# Whole genome sequencing of multiple myeloma genomes with a novel clinical assay enables identification of genetic alterations underlying immunotherapy resistance

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## Introduction

The detection of genetic abnormalities is required during diagnostic workup and for potential individualization of therapy selection in multiple myeloma (MM) and its precursor conditions. At present, this relies on invasive bone marrow (BM) biopsies, severely limiting early detection, frequent longitudinal monitoring, and the precise selection of therapy. The current standard for detecting genetic alterations in MM is fluorescence in situ hybridization (FISH), which cannot detect point mutations and other clinically relevant alterations. Consequently, the IMS-IMWG guidelines were recently updated to require next-generation sequencing for the classification of high-risk MM.

To address these needs, we recently launched GenoPredicta, a CLIA-approved LDT that enables routine monitoring, informing diagnosis, and treatment selection by comprehensively characterizing MM genomes with whole genome sequencing (WGS) from as few as 50 circulating tumor cells (CTCs) isolated from peripheral blood (PB) or tumor cells from BM.

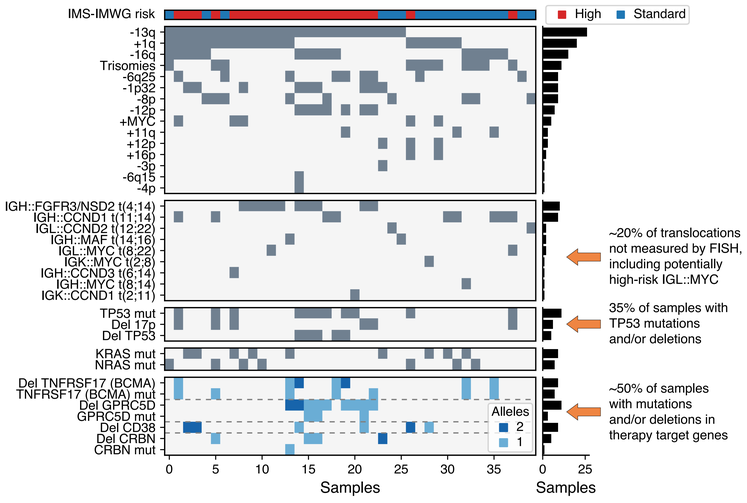
## Study design and methods

- Assay validation cohort: 68 viably frozen, paired BM and PB samples from a retrospective cohort of 34 patients. For a subset of samples, the BM was also characterized by FISH at Mayo Clinic Laboratories, using a standard clinical panel for characterizing MM.
- Clinical cohort: 40 samples spanning disease stages and risk categories.
- 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.

## Results

Summary of relevant alterations identified in a cohort of 40 clinical samples

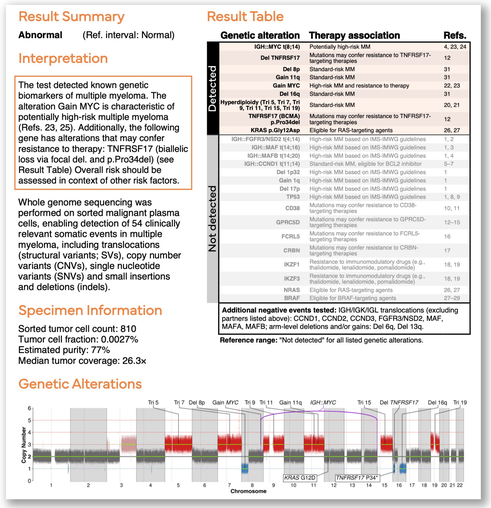
- 49% of identified alterations undetectable via FISH
- 90% of samples have ≥1 event undetectable by FISH (median: 2 events)



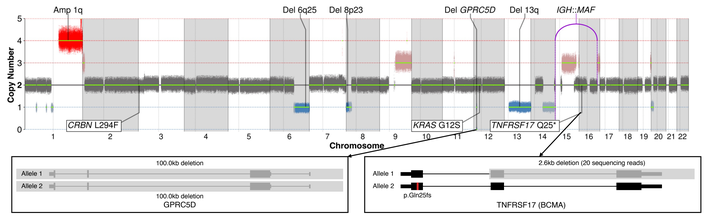

*GenoPredicta enables deep characterization of myeloma genomes and reveals genetic resistance mechanisms to therapy.*

## Clinical test enables identification of therapy resistance mechanisms from blood

- GenoPredicta from CTCs enables detection of deletions or mutations of immunotherapy targets.
- In a clinical blood sample from a patient with prior BCMA-targeting therapy (teclistamab), a biallelic alteration of BCMA was observed by WGS (p.Pro34del and focal deletion).
- Patient clinical report shown at right; physician-ready reports are automatically generated end-to-end from raw sequencing data in ~6h.

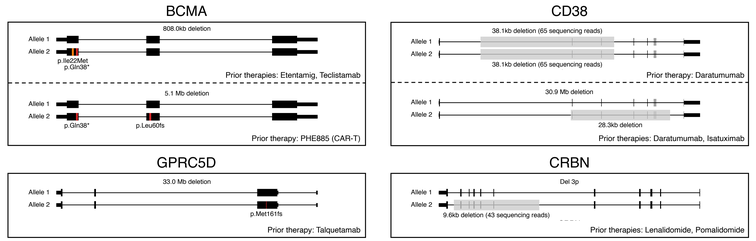



## Biallelic loss of BCMA and GPRC5D



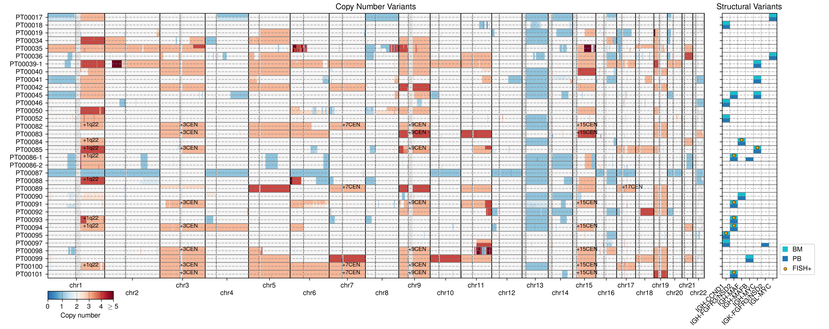

Alterations are consistent with progressive disease after therapy with talquetamab (anti-GPRC5D bispecific), and previous nonresponse to teclistamab (anti-BCMA bispecific) after prior therapy with Cilta-cel (BCMA-directed CAR T).

## Broad spectrum of resistance mechanisms only measurable by WGS




*Resistance mechanisms identified in clinical production samples range from point mutations and small focal deletions to large arm-level deletions.*

## GenoPredicta on blood recalls 100% of events observed with GenoPredicta on bone marrow, and is concordant with FISH



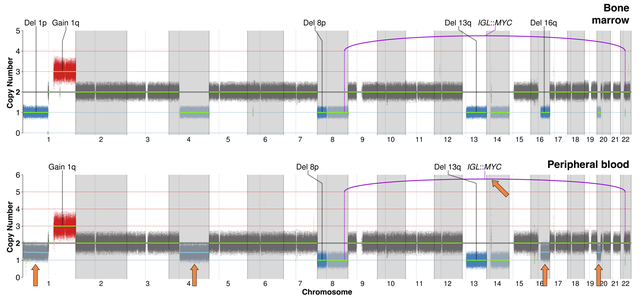

**100% recall** of 9 SVs and 30 CNVs observed by FISH, and **100% precision** across 50 SVs and 30 CNVs not detected by FISH.

| 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 CTC populations enables highly accurate variant detection.
- GenoPredicta can recover clinically actionable results at extremely low CTC counts and purities.

## GenoPredicta 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.*

## 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.
- GenoPredicta enables in-depth characterization of therapy resistance mechanisms.

For more information: tinyurl.com/genoprd
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Website

Poster