Short Talk – Precision Diagnostics in Hematological Malignancies

Next-Generation Karyotyping in Acute Myeloid Leukemia

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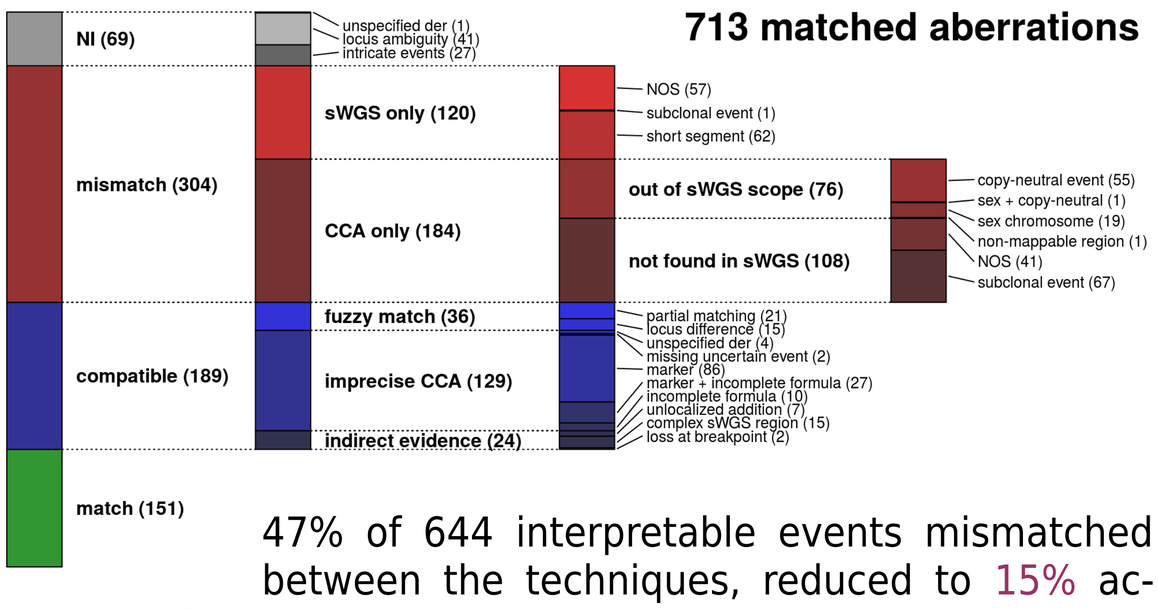
Introduction

The prognosis of patients diagnosed with Acute Myeloid Leukemia (AML) can be inferred based on several tumoral characteristics [1]. Such a stratification performed at diagnosis allows the identification of patients who could benefit from targeted (APLs, defined by the 15;17 translocation) or more intensive therapies (stem-cell transplantation, in higher risk patients). Along with a few mutations, the European Leukemia Network (ELN) consensual model [1] relies on several translocations and copy-number variations which are still assessed today by microscope-based techniques (Conventional Cytogenetic Analysis, CCA). The goal of this project was to compare this aging standard to modern high-throughput alternatives, and discuss a potential replacement of CCA.

This study relies on the ClinSeq nationwide population-based cohort, consisting of 286 unselected Swedish AML patients.

1. Copy-Number Variations (CNV)

CNVs were assessed by shallow Whole-Genome Sequencing (sWGS), producing ~10 million reads per sample (Illumina 1x51 bp) and thus an average depth of 0.2x. Reads were counted in 15 kbp wide bins using QDNASeq [2], providing a data set equivalent to a 167 000 probe CGH array.

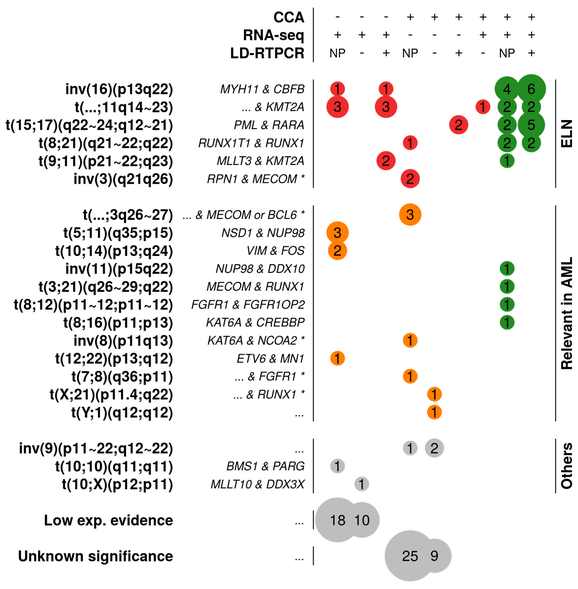


47% of 644 interpretable events mismatched between the techniques, reduced to 15% accounting for their respective weaknesses: 12% are copy-neutral or involve X/Y (out of sWGS scope), 10% are too short for CCA and 11% are potentially unstable subclonal events.

Regarding ELN prognostic events (below), CCA monosomies were found to be illegitimate in many cases, with missing chromosomes reported as markers. Karyotype complexity varied, with more short events but less translocations in sWGS. Sensitivity was also higher regarding the other ELN events.

References :
[1] Döhner et al, Blood. 2017 Jan 26; 129(4): 424–447.
[2] Scheinin et al, Genome Res. 2014 Dec;24(12):2022-32
[3] Nicorici et al, bioRxiv 011650 (2014)
[4] Ruminy et al, Leukemia. 2016 Mar;30(3):757-60

2. Gene fusions

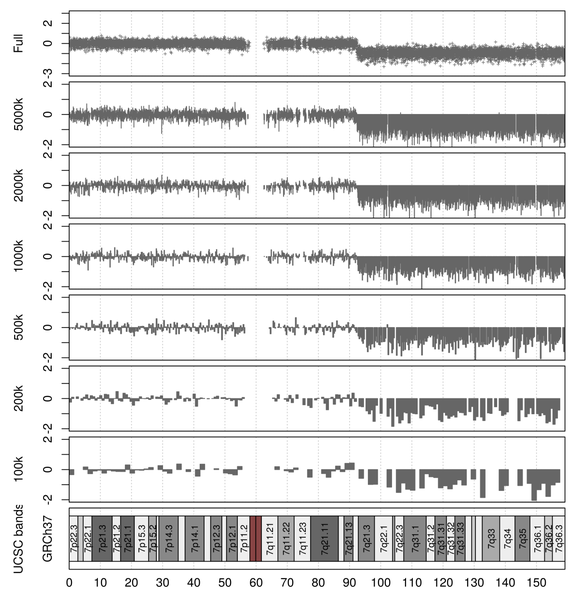


Gene fusions were assessed by whole-transcriptome seq-uencing (RNA-seq), producing ~70 million reads per sample (Illumina 2x126 bp) after rRNA depletion. Reads were analysed with FusionCatcher [3], focusing on known fusions.

28/43 ELN events matched between the techniques (left & footer figures). RNAseq demonstrated a shallower but broader detection span, missing 3 fusions due to insufficient read support while detecting 2 inv(16) and 8 t(KMT2A) missed in CCA. Two inv(3) were missed in RNA-seq, unsurprisingly considering they don't result in any chimeric transcript. Finally 20 Partial Tandem Duplications (PTD) of *KMT2A* were identified by RNA-seq, an adverse prognosis event cryptic in CCA.

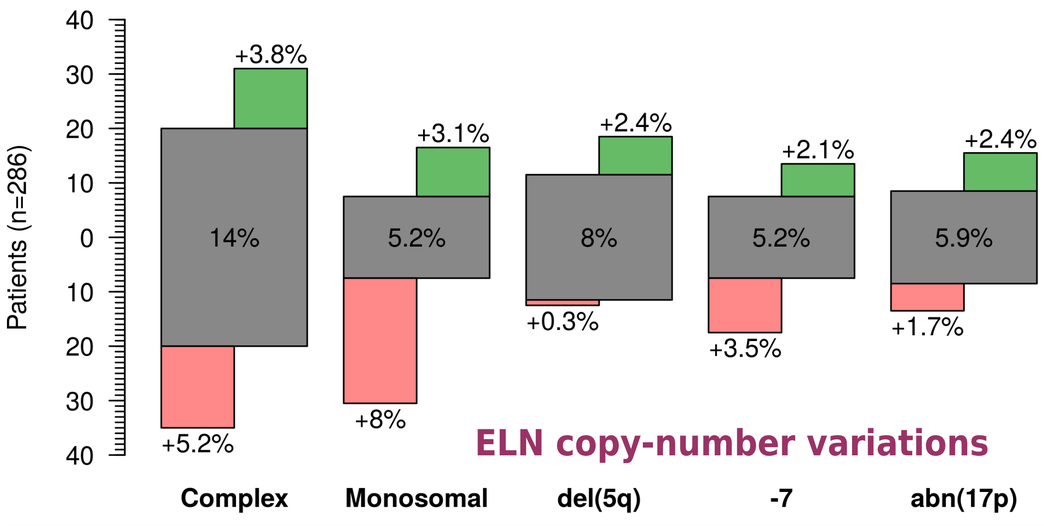
3. Toward more affordable tools

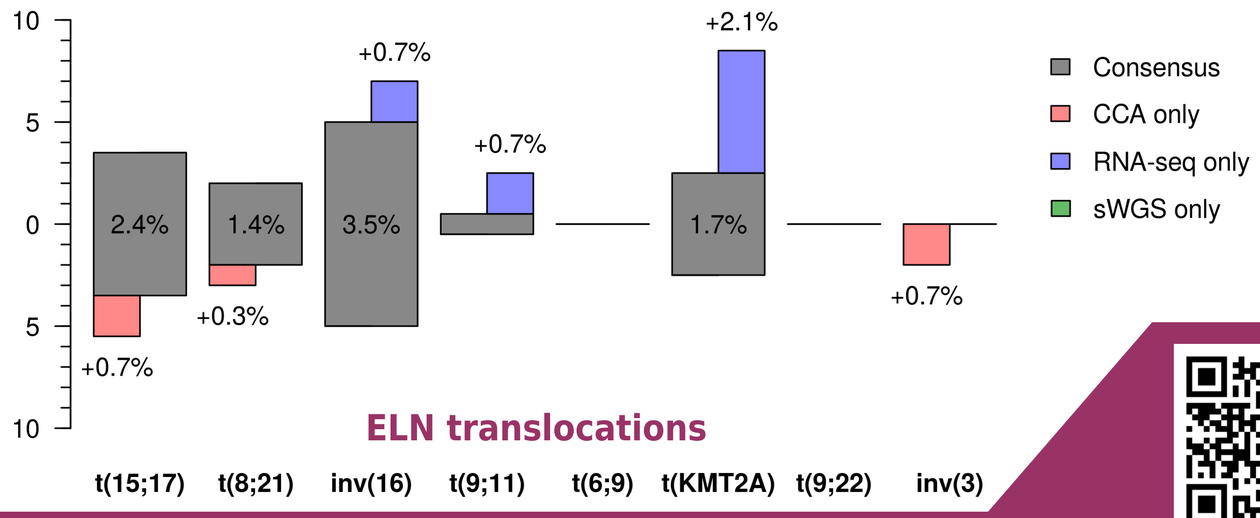
Regarding CNVs, sWGS has an the advantage of flexibility. While 10M reads per sample were used here, 1M reached the same accuracy. A resolution equivalent to CCA's (~10 Mbp) could be achieved with no more than 100k reads. This approach could moreover be incorporated in DNA mutation panels anyway required by ELN, providing CNVs as a by-product for minimal extra cost.



Ligation-Dependent RT-PCR [4] could fill the remaining gap represented by gene fusions. Using basic machines and reagents (PCR, Sanger sequencing), it allows to query 243 fusion points in a single experiment, multiplexing up to 30 samples in a single day. 74 noteworthy samples were analysed with this technique, showing a broad agreement with RNA-seq (21/22 ELN fusions) and even detecting 2/3 fusions below RNA-seq detection limit. A single t(KMT2A) was missed, due to unusual breakpoints not covered in the current design.

Overall the next-generation techniques provided a prognostic stratification equivalent to CCA's (EFS $p<10^{-5}$), with the opportunity to get full ELN classification in 48 hours, thus cutting both time and money costs.





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