Improved Success Rate of OGM to Reveal Comprehensive Genome-wide Genetic Profile in Multiple Myeloma

Jennifer Ghabrial, Melanie Klausner, Victoria Stinnett, Patty Long, Laura Morsberger, Katie Beierl, Christopher D. Gocke, Rena R. Xian, Jaclyn B. Murry, Ying S. Zou

Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, MD, USA

Introduction

Multiple Myeloma(MM) is a cancer of plasma cell, which are responsible for producing antibodies. It is characterized by the accumulation of abnormal plasma cells in the bone marrow. Multiple Myeloma occurs in 1-4 per 100K people per year with a median survival rate of 3-4 years.

Identification of genetic abnormalities are important for pathogenesis, prognosis, and therapy of MM. Recurring genetic abnormalities in MM include structural variants (SVs, such as rearrangements involving IGH and MYC genes), copy number variants [CNVs, such as 1q+, del(17p), del(13q)], hyperdiploidy (with gain of odd-number chromosomes), hypodiploidy, and various gene mutations (such as mutations in the *RAS* pathway, *BRAF*, *FAM46C*, *DIS3*, and *TP53*) [2].

Current Cytogenetic assays used to help in the diagnosis of MM are Karyotyping, Fluorescence *In Situ* Hybridization(FISH) and SNP Microarray. Karyotyping is used to look at the whole genome on a chromosomal level of cultured bone marrow cells. FISH is a targeted analysis used to determine a rearrangement in a specific gene or gain or loss of specific chromosomes. Microarray is a whole genome approach to determine copy number variants. Optical Genome mapping technology (OGM) is an innovative method to detect genetic abnormalities in MM.

Methods

Our study consisted of 29 cases, with various stages of MM, that underwent CD138+ plasma cell isolation prior to FISH and OGM analysis. The percentage of plasma cells in these samples ranged from 0.04% to 46%(median 4%). Immunomagnetic isolation of CD138+ plasma cell was performed on fresh Bone Marrow specimens and provided a purity of approximately 95% [CI:92%-97%] by Flow cytometry and FISH analysis. Then FISH studies were performed according to the following MM FISH panel and reflex protocols.

OGM DNA isolation and labeling of frozen plasma cell pellets were done using Bionano Frozen cell pellet G2 protocol for OGM. The throughput data was set to 1500Gbp. By using a customized MM BED File, we were able to perform a targeted analysis looking the SV's and CNV's used to determine gene rearrangements involved in Multiple Myeloma.

CD138+ Plasma Cell Isolation

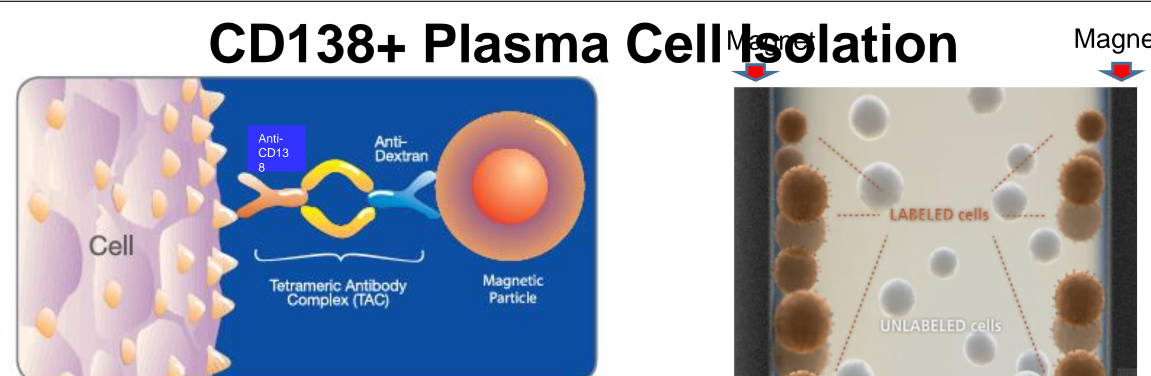

Figure 1: CD138+ plasma cell Isolation: attach magnetic micro-beads labeled with anti-CD138 to plasma cells then sample is run through a magnetic column and labeled plasma cells are then eluded into final enriched sample.

Multiple Myeloma FISH Panel

- 13/13q-, *RB1*/*LAMP1*
- t(11;14), *CCND1*/*IGH*
- 14q32 rearrangement, *IGH*
- del(17p), *TP53*/D17Z1
- 1q gain, *TP73*/*1q22*
- 8q24.1 rearrangement, *MYC*

When an IGH rearrangement is identified and the partner is not *CCND1*, reflex testing is used to identify the translocation partner using the dual fusion probes below.

- t(4;14)(p16.3;q32) *FGFR3*/*IGH*
- t(6;14)(p21;q32) *CCND3*/*IGH*,
- t(14;16)(q32;q23) *IGH*/*MAF*
- t(14;20)(q32;q12) *IGH*/*MAFB*.

Results

OGM revealed findings not found by FISH, which changes the Cytogenetic risk classifications. Among 11 loci by FISH, 97% of the results(113 of 116) displayed concordance between FISH and OGM. OGM had excellent concordance with FISH on CD138+ plasma cells. More clinical-related genetic abnormalities were revealed by OGM compared to karyotype(~30%) and FISH (75% for limited loci studied).

Hyperdiploidy was seen in 14 of the 29 cases(48%),4 of the 29 cases (14%) had Hypodiploidy with -13,-14 and -22 being the most significant. In 7 of the 29 cases (24%), *IGH* or *MYC* rearrangements were seen by OGM and not by FISH. FISH may not have revealed these rearrangements due to the limited loci tested. Some of the rearrangements seen by OGM include: *MAFA::IGH, MYC::IGH, MAFB::IGH, MYC::IGL,* and *CCND3::IGH.*

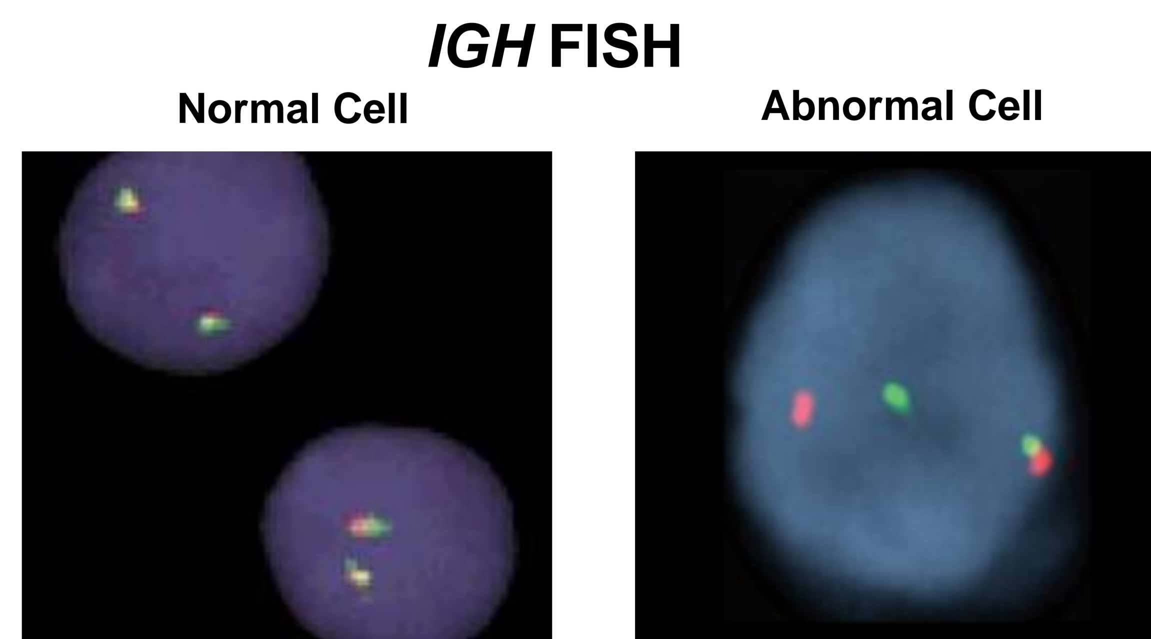

Figure 2: *IGH* break-apart FISH approach shows rearrangements, but no information of the gene partner.

IGH fusion partners

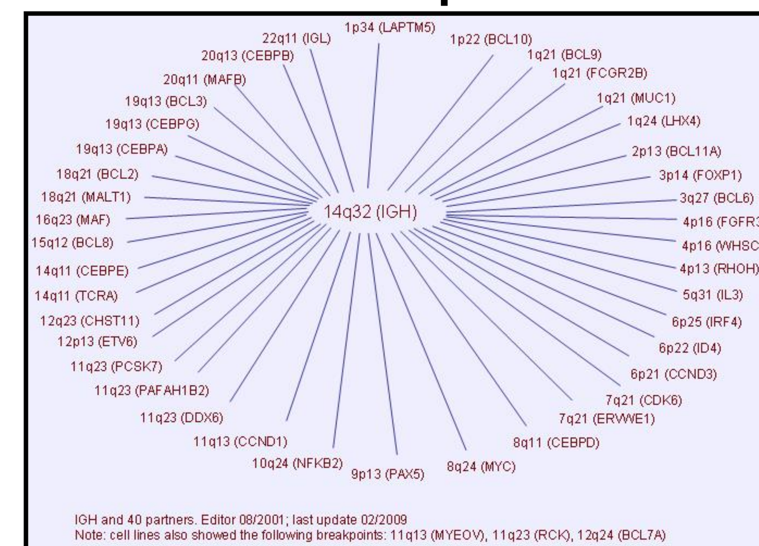

Figure 3: *IGH* gene can have over 150 fusion partners. Targeted FISH analysis makes it difficult to determine various translocation partners involved in cancers.

Staging System for Multiple Myeloma

Stage	Durie-Salmon Staging System	Revised International Staging System

I	**All of the following:**
	○ Hemoglobin concentration > 10.5 g/dL
	○ Serum calcium value normal or ≤ 12 mg/dL
	○ X-ray studies of bone showing normal bone structure (scale 0) or solitary bone plasmacytoma only
	○ Low M-component production rate
	  ○ IgG value < 5 g/dL
	  ○ IgA value < 3 g/dL
	○ Urine light chains < 4 g/24 hours
		○ Serum albumin > 3.5 g/dL
		○ Serum β_2-microglobulin < 3.5 mg/L
		○ No high-risk cytogenetic features
		○ Normal serum lactate dehydrogenase level

II	**Neither stage I nor stage III** ○ A—No renal failure (creatinine ≤ 2 mg/dL) ○ B—Renal failure (creatinine > 2 mg/dL)	**Neither stage I nor stage III**

III	○ Hemoglobin concentration < 8.5 g/dL
	○ Serum calcium value > 12 mg/dL
	○ X-ray studies of bone showing > 3 lytic bone lesions
	○ High M-component production rate
	  ○ IgG value > 7 g/dL
	  ○ IgA value > 5 g/dL
	○ Urine light chains > 12 g/24 hours
		○ Serum β_2-microglobulin > 5.5 mg/L
		○ AND one of the following:
		  ○ (a) High-risk cytogenetics t(4;14) t(14;16) del(17p)
		  ○ (b) Elevated serum lactate dehydrogenase level

Key: del = deletion; dL = deciliter g = gram; Ig = immunoglobulin; L = liter; M-component = monoclonal component; M protein = monoclonal (myeloma) protein; mg = milligram; t = a translocation between chromosomes.

Results continued

OGM Analysis of *MYC* and *IGH* Rearrangements Not Found by FISH

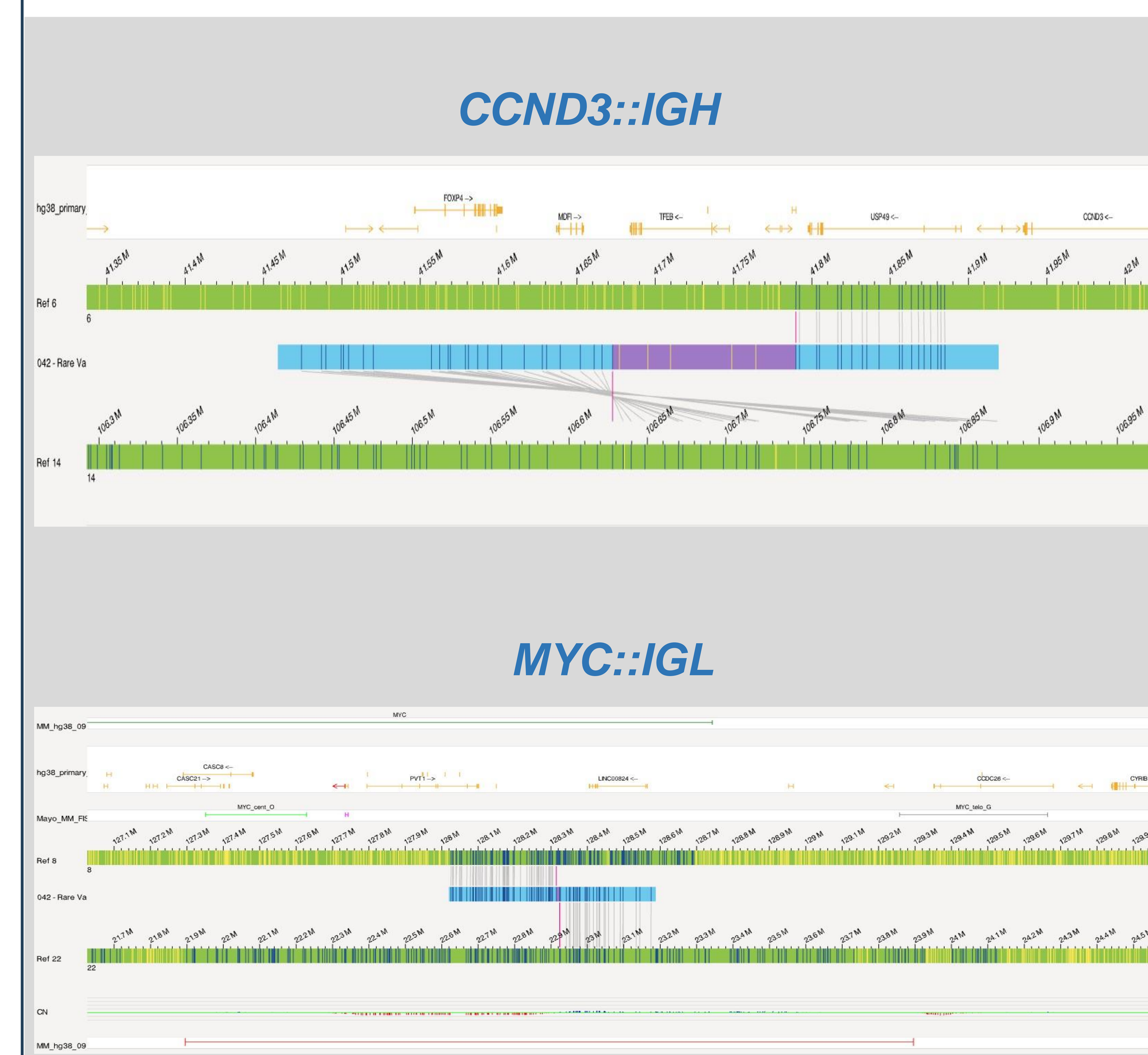

Figure 4: SV's called on OGM consisting of fusions involving *MYC* or *IGH* genes

Chromoanagenesis

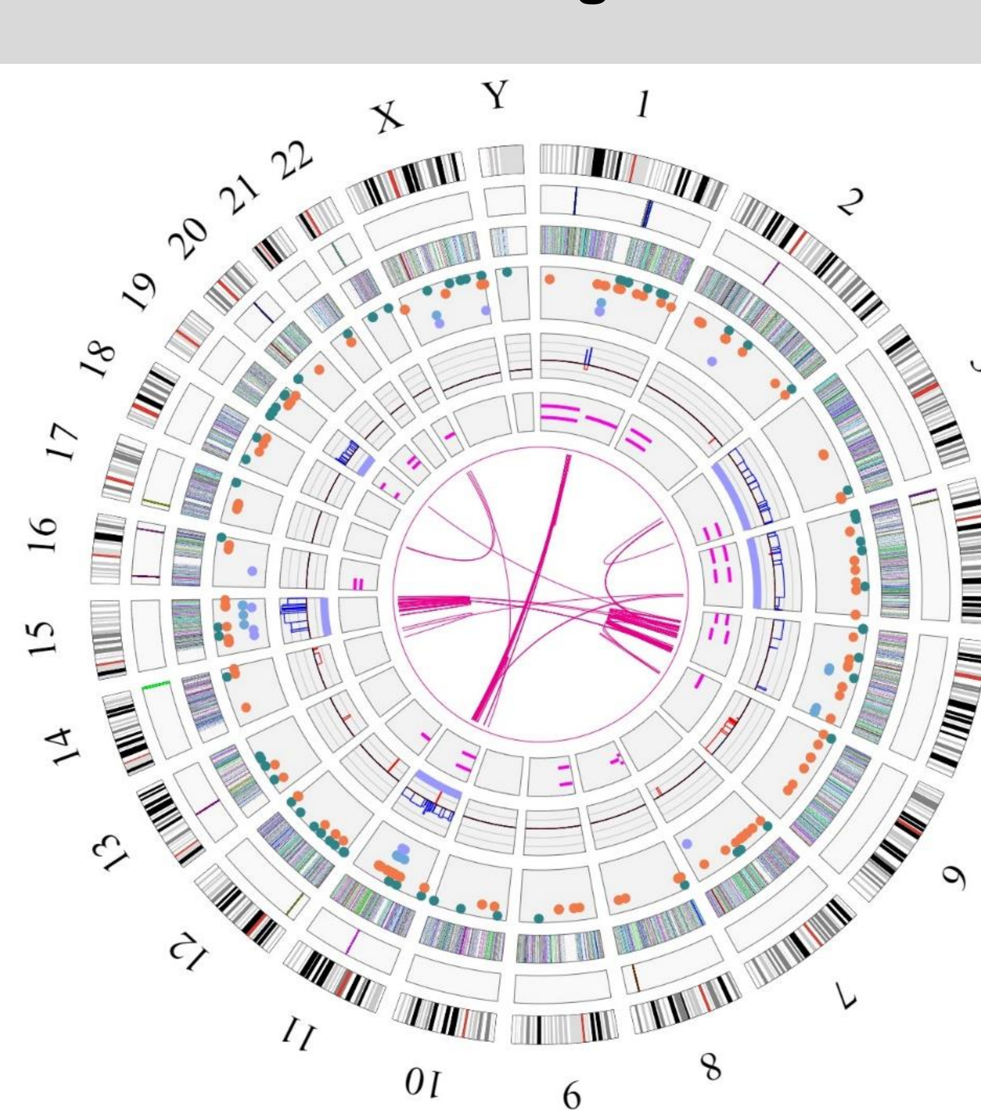

Figure 5: Chromoanagenesis indicative of chromosome fragmentation in High risk MM disease

Results continued

OGM Whole Genome view

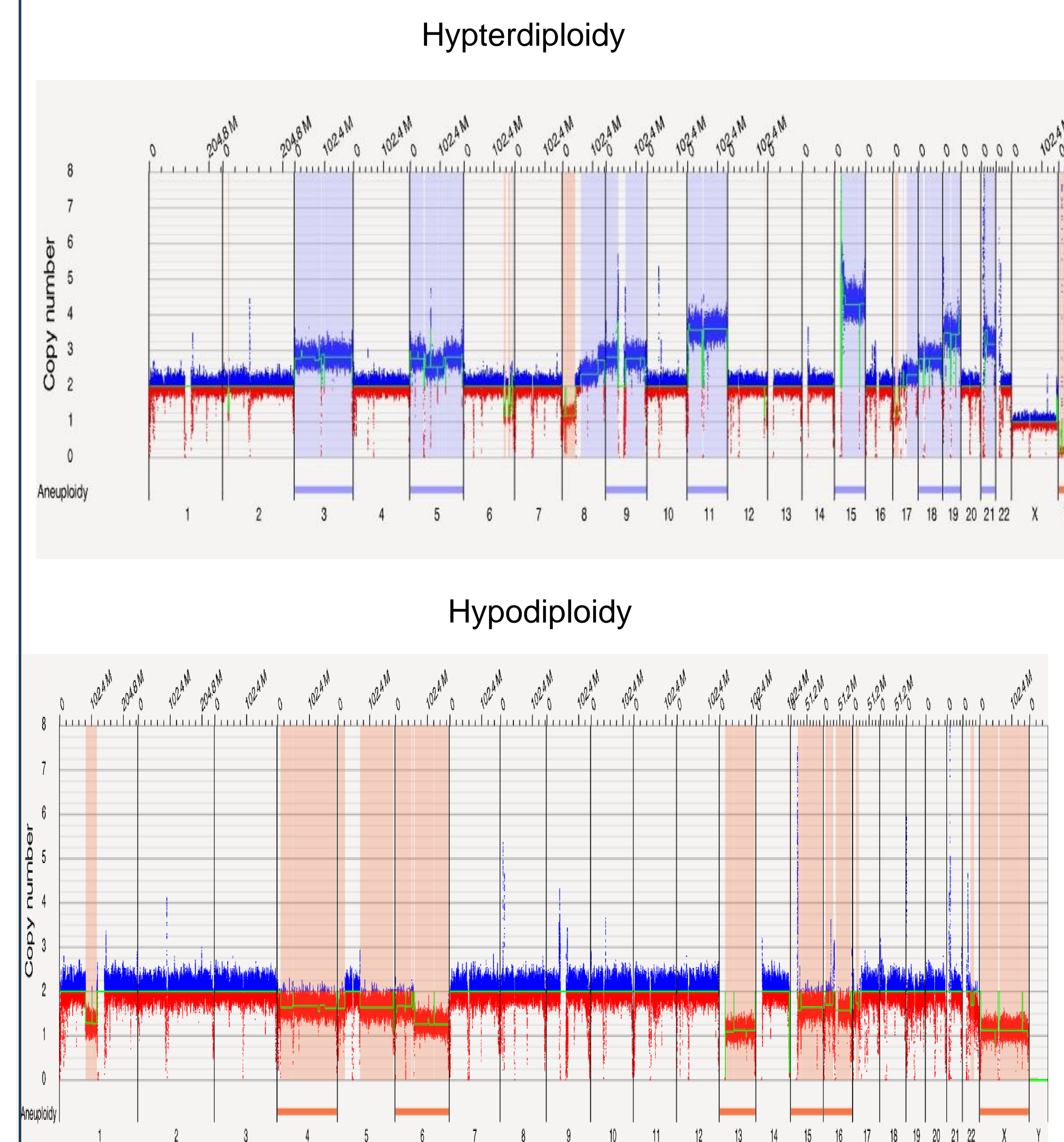

Figure 6: Hyper- and Hypodiploidy-changes Risk Classification

Conclusions

- Compared to routine chromosome analysis and FISH on CD138+ plasma cells, OGM improved the detection rate for genetic abnormalities. OGM had excellent concordance with FISH on CD138+ plasma cells.
- In-house OGM testing is preferred over sending out due to fragility of plasma cells during specimen transport.
- More clinical-related genetic abnormalities were revealed by OGM compared to karyotype (~30%) and FISH (75% for limited loci). OGM revealed genome-wide structural and copy number variants, which changed cytogenetic risk classification.
- OGM is recommended to become the first-tier cytogenetic test for patients with Multiple Myeloma.

References

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