Novel genomic findings in multiple myeloma identified through routine diagnostic sequencing

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ABSTRACT

Aims Multiple myeloma is a genomically complex haematological malignancy with many genomic alterations recognised as important in diagnosis, prognosis and therapeutic decision making. Here, we provide a summary of genomic findings identified through routine diagnostic next-generation sequencing at our centre.

Methods A cohort of 86 patients with multiple myeloma underwent diagnostic sequencing using a custom hybridisation-based panel targeting 104 genes. Sequence variants, genome-wide copy number changes and structural rearrangements were detected using an inhouse-developed bioinformatics pipeline.

Results At least one mutation was found in 69 (80%) patients. Frequently mutated genes included TP53 (36%), KRAS (22.1%), NRAS (15.1%), FAM46C/DIS3 (8.1%) and TET2/FGFR3 (5.8%), including multiple mutations not previously described in myeloma. Importantly we observed TP53 mutations in the absence of a 17p deletion in 8% of the cohort, highlighting the need for sequencing-based assessment in addition to cytogenetics to identify these high-risk patients. Multiple novel copy number changes and immunoglobulin heavy chain translocations are also discussed.

Conclusions Our results demonstrate that many clinically relevant genomic 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.

INTRODUCTION

Through large whole genome, whole exome and targeted sequencing efforts, multiple myeloma has become one of the most genomically well-characterised 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 and whole transcriptome sequencing are also publicly 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 chromosome aneuploidies and translocations involving the immunoglobulin loci, followed by the acquisition of driver mutations in the RAS/MAPK pathway, MYC dysregulation (through translocation with multiple partners) and acquisition of TP53 mutation and copy number loss.

Despite the extensive myeloma genomic data sets 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 potential 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 hybridisation-based next-generation sequencing panel that detects sequence variants, genome-wide copy number changes and immunoglobulin translocations (the Peter MacCallum Cancer Centre PanHaem panel) with a focus on novel genomic findings.

MATERIALS AND METHODS

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 (online supplementary table 1). This includes a cohort of patients with deletion of 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).

Mutation screening

We performed targeted next-generation sequencing of 104 genes (listed in online supplementary table 2) selected from previous myeloma sequencing studies2–5 or known to be relevant in other haematological malignancies. The custom SureSelect hybridisation-based capture panel (Agilent, California, USA) also included the immunoglobulin heavy chain (IGH) locus, including the entire constant region and covering the switch and enhancer regions, and V, D and J segments as described previously.9 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 was used to align reads to the human genome (GRCh37 assembly), followed by local indel realignment (using the GATK software) and marking of duplicate alignments (using Picard; http://broadinstitute.github.io/picard/). Captured regions were sequenced to a mean depth of 700×, which was sufficient data for 98% of target regions to achieve at least 100-fold coverage.

Variants were called using Haplotype caller 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 Exome Variant Server databases) and by manual review in Integrative Genomics Viewer (IGV). Variants overlapping coding regions and canonical splice sites were curated for pathogenicity, including previous description in the literature and cancer databases (eg, the Catalogue of Somatic Mutations in Cancer (COSMIC)) and in silico prediction. Only variants considered pathogenic are reported and are listed in online supplementary table 3.

Copy number analysis and translocation detection
Copy number variants were estimated by comparing read counts from on-target and off-target reads with a pooled reference to correct for enrichment and sequencing biases (I Markham, 2018, manuscript in preparation). All samples were assessed for common copy number aberrations in myeloma (loss of 1p, gain of 1q, monosomy 13, loss of 17p), as well as novel changes. The Genomic Rearrangement IDentification Software Suite (GRIDSS) was used to call translocations involving the IGH locus using split reads, discordant pair reads and breakpoint assembly post genome-wide alignment.

RESULTS
Samples from 86 patients were identified (clinical parameters are summarised in online supplementary table 1). Sixty-four per cent (55/86) of the 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 deletions detected by fluorescence in situ hybridisation (FISH) (n=47), 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 with TP53 mutation alone (7/86, 8%).

Novel sequence variant findings
A list of all sequence variants detected is shown in online supplementary table 3. Overall, we detected 146 mutations in 31 of the 104 genes analysed and found at least one mutation in 80% of the cohort (69/86 cases, median of 2 mutations per case, range 0–6 mutations per case). Among the most frequently mutated genes were TP53 (36%), KRAS (22.1%), NRAS (15.1%), PTPRD (8.1%), DIS3 (8.1%), TET2 (5.8%) and FGFR3 (5.8%). Despite this, specific variants that have not previously been described in myeloma were detected. As expected, KRAS mutations involved canonical hot spot codons Gln61 and Gly12/13. We also detected an Ile24Asn and Tyr64Asp, and mutations involving Ala59 and Lys117 in each of two cases, which are only rarely described in larger cohorts (ComPASS Interim Analysis 12 (IA12) data set).

In addition, we detected novel mutations in RAS2 and PTPN11 previously undescribed in myeloma. Collectively, the incidence of RAS/MAPK pathway sequence variants (in KRAS, NRAS, BRAF, RAS2 and PTPN11) was 46.5% (40 of 86 patients).

Three CRBN mutations (Thr361Cys*7, Gly151*, Pro382Arg) were detected in two patients, and one of these patients had relapsed/refractory disease and had been extensively treated with immunomodulatory drugs (IMiDs). Cereblon (encoded by CRBN) is important in mediating 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. 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. No mutations in other genes associated with IMiD resistance (IKZF1, IKZF3 or IRF4) were identified.

Novel copy number changes
A diverse range of recurrent gains and losses were detected in the cohort (figure 2), including numerous novel focal copy number changes (table 1 and online supplementary table 4).

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, as well as novel bialleic losses in AVV3 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 (exons 23–39), the three RB1 deletions (exons 5–17, exons 18–23, exons 8–11) and PTPRD (exons 15–26).

Novel structural variants
Fifty per cent (43/86) of patients had a structural variant (SV) involving the IGH locus detected by sequencing, which are listed in online supplementary table 5. 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 (3 cases), CCND3 (2 cases), CCND2 (1 case) and MAFB (1 case). Two cases also harboured a SV involving MYC. Multiple SVs were detected involving the IGH locus and non-canonical candidate partner genes (selected based on proximity to the translocation breakpoint), and these are listed in table 2.

DISCUSSION
Genomic characterisation in multiple myeloma is becoming increasingly adopted in the diagnostic laboratory in order to enhance diagnosis, prognosis and choice of therapy. Despite the genetically 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.

Dysregulation of the RAS/MAPK pathway is an established mechanism in malignant plasma cells. While this is typically achieved with activating NRAS, KRAS and BRAF mutations, we have described mutations in RAS2 not previously described in myeloma. Interestingly, despite the general effect of mutations in RAS2 being loss of function (encoding for a GTPase-activating protein that promotes the conversion of active RAS to an inactive state, thus negatively
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 online supplementary tables 3–5. CNV, copy number variation; IGH, immunoglobulin heavy chain.

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 oesophageal cancers). Therefore our data provide further evidence for this codon as a recurrently mutated hot spot 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 signalling conventionally associated with the autosomal disorder Noonan syndrome as well as juvenile myelomonocytic leukaemia.

From a clinical management perspective, we make the important observation that of patients with TP53 abnormalities, 8% 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, FISH for 17p deletion alone is most commonly performed as part of routine prognostic investigation. Importantly, our analysis of patients in the CoMMpass data set with deletion of 17p and those with TP53 mutation only (ie, without deletion of 17p) demonstrated no statistically significant difference in progression-free or overall survival. Therefore, in order to identify all patients with abrogated p53 function (and thus those at risk of inferior outcomes and potentially requiring novel treatment approaches), both mutations and copy number changes need to be assessed.

In terms of 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; see online supplementary table 4). The IL6-IL6R axis is important in multiple myeloma pathogenesis, and IL6R is part of the typical 1q gain seen in multiple myeloma and has been previously implicated in high-level amplifications. Lower level gains are described in the CoMMpass IA12 data set and are associated with increased IL6 expression by RNA sequencing.

A focal amplification was detected involving 17p11.2 in three patients. This copy number amplification has not been specifically described in the literature to date; however, one patient in the CoMMpass IA12 data set is documented as having high-level copy number gain at the same locus. While 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. RNA sequencing data from the CoMMpass IA12 data set demonstrate 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. In addition to focal copy number amplifications, our observation of partial deletion of prognostic markers such as RB1 is important as these are possibly below the resolution of FISH and require sequencing approaches to detect.

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

- **CD46**, which has a role in innate immune recognition and has been demonstrated to have increased expression in myeloma cell lines and primary myeloma cells particularly in association with 1q copy number gains.

- **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 a previous report.

- **TRAP1**, which is a member of the Hsp90 family that functions as an antiapoptotic protein by controlling ubiquitination of several mitochondrial proteins.

- **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 (ie, increase DNA damage at common fragile sites).

- **RCC2**, which is involved in cell cycle regulation.

- **BCL7A**, which is expressed throughout the B cell lineage but tends to be downregulated in normal plasma cells.

Coding and non-coding mutations have been reported in BCL7A previously in myeloma. 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 data set (non-coding mutations, relatively high proportion of inframer 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 is atypical for classical tumour suppressor biology, suggesting further study is warranted to clarify its potential biological role in myeloma.

Despite extensive genomic characterisation 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.

### Table 2 Novel immunoglobulin heavy chain structural variants

<table>
<thead>
<tr>
<th>Sample</th>
<th>Candidate partner gene</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>MM16</td>
<td>7p15.3 (chr7:20015001–23940000)</td>
<td>30 Mb</td>
</tr>
<tr>
<td>MM13</td>
<td>17p11.2 (chr17:6540001–17050000)</td>
<td>27 765 kb</td>
</tr>
<tr>
<td>MM14</td>
<td>17p11.2 (chr17:6225000–18230000)</td>
<td>6 2 Mb</td>
</tr>
<tr>
<td>MM15</td>
<td>17p11.2 (chr17:16175000–18105000)</td>
<td>6 2 Mb</td>
</tr>
</tbody>
</table>

* Coding genes within this interval are listed in online supplementary table 4.
but also illustrate the importance and utility of collaborative initiatives such as the CoMMpass study in order to centralise 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 a diversity of genomic lesions from simple mutations to structural variations in myeloma can be performed efficiently with a single hybridisation-based assay in the diagnostic laboratory.

Take home messages

► Comprehensive genomic characterisation of sequence variant, copy number changes and immunoglobulin heavy chain (IGH) translocation is possible in the diagnostic department with a single hybridisation, next-generation sequencing-based assay and yields novel genomic findings.

► Sequence variant detection in myeloma is important in order to completely capture TP53 abnormalities and those with inferior outcomes.

► Novel genomic changes giving clues to pathogenesis are still identifiable in myeloma despite its extensive published genomic characterisation.

► We have detected multiple novel candidate IGH partners including RCC2, TRAP1, CD46 and BCL7A.

Handling editor Runjan Chetty.

Contributors GLR, KJ, MD, HMP and PB contributed to study concept. GLR, KJ and JS performed the experiments. GLR, KJ, JM, YK and PB analysed the data. MEC, MacC and KY contributed clinical samples and collected clinical data. GLR and PB drafted the manuscript. All authors approved the final version of the manuscript.

Funding We gratefully acknowledge funding support from The Snowdome Foundation (http://snowdome.org.au/) and Vision Super (https://www.visionsuper.com.au/).

Competing interests None declared.

Patient consent Not required.

Ethics approval This study was performed with approval from the ethics committee of the Peter MacCallum Cancer Centre and the National Research Ethics Service Committee London.

Provenance and peer review Not commissioned; externally peer reviewed.

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REFERENCES