CORRESPONDENCE

Comprehensive genomic testing is required to assess for markers of poor prognosis in multiple myeloma

Sir,

Plasmacytoma is a relatively rare plasma cell dyscrasia where monoclonal plasma cells grow in discrete clusters in either bone or soft tissue. The median survival of patients with plasmacytoma is 10 years; however, multiple factors are reported to affect prognosis.¹ Features of a less favourable prognosis include solitary plasmacytoma of bone (SBP), older patients, bone marrow involvement, lesions greater than 5 cm or persistence of M protein for greater than 12 months following treatment with localised radiotherapy.¹

Genetic testing is being increasingly used to direct management and predict prognosis in myeloma. The International Myeloma Working Group (IMWG) classification based on cytogenetics and fluorescence *in situ* hybridisation (FISH) has identified t(14;16)(q32;q23); t(14;20)(q32;q11); del(17)(p13); t(4;14)(p16;q32); non-hyperdiploid state; and gain of 1q as poor prognostic markers.² Traditionally, copy number changes (CNC) have been screened for by conventional karyotyping and FISH. Single nucleotide polymorphism (SNP) chromosome microarray (CMA) is a relatively new technique performed on DNA extracted from CD138 enriched cells which provides a global measure of CNC. It overcomes the technical challenges of growing quiescent plasma cells for conventional karyotyping and missing abnormalities that lie outside of the discrete FISH probe regions. Relying on conventional karyotyping and FISH alone has the potential to miss important genomic changes and misinterpretation of results. We present a patient with plasmacytoma and multiple myeloma who required multiple techniques to identify genomic changes associated with poor prognosis.

A 76-year-old woman with a background history of stage 3 kidney disease, pacemaker for heart block, hypertension and gout presented with backache and computed tomography (CT) scan showed a L3 vertebral lesion. Core biopsy revealed sheets of plasma cells expressing CD45, CD138 and CD20, consistent with plasmacytoma. Skeletal survey showed multiple lytic lesions in the skull, spine, ribs, sternum and right scapula. The previously noted lesion at L3 was associated with a soft tissue component. Serum protein studies showed no intact paraprotein and serum free light chains showed raised lambda light chains of 284.3 mg/L and kappa light chains of 98.3 mg/L with ratio of 0.35. Normocytic normochromic anaemia was present; creatinine was 256 µmol/L and eGFR was 15 mmol/L. Calcium levels were normal. Bone marrow biopsy showed marked plasmacytosis (85%) consistent with a diagnosis of light chain multiple myeloma.

Conventional karyotyping and FISH were performed on the bone marrow aspirate. Both 24 hour synchronised and 72 hour unsynchronised cultures stimulated with B-cell mitogens were initiated and harvested as per standard recommendations.³ FISH was performed on a purified population of CD138+ plasma cells which was achieved using magnetic cell separation (MACS) beads and columns (Miltenyi Biotec,

Germany). The FISH panel used was based on IMWG recommendations. Probes were used in accordance with the manufacturer's protocol and included XL *CDKN2C/CKS1B* Amplification/Deletion Probe, XL CLL Probe Kit 1 (*ATM/TP53*) Deletion/Enumeration Probe and XL *IGH* BA Break Apart Probe (MetaSystems, Germany). DNA was extracted from the purified CD138+ cells and was processed for SNP CMA using the Core Exome Array (Illumina, USA). DNA was also referred to an external laboratory for massively parallel sequencing (MPS) using a custom designed lymphoid amplicon gene panel.

G-banded chromosomes showed a complex, female, hyperdiploid karyotype with multiple structural and numerical changes including gains of odd number chromosomes, structural abnormalities of chromosomes 11 and 14 and the presence of marker chromosomes and double minutes (Fig. 1A). FISH showed a signal pattern consistent with gains of two copies of *ATM* (11q13). All other FISH probes showed normal hybridisation patterns (two copies). Additional FISH testing using the *MYC* Break Apart probe (MetaSystems) showed that the double minutes were derived from amplification of c-*MYC* (Fig. 1B). SNP CMA identified an amplified region spanning approximately 2.25 MB (genomic coordinates 127782767_130031959) on chromosome 8q24 including the *MYC* gene (Fig. 1C). On visualisation of this region using the UCSC browser, *PVT1* was also identified within this amplified region. In addition to confirming the FISH identification of *MYC* positive double minutes, SNP CMA also identified multiple chromosomes with complete loss of heterozygosity consistent with doubling of a hypodiploid clone (Fig. 1D,E). MPS detected a 14 base pair deletion in the *TP53* gene [NM000546.5:c.157_170del:p.(Trp53 Profs*5)] which is predicted to result in a truncated protein product. This variant was observed at a variant allele frequency of 10–15%.

The patient was treated with four cycles of cyclophosphamide, bortezomib and dexamethasone with weekly doses. The fourth cycle was complicated by development of cytopenias for which treatment was deferred for 2 weeks. When counts did not improve, a follow-up bone marrow biopsy was performed which showed markedly hypercellular marrow with suppressed trilineage haemopoiesis due to extensive malignant plasma cell infiltration (~80%) refractory to treatment. The patient opted for palliation and died within a couple of weeks of progressive disease.

The abnormalities observed in our case were identified due to additional testing including SNP CMA and MPS performed for research purposes. This highlights that multiple genomic techniques were required to comprehensively identify cytogenetic prognostic factors in plasma cell disorders. This patient had (1) double minutes which were identified on routine karyotyping and confirmed to be derived from c-*MYC* amplification using FISH and SNP CMA, (2) *MYC* and *PVT1* amplification identified using SNP CMA, (3) hypodiploidy which was identified using SNP CMA, and (4) *TP53* dysregulation identified using MPS.

Double minutes are small chromatin bodies that are acentric, atelomeric fragments containing 1–2 MB of DNA in multiple copies within a single cell, often found to contain oncogenes.⁴ They are commonly observed in solid tumours,

Fig. 1 (A) Metaphase cell showing multiple double minutes. (B) FISH showing MYC positive double minutes. (C) SNP CMA showing amplification of 8q24 containing MYC. (D) Four copies of chromosome 3 and two copies of chromosome 17 in a metaphase. (E) SNP CMA from chromosome 3 indicating copy number neutral despite four copies on conventional karyotype. (F) SNP CMA from chromosome 17 showing loss of heterozygosity resulting in complete loss of function of *TP53*.

but are a rare finding in haematological neoplasms such as acute myeloid leukaemia and myelodysplastic syndrome, where they are often found to contain *MYC* and are associated with poor overall survival.⁵ Double minutes in plasma cell disorders has been rarely reported. Yamamoto *et al.*⁶ described a patient with plasma cell leukaemia who had the same dismal prognosis and a short overall survival after diagnosis as our patient. In our patient, the double minutes were noted on routine karyotyping and their derivation from *MYC* was identified using FISH and SNP CMA.

MYC is an oncogene which codes a transcription factor with roles in cell proliferation, cell cycle progression and apoptosis. Abnormalities involving *MYC* such as translocations and copy number gains have been reported in multiple myeloma.⁷ *MYC* overexpression is associated with higher proliferation rate, aggressive clinical course and short event-free and overall survival following allogeneic stem cell transplant.⁸ However, it is uncertain if *MYC* copy number gains which result from the *MYC*-containing double minutes as in our patient, are sufficient to result in constitutive activation of the downstream pathway.

Interestingly, the double minutes in our patient were also found to contain *PVT1*, which is an oncogene. Amplification or increased copy number of *PVT1* has also been reported to be observed in many cancers including plasmacytoma where it is able to regulate *MYC* expression.⁹ Functional studies have shown that when both *MYC* and *PVT1* are amplified they result in positive feedback with *PVT1* stabilising *MYC* and *MYC*

upregulating the promoter of *PVT1*.⁹ This could have potentially played a role in the poor prognosis in our patient, and would not have been detected without the use of SNP CMA.

SNP CMA was able to unmask a poor prognostic marker (doubling of a hypodiploid clone) which had previously been considered hyperdiploidy by conventional karyotyping which is favourable prognostic marker.² Recurrent copy number changes such as 1p loss, 1q gain and 17p loss can also be identified by SNP CMA as well as other copy number changes that exist across the genome and outside of the specific loci targeted by FISH.

Low-hypodiploidy is defined as 31–39 chromosomes, is rarely reported in MM¹⁰ and is associated with an extremely poor prognosis.¹¹ Doubling of a low-hypodiploid clone can mask the true abnormality and confuse it with a hyperdiploid cell line which has a favourable prognosis.² SNP CMA was able to unmask the low-hypodiploid clone in this patient by the presence of multiple whole chromosome copy number neutral losses of heterozygosity (LOH). The conventional karyotype in our case had four copies of chromosomes that were not lost in the low-hypodiploid clone and two copies of the chromosomes that were lost in the low-hypodiploid clone (Fig. 1F).

A loss of function mutation in the *TP53* tumour suppressor gene was demonstrated by MPS. *TP53* dysregulation is important in the pathogenesis of plasmacytoma/multiple myeloma and is associated with high risk disease.^{2,12} Together with the copy number neutral LOH for chromosome 17 shown

by SNP CMA, we were able to identify that our patient had no functional copy of the *TP53* gene.

The poor prognosis in this patient was likely driven by *MYC* and *PVT1*-containing double minutes together with low-hypodiploidy and bi-allelic loss of function of *TP53*. These changes could be comprehensively delineated because of the use of multiple complementary genomic techniques. In clinical practice, identification of a comprehensive snapshot genomic changes associated with poor prognosis in multiple myeloma aids in communication of a personalised risk profile allowing the clinical team and patient to make an informed decision regarding treatment.

While IMWG does not specifically recommend SNP CMA, we suggest that subsequent editions of the guidelines include SNP CMA to replace conventional karyotyping for identification of copy number changes across the genome. SNP CMA is the only assay that can accurately detect doubling of a hypodiploid clone; however, it must be used with FISH to detect IGH rearrangements as an efficient and cost-effective way of identifying cytogenetic changes in patients with multiple myeloma. This case study also provides evidence for inclusion of additional genomic changes such as hypodiploidy and *MYC-PVT1* containing double minutes as poor prognostic markers.

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Evaluation of direct antimicrobial susceptibility testing using positive blood culture broth in an Australian laboratory

Sir,

Sepsis continues to cause significant mortality worldwide, with evidence that early institution of appropriate antibiotic therapy has a mortality benefit.^{1,2} The microbiology laboratory plays an important role in providing timely identification of organisms and their susceptibility profiles to enable tailoring of antimicrobial therapy. Methods that significantly reduce the time to identification of isolates have been shown to reduce the time to effective and optimal antibiotic therapy and contribute to hospital anti-microbial stewardship programs.^{3,4} Using methods described in previous studies we investigated whether using positive blood culture bottles to directly inoculate the Vitek-2 cards to perform susceptibility testing correlated well with traditional methods and enabled significantly faster results.^{5–7}

This study was conducted at the Mater Pathology laboratory in Brisbane, Australia, from July to November 2018. Samples from the Mater public hospital, Mater mothers hospital and the Mater private hospital were included in the study. Blood specimens were incubated in the BacT/Alert 3D automated microbial detection system (bioMérieux, France). After blood culture bottles flagged positive a Gram stain was performed. All blood cultures that flagged positive between 08:00 hour Monday to 16:00 hour on Friday with Gram stains that showed Gram-positive cocci or Gram-negative bacilli were included in the study. Exclusion criteria were: any blood culture bottles that were from the same patient episode with the same organism that had already been included in the study; those that flagged positive outside of the hours stipulated above; those in which the organism identified was not validated for Vitek-2 antimicrobial susceptibility testing; those which showed Gram-positive bacilli or Gram-negative cocci on Gram stain; and those in which more than one organism was cultured (polymicrobial cultures). Identification was performed on isolates from solid growth media primarily using the matrix assisted laser desorption/ionisation mass spectrometry (MALDI-TOF MS) BioTyper (Bruker Daltonics, Germany). Routine susceptibility testing was performed pure growth of isolates on solid media using a Vitek-2 automated system (bioMérieux) according to the manufacturer's instructions for the determination of minimum inhibitory concentration (MIC) values. The values were then categorised according to European Committee for