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Novel genomic findings in multiple myeloma identified through routine diagnostic sequencing

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Abstract: Multiple myeloma is a genomically complex haematological malignancy with many genomic alterations recognized as important in diagnosis, prognosis and therapeutic decision making. We provide a summary of novel genomic findings including sequence variants, genome-wide copy number changes and structural rearrangements identified through routine diagnostic next generation sequencing at our center. Our results highlight that many clinically relevant findings remain in multiple myeloma which have not yet been identified through large-scale sequencing efforts, and provide important mechanistic insights into plasma cell pathobiology.

Keywords: multiple myeloma; genomics; mutation; high throughput sequencing; copy number; immunoglobulin heavy chain; translocation

1. Introduction

Through large whole genome, whole exome and targeted sequencing cohorts, multiple myeloma has become one of the most genomically well-characterized haematological malignancies [1-4]. In addition to published cohorts, important resources such as data from approximately 1000 patients with myeloma that have undergone whole exome sequencing and whole transcriptome RNA-sequencing are also publically available as part of the Multiple Myeloma Research Foundation Personalized Medicine Initiative CoMMpass℠ study (https://research.themmrf.org and www.themmrf.org). The molecular data to date demonstrate that myeloma is a temporally and spatially heterogeneous malignancy characterised by early onset chromosomal aneuploidies and translocations involving the immunoglobulin loci followed by the acquisition of driver mutations in the RAS/MAPK pathway, MYC dysregulation (through MYC translocation with multiple partners) and later acquisition of TP53 mutations and copy number changes.

Despite the extensive myeloma genomic datasets available, novel genomic lesions continue to be discovered with potential further insights into the biology of this disease [5]. The increase in routine genomic analysis in the diagnostic laboratory in patients with myeloma provides another source of novel genomic findings. We aimed to review data from myeloma patient samples referred to our diagnostic service (Peter MacCallum Cancer Centre, Molecular Haematology Laboratory) that have been sequenced using a hybridization-based next generation sequencing panel that detects sequence variants, genome wide copy number changes and immunoglobulin translocations (the Peter MacCallum Cancer Centre PanHaem panel [6]) with a focus on novel genomic findings.

2. Results and Discussion

Samples from 86 patients were identified. 64% (55/86) of patients were male, the median age was 62 years and the majority of patients (81%, 70/86) were identified as having relapsed/refractory disease. A summary of the recurrent genomic abnormalities is shown in Figure 1. Overall the genomic landscape of the cohort was consistent with the literature published to date. Of note, the cohort was enriched for TP53 abnormalities (with sequence variants and/or copy number changes detected in 57% (49/86) of patients) reflecting the inclusion of a previous cohort that had been selected for 17p deletion detected by FISH [7] as well as a high proportion of patients with relapsed/refractory disease. Both TP53 copy number loss (18/86, 21%) and TP53 mutation plus copy number loss (24/86, 28%) were more common compared to TP53 mutation alone (7/86, 8%).

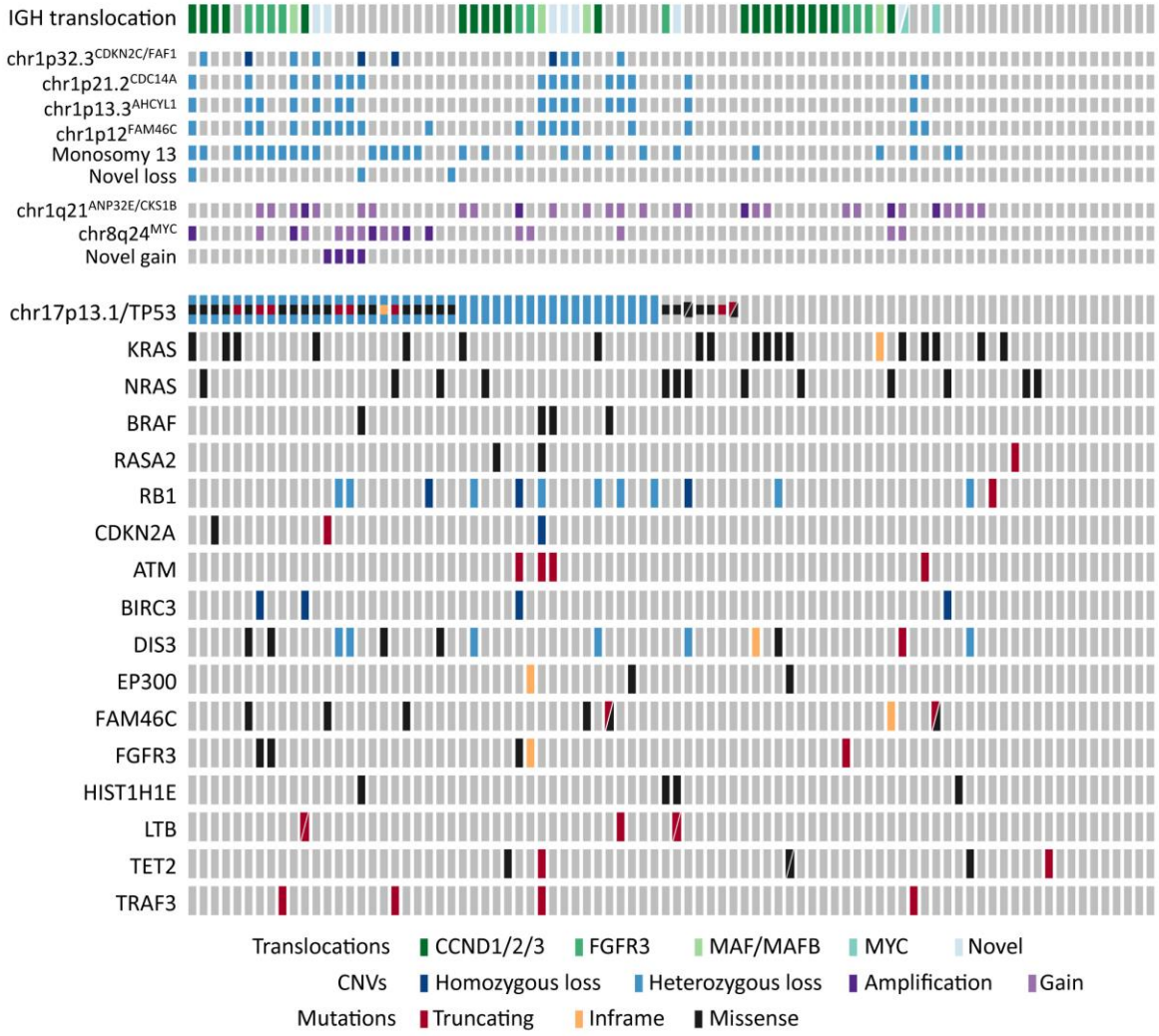

Figure 1. Genomic alterations in 86 multiple myelomas detected by targeted sequencing. Each column represents an individual sample and each row represents a gene or genomic location. Key copy number changes including loss of chromosomes 1p, 13 and 17p, and gain of chromosomes 1q and 8q are indicated, as well as novel gains and losses detected by this study. Recurring mutations (mutated in at least three cases) and structural variants involving the IGH locus are also shown. A complete list of mutations and IGH translocations are found in Table S3 and Table S4.

2.1. Novel sequence variant findings

A list of all sequence variants detected is included in Table S2. Overall, we detected 146 mutations in 31 of the 104 genes analysed and found at least one mutation in 69 cases (80% of the cohort). The gene list included for analysis comprised those that were known to be mutated in myeloma and therefore our analysis was not placed to detected novel mutated genes; among the other most frequently mutated genes were TP53 (36%), KRAS (22.1%), NRAS (15.1%), FAM46C/DIS3 (8.1%) and TET2/FGFR3 (5.8%). Despite this, specific variants that have not previously been described in myeloma were detected. As expected KRAS mutations involved canonical hotspot codons Gln61 and Gly12/13. We also detected an Ile24Asn and Tyr64Asp, and mutations involving Ala59 and Lys117 each in two cases, which are only rarely described in larger cohorts (CoMMpass℠ IA12 dataset). In addition, we detected novel mutations in *RASA2* previously undescribed in myeloma. Interestingly, despite the general effect of mutations in *RASA2* being loss-of-function (encoding for a GTPase-activating protein that promotes the conversion of active RAS to an inactive state thus negatively regulating this pathway), we detected two missense mutations occurring in the same codon (Arg511Cys and Arg511His). The COSMIC database (http://cancer.sanger.ac.uk/cosmic) indicates this codon to be the most frequently mutated codon in *RASA2* (however with only six cases reported in skin, gastric and esophageal cancers). Therefore our data provides further evidence for this codon as a recurrently mutated hotspot in *RASA2* and that *RASA2* suppression provides an alternative mechanism for the constitutive activation of RAS signalling in myeloma. Likewise, we also detected activating mutations in *PTPN11* (Asp61His and Ala72Val), an oncogene encoding for the SHP2 protein leading to increased RAS/MAPK pathway signaling conventionally associated with the autosomal disorder Noonan syndrome as well as juvenile myelomonocytic leukemia [8]. Collectively, the incidence of RAS/MAPK pathway sequence variants (in *KRAS*, *NRAS*, *BRAF*, *RASA2* and *PTPN11*) was 46.5% (40 of 86 patients).

Three CRBN mutations (Thr361Cysfs*7, Gly151*, Pro382Arg) were detected in two patients, both with relapsed/refractory disease that had been extensively treated with immunomodulatory drugs (IMiDs). Cereblon (encoded by CRBN) mediates the anti-myeloma activity of the IMiD class of therapeutics and therefore the pattern of mutations seen in CRBN is truncation or deleterious missense mutation in critical functional domains [9]. The missense mutation we detected (Pro382Arg) has not been previously described in myeloma but is in the IMiD-binding domain next to a previously described missense mutation [10]. No mutations in IKZF1, IKZF3 or IRF4 were identified.

2.2. Novel copy number changes

A diverse range of recurrent gains and losses were detected in the cohort (Figure 2), including numerous novel copy number changes. We focused on high-level focal amplifications and biallelic deletions due to their ability to give insight into potentially important oncogenic and tumour suppressor pathways. A high level focal copy number gain was detected on chromosome 7p involving *IL6* (as well as numerous other genes on 7p15.3, see Table 3). The IL6-IL6R axis is important in multiple myeloma pathogenesis [11] and *IL6R* is part of the typical 1q gain seen in multiple myeloma and has been previously implicated in high level amplifications [12]. Lower level gains are described in the CoMMpass℠ IA12 dataset and are associated with increased *IL6* expression by RNA-sequencing.

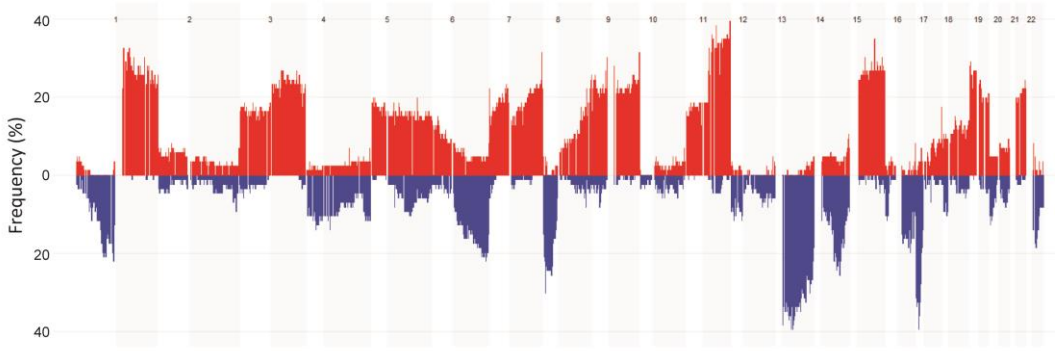

Figure 2. Genome-wide frequency plot of copy number gains (red) and losses (blue) in 86 multiple myelomas.

A focal amplification was detected involving 17p11.2 in three patients (Table 1). This copy number amplification has not been specifically described in the literature to date however one patient in the CoMMpass℠ IA12 dataset is documented as having high-level copy number gain at the same locus. Whilst this amplification involves many genes, one gene of particular interest inside the common amplified region is *TNFRSF13B* which encodes TACI, the receptor for BAFF/APRIL which has been shown to be variably expressed in myeloma and is associated with plasma cell differentiation [13]. RNA-sequencing data from the CoMMpass℠ IA12 dataset demonstrates that the patient with amplification of a similar region had increased expression of *TNFRSF13B* relative to the rest of the cohort. Interestingly, both 7p15.3 and 17p11.2 have also been shown to contain germline variants associated with a genetic susceptibility to myeloma [14,15].

Table 1. Novel copy number amplifications.

Sample	Approximate genomic location[1]	Median copies	Approximate size
MM16	7p15.3 (chr7:20015001-23940000)	30	4MB
MM13	17p11.2 (chr17:16540001-17305000)	27	765kb
MM14	17p11.2 (chr17:16225000-18230000)	6	2MB
MM15	17p11.2 (chr17:16175000-18105000)	6	2MB

[1]Coding genes within this interval are listed in Table S2.

We observed previously described rare biallelic losses in tumour suppressors *BIRC2*/*BIRC3*, *CDKN2A*, *PTPRD*, *FAF1*/*CDKN2C* and *RB1* consistent with the relapsed/refractory nature of the cohort [3,16,17], as well as novel biallelic losses in *VAV3* and *MYH4*. Two of the *BIRC2*/*BIRC3* biallelic losses occurred in patients with t(4;14), an association that has been previously noted. In addition, we observed that the biallelic loss was intragenic for *MYH4* (exon 23-39), the three *RB1* deletions (exon 5-17, exon 18-23, exon 8-11) and *PTPRD* (exon 15-26). The observation of partial deletion of prognostic markers such as *RB1* is important, as these are potentially below the resolution of FISH and require sequencing approaches to detect.

2.3. Novel structural variants

50% (43/86) of patients had a structural variant (SV) involving the immunoglobulin heavy chain (IGH) locus detected by sequencing, which are listed in Table S4. These SVs included those with breakpoints within the vicinity of genes known to be involved in chromosomal translocations in myeloma including *CCND1* (18 cases), *FGFR3*/*WHSC1* (10 cases), *MAF* (three cases), *CCND3* (two

cases), *CCND2* (one case) and *MAFB* (one case). Two cases also harbored a SV involving *MYC*, both of which also had a *KRAS* mutation.

In addition to recurrent IGH SVs, multiple SVs were detected involving the IGH locus and non-canonical candidate partner genes (see Table 2) selected based on their proximity to the translocation breakpoint including

- *CD46* which has been demonstrated to have increased expression in myeloma cell lines and primary myeloma cells particularly in association with 1q copy number gains [18],
- *TXNDC5* which has recently been described as a rare but recurrent translocation in myeloma and in this study was detected in a patient with a high hyperdiploid karyotype consistent with the previous report [5],
- *TRAP1* which is a Hsp90 that functions as an anti-apoptotic protein through controlling ubiquitination of several mitochondrial proteins [19],
- *ZBTB38* which is a transcriptional repressor that regulates DNA replication with overexpression expected to have the same functional effect as loss of the tumour suppressor gene *RBBP6* (i.e. increase DNA damage at common fragile sites) [20],
- *RCC2* which is involved in cell cycle regulation [21]
- *BCL7A* which is expressed throughout the B-cell lineage but tends to be downregulated in normal plasma cells [22].

Coding and non-coding mutations have been reported in *BCL7A* previously in myeloma [1,23]. However despite *BCL7A* being initially described as a putative tumour suppressor in other B-cell malignancies the spectrum of genomic lesions seen in *BCL7A* to date from the literature, the CoMMpass℠ IA12 dataset (non-coding mutations, relatively high proportion of inframe changes and loss of start codons, absence of nonsense/frameshift mutations and copy number losses) and our finding of *BCL7A* as a potential immunoglobulin fusion partner suggests further study is warranted to clarify its role in myeloma.

Table 2. Novel IGH structural variants.

Sample	Candidate partner gene	Description
MM44	*RCC2*	Previously undescribed. RNA-sequencing confirmed *RCC2* overexpression
MM33	*TRAP1*	Previously undescribed
MM34	*ZBTB38*	Described in CoMMpass IA12
MM35	*CD46*	Previously undescribed
MM13	*GABRG1/GNPDA2*	Previously undescribed
MM64	*TXNDC5*	Rare previously described translocation [5]
MM12	*BCL7A*	Previously undescribed

Genomic characterization in multiple myeloma is become increasingly adopted in the diagnostic laboratory in order to enhance diagnosis, prognosis and choice of therapy. Despite the genomically well-characterised nature of multiple myeloma, review of clinical sequencing data from our diagnostic laboratory has detected multiple novel genomic findings including novel sequence variants, copy number changes and SVs involving the IGH locus.

Importantly, from a clinical management perspective we make the observation that of patients with *TP53* abnormalities, 8% of patients had *TP53* mutations only without copy number change. Despite both deletion of 17p detected by FISH and *TP53* mutations being associated with inferior outcomes in myeloma [7], FISH for 17p deletion is most commonly performed as part of routine prognostic investigation compared to *TP53* mutation testing. However, our data shows that in order to identify patients with abrogated *TP53* function, both mutations and copy number changes need to

be assessed in patients with myeloma to identify all patients at risk of inferior outcomes and requiring potential novel treatment approaches.

Despite extensive genomic characterization of patients with myeloma in the literature and the public availability of more than 1000 exomes and RNA-sequencing data, we have demonstrated that there are still novel genomic findings to detect even in routine diagnostic sequencing. Findings such as ours contribute to the overall documentation of the genomic landscape of myeloma and provide insights into plasma cell pathobiology but also illustrate the importance and utility of collaborative initiatives such as the CoMMpass℠ study in order to centralize and aggregate genomic data with an aim to better understand the genomic landscape that underpins this disease. In addition, we have shown that comprehensive clinically relevant genomic assessment detecting the full range of genomic lesions in myeloma can be performed efficiently with a single hybridization based assay in the diagnostic laboratory.

3. Materials and Methods

3.1. Patient samples and DNA extraction

Samples received for diagnostic testing by the Molecular Haematology Laboratory (Peter MacCallum Cancer Centre) from patients with myeloma were included in this study. This includes a cohort of patients with del 17p, the outcomes of which have been previously described [7]. DNA was extracted from bone marrow aspirate samples with at least 30% plasma cells (32 by morphological assessment and 54 enriched by immunomagnetic selection using CD138+ beads post mononuclear cell isolation by density centrifugation) using DNeasy blood and tissue kit reagents (Qiagen, Hilden, Germany). This study was performed with approval by the ethics committees of the Peter MacCallum Cancer Centre and National Research Ethics Service Committee London and was conducted in accordance with the Helsinki Declaration.

3.2. Mutation screening

We performed targeted next generation sequencing of 104 genes (listed in Table S1) selected from previous myeloma sequencing studies [2,24] or known to be relevant in other haematological malignancies. The custom SureSelect hybridisation-based capture panel (Agilent, California, USA) also included the IGH locus including the entire constant region and covering the switch and enhancer regions, and V, D and J segments as described previously [25]. DNA (200-300 ng) was sheared by focused acoustic sonication (Covaris, Massachusetts, USA) and fragment libraries prepared using the KAPA Hyper Prep Kit according to standard protocols (KAPA Biosystems, Massachusetts, USA). Hybridisation capture was performed according to the Agilent SureSelectXT protocol followed by sequencing of indexed libraries on an Illumina NextSeq (paired-end 75 bp reads). After de-multiplexing and base calling, BWA-MEM [26] was used to align reads to the human genome (GRCh37 assembly), followed by local indel realignment (using GATK software [27]) and marking of duplicate alignments (using Picard, http://broadinstitute.github.io/picard/). Captured regions were sequenced to a mean depth of 700x which was sufficient data for 98% of target regions to achieve least 100-fold coverage.

Variants were called using Haplotype caller [27] and annotated using Ensembl Variant Effect Predictor (v78). Known polymorphisms and common sequencing artefacts were excluded by filtering for minor allele frequencies <0.05% (in the 1000 Genomes, ExAC, gnomAD or EVS databases) and by manual review in IGV. Variants overlapping coding regions and canonical splice sites were curated for pathogenicity including previous description in the literature and cancer databases (e.g. COSMIC) and *in silico* prediction. Only variants considered pathogenic are reported and are listed in Table S2.

3.3. Copy number analysis and translocation detection

Copy number variants were estimated by comparing read counts from on and off target reads to a pooled reference to correct for enrichment and sequencing biases. All samples were assessed for

common copy number aberrations in myeloma (loss of 1p, gain of 1p, monosomy 13, loss of TP53), as well as novel changes. GRIDSS was used to call translocations involving the IGH locus using split reads, discordant read pairs and breakpoint assembly post genome-wide alignment. Detailed methods for the analysis of copy number and translocation detection are provided in the Supplementary Methods.

Supplementary Materials: Supplementary materials can be found at www.mdpi.com/link.

Supplementary Methods

Table S1: Genes analysed by targeted sequencing

Table S2: Variants detected by targeted sequencing

Table S3: Copy number variants detected by targeted sequencing

Table S4: Structural variants involving the IGH locus

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Author Contributions: G.L.R., M.D., M.P. and P.B. conceived and designed the experiments; G.L.R, K.J and J.G. performed the experiments; G.L.R, K.J, J.M., Y.K, and P.B. analyzed the data; M.C., M.C. and K.Y. contributed clinical samples and collected clinical data; G.L.R and P.B. wrote the paper.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

IGH	Immunoglobulin heavy chain
IMiDs	Immunomodulatory drugs
SV	Structural variant

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