A novel clinical assay comprehensively characterizes myeloma genomes from bone marrow and peripheral blood

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Introduction

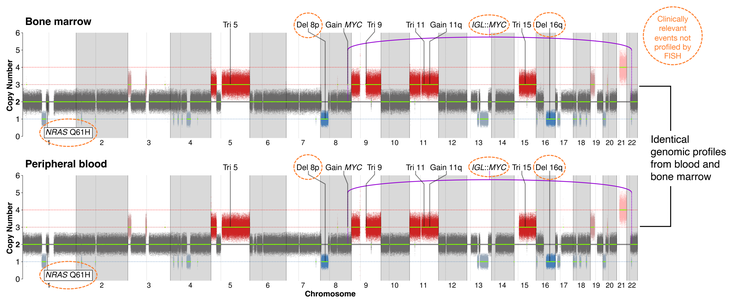
Detecting genetic abnormalities is crucial for risk stratification and tailored interventions in multiple myeloma (MM) and its precursor conditions. This currently requires invasive bone marrow (BM) aspirates, which poses a challenge for serial monitoring of genomic evolution and causes discomfort to patients. The current standard for detecting genetic alterations in MM is fluorescence in situ hybridization (FISH), which cannot detect mutations and other clinically relevant events. As a result, the recently updated IMS-IMWG guidelines require next-generation sequencing for the classification of high-risk MM. Moreover, identification of mutations/deletions in therapeutic targets (e.g., BCMA, GPRC5D) is critical for guiding immunotherapies. Here, we enable routine assessment of MM genomes by comprehensively and robustly characterizing with whole genome sequencing (WGS) a minimum of ~50 circulating tumor cells (CTCs) isolated from peripheral blood (PB), in a novel CLIA-approved Laboratory Developed Test called GenoPredicta.

Study design and methods

- 68 viably frozen, paired BM and PB samples from a retrospective cohort of 34 patients were used for validation.
- Tumor cells were isolated from BM or PB samples using fluorescence-activated cell sorting (FACS) (after CD138+ enrichment for PB) and subjected to WGS.
- Copy number alterations, structural variants, and single nucleotide variants and indels were called from WGS using a combination of open-source and proprietary bioinformatics methods.
- For a subset of samples, the BM was also characterized by FISH at Mayo Clinic Laboratories, using a standard clinical panel for characterizing MM.

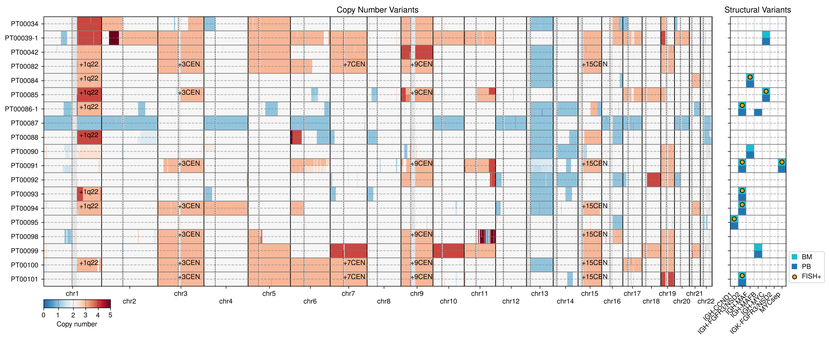
Results

GenoPredicta enables comprehensive characterization of multiple myeloma genomes



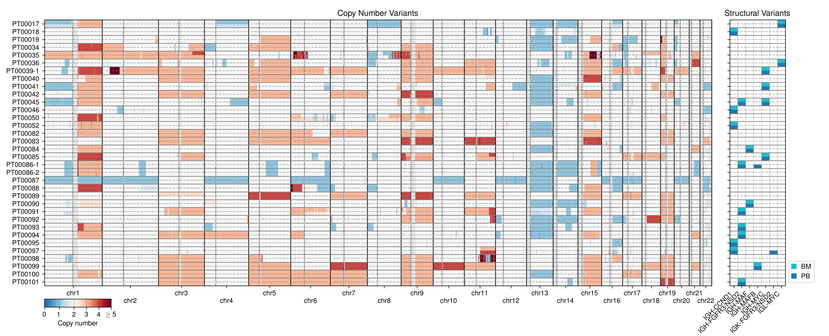
GenoPredicta enables detection of genomic driver events not captured by current diagnostics (e.g., *NRAS* mutation, *IGL::MYC* translocation).

Complete concordance between GenoPredicta and FISH

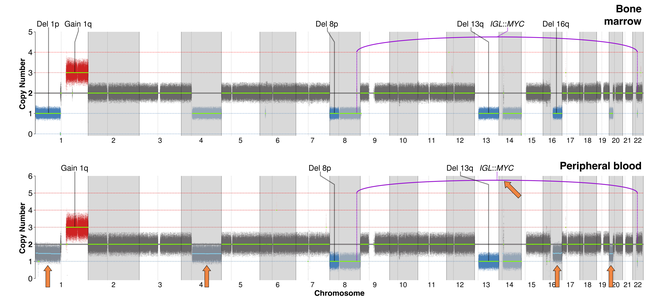


100% recall of 9 SVs and 30 CNVs observed by FISH, and **100% precision** across 50 SVs and 30 CNVs not detected by FISH.

GenoPredicta on blood recalls 100% of events observed with GenoPredicta on bone marrow



GenoPredicta identifies and characterizes subclonal events from blood



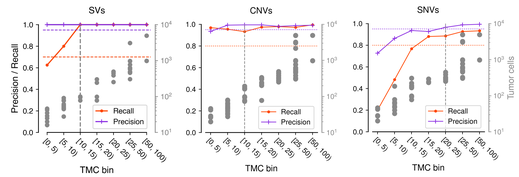
- GenoPredicta enables accurate detection of subclonal events.
- Subclonal characterization can elucidate tumor evolutionary history and thus predict evolution in response to treatment.
- Subset of copy number changes that are clonal in sampled marrow lesion are subclonal in blood.

Subclonal alterations detected in blood

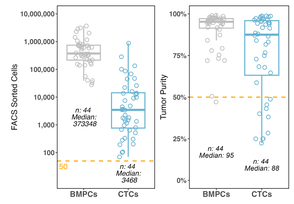
Blood can capture signal from multiple lesions, including (subclonal) events originating from unbiopsied bone marrow lesions or extramedullary disease, thus providing more complete information about the tumor.

Analytical validation of GenoPredicta shows exceptional sensitivity and specificity

Variant calling performance



CTC counts and purities



Performance summary

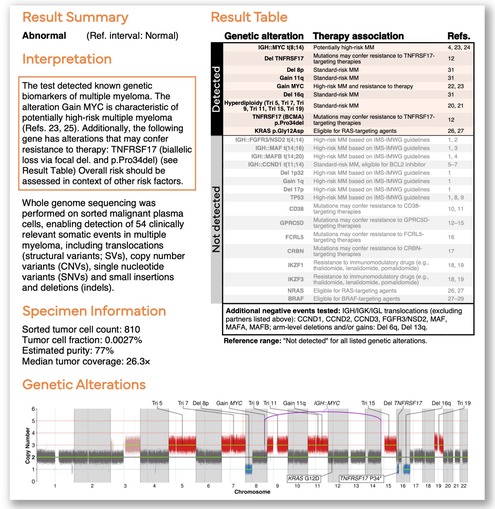
Tumor median coverage	CNV	SV	SNV/indel
10-15x	100% / 100%	100% / 100%	76% / 94%
15-20x	100% / 100%	100% / 100%	88% / 93%
≥20x	100% / 100%	100% / 100%	91% / 98%
Overall	100% / 100%	100% / 100%	87% / 96%

Sensitivity: Recall; Specificity: PPV. CNVs and SVs were evaluated for reportable events, SNVs/indels genome-wide.

- The ability of GenoPredicta to recover highly pure populations of CTCs enables highly accurate variant detection.
- GenoPredicta can recover clinically actionable results at extremely low CTC counts and purities.

Clinical test enables identification of therapy resistance mechanisms from blood

- GenoPredicta from CTCs enables detection of deletions or mutations of immuno-therapy targets.
- In a clinical blood sample from a patient with prior BCMA-targeting therapy (teclistamab), a biallelic alteration of BCMA was observed by WGS (P34del and focal deletion).
- Patient clinical report shown at right; clinical reports are automatically generated end-to-end from raw sequencing data in ~6h.



Conclusions

WGS-based characterization of MM from BM or CTCs is a viable replacement for FISH for clinical diagnosis, with blood-based measurements enabling more dynamic and minimally invasive monitoring of the myeloma cancer genome.

For more information: tinyurl.com/genopr
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