with a normal karyotype. Interestingly, FISH analysis of the same case suggested the presence of a hypodiploid clone. This demonstrates the limitation of DNA-based techniques to accurately determine ploidy when allele information is not available.

There were some discrepancies between the results of cytogenetics and Bionano:

- Bionano could not identify the presence of multiple clones, a common limitation for DNA based techniques.
- Bionano did not confirm some abnormalities identified by karyotype. Chromosome analysis of ALL samples is notoriously difficult due to low chromosome resolution explaining some of these differences. Some of the discrepant abnormalities were often marked as uncertain or were present only in a subclone (<10%).
- Bionano allowed a correction of the karyotype in some cases and a refinement of the breakpoints of structural abnormalities.
- Genome imaging revealed several additional intra- and inter-chromosomal translocations including (clonal) rearrangements of the *TCR* or *IGH* locus.

Conclusion:

Overall our data demonstrate that genome imaging is a promising technique to identify structural and numerical abnormalities in ALL. Bionano provided an informative result in each case and results were concordant with other results in the majority of cases.

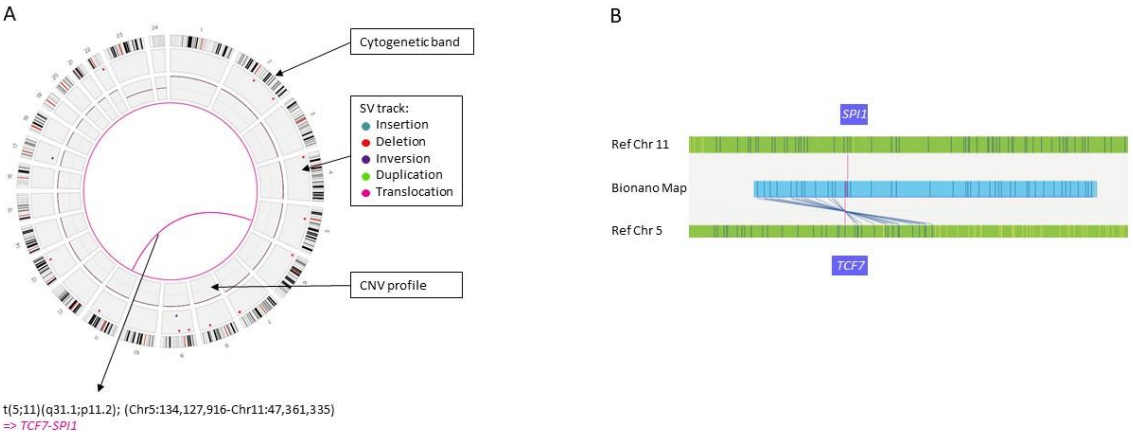

Figure 1: Bionano result for a T-ALL case with failed karyotype.
The Bionano RVP pipeline identifies a t(5;11)(q31.1;p11.2) *TCF7-SPI1* fusion. A/ Whole genome circus plot illustrating the structural variants (SV track) and the copy number variant (CNV) profile. B/ Consensus map that supports the translocation and illustrates the mapping of the breakpoints to the *SPI1* gene (chromosome 11) and the *TCF7 gene* (chromosome 5).