Genomic profiling of plasma cell disorders in a clinical setting: integration of microarray and FISH, after CD138 selection of bone marrow

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ABSTRACT

Aim To evaluate the role of whole genome comparative genomic hybridisation microarray (array-CGH) in detecting genomic imbalances as compared to conventional karyotype (GTG-analysis) or myeloma specific fluorescence in situ hybridisation (FISH) panel in a diagnostic setting for plasma cell dyscrasia (PCD).

Methods A myeloma-specific interphase FISH (i-FISH) panel was carried out on CD138 PC-enriched bone marrow (BM) from 20 patients having BM biopsies for evaluation of PCD. Whole genome array-CGH was performed on reference (control) and neoplastic (test patient) genomic DNA extracted from CD138 PC-enriched BM and analysed.

Results Comparison of techniques demonstrated a much higher detection rate of genomic imbalances using array-CGH. Genomic imbalances were detected in 1, 19 and 20 patients using GTG-analysis, i-FISH and array-CGH, respectively. Genomic rearrangements were detected in one patient using GTG-analysis and seven patients using i-FISH, while none were detected using array-CGH. I-FISH was the most sensitive method for detecting gene rearrangements and GTG-analysis was the least sensitive method overall. All copy number aberrations observed in GTG-analysis were detected using array-CGH and i-FISH.

Conclusions We show that array-CGH performed on CD138-enriched PCs significantly improves the detection of clinically relevant and possibly novel genomic aberrations observed in GTG-analysis, and thus could be considered as a standard diagnostic technique in combination with IGH rearrangement i-FISH.

Genomic imbalances are the predominant genetic abnormality in monoclonal plasma cell disorders (PCD), a group of disorders that include monoclonal gamopathy of undetermined significance, asymptomatic myeloma and symptomatic myeloma (commonly known as multiple myeloma).1

Rearrangements of the Immunoglobulin heavy locus (IGH) gene are a common feature of PCDs and are of prognostic importance.2 3

PCDs have heterogeneous clinical and biological features. Chromosomal abnormalities detected at diagnosis provide important prognostic information, and predict initial response to chemotherapy, remission duration and overall survival. Genetic risk stratification can assist in guiding specific chemotherapeutic interventions, such as bortezomib and high-dose therapy for patients categorised into high-risk groups.4-8 Genetic results are not currently included in the diagnostic criteria for PCDs.4-8

Array-comparative genomic hybridisation (CGH) overcomes the need for dividing PCs for conventional GTG-analysis as well as the highly targeted detection of changes in regions by interphase fluorescent in situ hybridisation (i-FISH). Array-CGH, similar to G-banding, detects regions of chromosomal gain or loss throughout the whole genome. Furthermore, array-CGH allows for the detection of gains and losses of genetic material at a much higher resolution. In this study, we demonstrate the superiority of combining the screening methods of array-CGH and IGH translocation i-FISH in detecting genomic changes in PCDs.

PATIENTS AND METHODS

Patients We studied 20 patients (50% male) with PCD based on WHO criteria.1 Bone marrow (BM) aspirates were collected after informed consent and were selected based on availability of sufficient sample material.

GTG-analysis GTG-analysis was performed on unstimulated BM cells after long-term (72h and 120h) culture. Chromosomes were banded using trypsin digestion pretreatment followed by staining with Giemsa solution. Karyotypes were described according to ISCN 2013.9

Immunomagnetic enrichment of CD138 positive cells PCs were selected to a purity of >90% (as previously tested on a subcohort (results not shown)) by immunomagnetic enrichment of CD138 positive cells using the human CD138 positive selection kit (STEMCELL Technologies Melbourne, Australia). Selected cells were used for both array-CGH and i-FISH.

Interphase fluorescent in situ hybridisation i-FISH was performed on all samples using a mixture of locus-specific probes for 1q21(S100A10)/8p21(PNOC), 11q23(ZBTB16)/13q14(DLEU1) and 17p13(TP53)/19q13.3(CD37) (Kreatech, The Netherlands) and a break-apart probe for 14q32 (IGH). An additional panel of dual fusion probes was applied to the samples that were positive for an IGH rearrangement (4p16(FGFR3)/14q32(IGH), 11q13(MYEV)/14q32(IGH) and 14q32(IGH)/16q23(MAF) (Cytocell, UK). According to the UK Haematology-Oncology Best Practice Guidelines, a minimum of 100 interphase nuclei were scored wherever possible. Cut-off values were set at the levels recommended by the European Myeloma Network.10
Array-CGH
Whole genome microarray was performed using an oligonucleotide array (8×60 k oligonucleotide array, CCMC design) (BlueGnome, UK). Labelling and hybridisation of patient DNA was performed as per the Agilent Oligonucleotide Array-Based CGH for Genomic DNA analysis-Enzymatic protocol user manual. Sex-matched reference DNA supplied by Agilent Technologies was used (Agilent Technologies, USA). Scanning of the array was performed on an Agilent Technologies DNA Microarray Scanner. Analysis was performed using BlueFuse Multi V2.5 software (BlueGnome, UK).

RESULTS
Genomic imbalances and rearrangements were detected in 1 (15%) patient using GTG-analysis, 19 (95%) patients using i-FISH and 20 (100%) patients using array-CGH and the combination of array-CGH and IGH rearrangement i-FISH.

The total number of abnormalities detected using array-CGH was 248 (figure 1). Seventy abnormalities were detected using i-FISH and 14 by GTG-analysis. On integration of array-CGH and IGH rearrangement i-FISH techniques, the detection of genomic imbalances and rearrangements reached a total of 257 aberrations.

Comparison of techniques for discordance
GTG-analysis compared with iFISH and array-CGH
GTG-analysis provided metaphases for all cases, of which 95% (19/20) produced apparently ‘normal’ results (see online supplementary table S1). One case was found to be abnormal in which two clonal populations were identified in a low number of cells (4/51). GTG-analysis detected a rearrangement involving 8q and an unknown partner chromosome. This rearrangement was not completely defined using GTG-banding, but was identified as two derivative 8 chromosomes with the loss of the 8p and a gain of unidentified genetic material (+der(8)t(8;?)(p11.2;?)×2). In this instance, the i-FISH probe located at 8p21(PNOC) identified the loss of this loci in 98% of cells scored, but did not detect a rearrangement. Array-CGH clarified the findings of GTG-analysis and identified the additional material on chromosome 8 as being that of 19p, which included the 19 centromere to produce +der(19)t(8;19)(q11.1;q12)×2. Array-CGH did not detect the rearrangement (see online supplementary table S1).

i-FISH compared with array-CGH
I-FISH detected only 62 copy number aberrations (20% cut-off value) and a total of eight IGH rearrangements (10% cut-off value) in seven cases. Array-CGH identified 241 genomic aberrations at a 1 Mb resolution and a further seven aberrations at the FISH probe loci that were <1 Mb to give a total of 248, of which 69 were whole chromosome changes. The seven copy number changes detected at <1 Mb all involved the IGH variable region at 14q32.3 (see online supplementary table S1).

Three copy number change aberrations detected by i-FISH in greater than 20% of cells were not detected using array-CGH. These aberrations were present in 21%, 23% and 28% of cells in cases 2, 6 and 11, respectively, and constituted 1.33% (3/226) of the total loci tested by i-FISH.

Interestingly, case 10 was identified as having a near-tetraploid clone by i-FISH (see online supplementary table S1). The array software normalised the data at a copy number of four (Log2=0), hence all copy numbers greater or less than four were seen as either a gain or a loss. Near-tetraploid clones generally represent a doubling of the original abnormal clone, hence, all aberrations of the original clone were detected.

Combining IGH rearrangement i-FISH and array-CGH
Predictably, array-CGH analysis did not detect IGH rearrangements. However, i-FISH detected rearrangements in 7/20 cases. Of the 7 cases with an IGH rearrangement, 3 involved MYEOV (11q13), 1 FGFR3(4p16), 1 MAF(16q23) and 2 with an unidentified partner chromosome (see online supplementary table S1).

DISCUSSION
GTG-analysis detection rates in myeloma
GTG-analysis for monoclonal PC disorders is inadequate as only one patient showed an abnormal karyotype (case 8) (see online supplementary table S1). This has also been demonstrated in several other studies and is most likely related to the low proliferation rate of PCs.11–13 The production of abnormal karyotypes has also been associated with disease progression. Studies have indicated that the earlier stages of disease will be less likely to produce abnormal karyotypes as the abnormal PCs are still stromal dependant and are not able to replicate in situ.14–16 This may impact on the availability of dividing PCs for GTG-analysis. Some studies show that stimulation of cultures using various agents such as interleukins, 12-0-tetradecanoylphorbol-13-acetate (TPA), phytohaemagglutinin and granulocyte-macrophage colony-stimulating factor (GM-CSF) may increase the yield of genetically abnormal karyotypes in some cases.17–19

The use of stimulants to increase the production of metaphases
by PCs is one methodology that may have increased the mitotic yield of abnormal PCs.

Interestingly, a cryptic translocation involving chromosome 8q and unidentified genetic material in case 20 was detected using GTG-analysis, and was further classified with array-CGH to show involvement with chromosome 19p and centromeric regions. This was refined from +der(8)(8:?)(p11.12:?)x2 to +der(19?)(8;19)(q11.1;q12)x2. To the best of our knowledge, this t(8;19) appears to be a novel translocation in myeloma and there are no known prognostic associations with a gain of 19p in myeloma (see online supplementary table S1).

**Genetic risk categorisation of monoclonal PC disorders**

Myeloma typically falls into one of two main categories hyperdiploidy or non-hyperdiploidy depending upon the genetic signature of the disease. The hyperdiploid group of myeloma patients (approximately 45% of cases) is associated with standard risk and improved outcome according to the International Myeloma Working Group (IMWG) and Mayo Clinic stratification for myeloma and risk-adapted therapy (mSMART) categorisation.23 24 25 The non-hyperdiploid patients (approximately 40%) generally exhibit a translocation involving IGH and losses of either whole chromosomes or chromosome arms. Risk involved in these patients ranges from standard to intermediate to high, depending largely on the translocation partner of the IGH gene. The remaining 15% of cases exhibit traits of both hyper and non-hyperdiploid categories and their associate risk varies depending on which genetic abnormalities are detected.9 20 21

The ploidy status of a patient is achievable using array-CGH; however, the complete risk categorisation can only be determined when array-CGH is combined with IGH rearrangement i-FISH.3 8 In our cohort, we determined 7 cases to be hyperdiploid, 12 cases non-hyperdiploid and 1 case to be a combination of both based on copy number changes and the IGH rearrangement status in accordance with the IMWG guidelines. The ability to determine ploidy when using a myeloma-specific FISH panel is somewhat limited, as it is unable to determine if the gain or loss is part of a whole chromosome or a segmental change. Used alone, it is an inaccurate tool in assessment of risk based on current IMWG guidelines.

In addition to the mSMART classification, the IMWG reports that some genetic features are considered to be ‘secondary genetic events’ being indicators of disease progression, including deletion of TP53 at 17p13 and abnormalities of chromosome 1, genetic events that some genetic features are considered to be based on current IMWG guidelines.

Change. Used alone, it is an inaccurate tool in assessment of risk however, reports vary as to percentage of cells involved before demonstrated that TP53 deletions are associated with high risk; associated with PCDs and other cancers.28 29 This shattering of chromosomes is not reliably detectable by either GTG-analysis or iFISH, and in some cases may be missed at a 1 Mb resolution. It is expected that this array platform will detect chromothripsis, however, there was no evidence of this in the current cohort of patients to discuss. The current prognostication guidelines set out by the IMWG and the Mayo Clinic do not refer to chromothripsis as this is a very recent finding in genomics. The mechanisms by which it occurs are not yet understood and there has only been one prospective study of a large cohort associating it with patient outcomes in myeloma.30 31 32 33 As this massive genomic instability generally occurs as a secondary genetic event, it can be assumed to be associated with disease progression, however, it is evident that more studies are required to understand the impact of this phenomenon.

**The effect of combining array-CGH with IGH rearrangement i-FISH**

In this study, the use of array-CGH increased the aberration detection rate (248) significantly in contrast with i-FISH (70) and GTG-analysis (14) alone. Upon combining the IGH rearrangement i-FISH results with the array-CGH results the detection rate improved (257) and added significant prognostic information. However, while array-CGH is a superior screening method for the identification of copy number changes in PCDs, IGH rearrangement i-FISH is still needed to provide prognostic information.

When comparing the sensitivity of i-FISH to array-CGH, only three aberrations were not detected by array-CGH. These were relatively close to the 20% cut-off value (21–28%). This may be explained by the scoring of single cells while excluding clumps of cells by i-FISH; on the other hand, the DNA from clumps of cells and singular cells were tested on the microarray. This slight difference in cell population may have changed the ratio of normal to abnormal clonal populations enough to mask the aberrations present on the microarray. The introduction of single-nucleotide polymorphism (SNP)-microarrays may overcome some of the sensitivity problems uncovered here, as low mosaic array-CGH calls may be confirmed by SNP calls. They will also provide information on copy-neutral genomic loss of heterozygosity, which may be of prognostic importance.16 25

We show that array-CGH significantly improves the detection of clinically relevant and possibly novel genomic abnormalities in PCD, and thus could be considered as a diagnostic technique in combination with IGH rearrangement i-FISH. However, further prospective studies analysing the prognostic significance of these array-CGH findings are needed.

**Take home messages**

- Comparative genomic hybridisation (CGH) arrays are a useful and superior screening method for the identification of copy number changes in plasma cell dyscrasia in a clinical setting.
- However, due to the prognostic significance of IGH(14q32) rearrangements, it is highly recommended that array-CGH be complemented with IGH translocation FISH.
- Array-CGH has significantly increased the ability to decipher the complex myeloma genome.

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Competing interests None.

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Data sharing statement An additional cohort of 10 patients was tested to provide information regarding the effectiveness of the CD138 plasma cell enrichment process. The results yielded >90% plasma cell purity as detected by flow cytometry in all 10 cases. This is stated in the methodology text and all data is available upon an email request to the corresponding author: Nadine Berry nadine.berry@hnehealth.nsw.gov.au.

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REFERENCES
13 International Myeloma Working Group Molecular Classification of Multiple Myeloma. 2011 [cited 2013]; 18 Apr 2013), http://myeloma.org/ArticlePage.action?tabId=0&menuId=0&articleId=3069&tab=1&ParentType=nugget&ParentId=188&parentIndexPageId=284