An Interphase Chromosome Profiling Assay to Determine the Need for CD 138 Enrichment in the Genetic Workup of Multiple Myeloma

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Interphase Chromosome Profiling

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INTRODUCTION

Plasma cell neoplasm, a B-cell malignancy is very common in elderly population and is currently incurable despite multiple treatment strategies. Genetic characterization, especially karyotype plays an important role in the diagnosis, prognosis as well as in the follow-up during treatment. In general, plasma cells are non-dividing and don't cooperate in tissue culture and as a result, over 90% of all standard cytogenetic studies end up having a "normal" karyotype. FISH testing using several probes has proven very useful in detecting the clonal abnormalities.

It has been suggested and some laboratories do use CD138 antibodies to enrich the plasma cells in an effort to increase the detection rate of clonal abnormalities. However this additional step adds cost to the overall testing and the efficacy of this enrichment and the clinical utility is somewhat controversial. Many institutions don't enrich the plasma cells and still can detect the clonal abnormalities using FISH probes. It would be of interest if the need for enrichment is clarified and if the results from un-enriched studies are comparable to those of enriched, then the cost savings will be obvious.

FISH testing, while extremely useful in increasing the detection of clonal abnormalities on the "normal" cytogenetic samples, has limitations in the sense that it can only detect the common changes targeted in the panels. Approximately 25% of all abnormal cases do have complex karyotypes harboring changes both numerical as well as structural that are beyond the scope of detection utilizing the current FISH panel of probes. These additional clonal changes have prognostic significance and it is well established that the greater the complexity of the karyotype, the worse is the prognosis. Therefore, it is imperative, from a clinical management standpoint that the testing laboratories use technologies that will detect all chromosomal abnormalities given the dismal culture success rate of traditional cytogenetic methods in detecting the abnormal clones.

Interphase chromosome Profiling (ICP) is a new novel molecular cytogenetic technology which is capable of producing a complete molecular karyotype from interphase nuclei of any tissue. ICP is failure proof and more sensitive than classical cytogenetics and FISH, and it can characterize the marker chromosomes and material of unknown origin in cytogenetics preparations (Cytogenet Genome Res 2014;142:226, Abstract #22; manuscript in preparation).

OBJECTIVES

To test the efficacy of ICP on unenriched samples from multiple myeloma patients to detect clonal abnormalities.

MATERIALS & METHODS

A total of ten patients with multiple myeloma were selected for this study. All ten bone marrow samples had the standard cytogenetics and Multiple myeloma FISH panel testing done. ICP was done on all samples in a blinded fashion. The ICP protocol is as described below.

The Interphase Chromosome Profiling design is based on the equidistant concept of placing the FISH probes along the whole length of the chromosome as depicted in the next section – ICP Illustrations. The total number of bands in any chromosome arm was largely dependent on the overall length of that arm. Each chromosome arm consisted of a minimum of one and a maximum of six bands. Telomeres and centromeres were given pure color band and the interstitial bands were either pure or hybrid color as depicted in the next section. This configuration provides approximately a 600 band resolution and each band on any given chromosome is molecularly distinct from its adjacent band or any other band on that chromosome. Therefore, any deviation of the expected number and/or position of the bands signifies an abnormality. Based on the specific characteristics of an abnormality, it is classified either numerical or structural and further classified into particular category of abnormality.

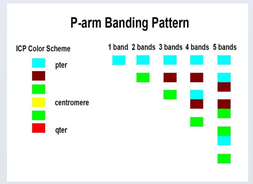
Individual chromosome hybridizations were done on four slides with six areas of hybridization on each slide, as per established standard FISH protocols. Appropriate filter sets were used to detect fluorochromes DEAC, Fluorescein-12, Cyanine555, Cyanine647, and CF594. A minimum of 20 interphase cells were analyzed for each chromosome. Since entire chromosome was profiled as opposed to targeted standard FISH, the usual guidelines of metaphase analysis were followed with minor adjustments in defining the abnormal clone – four cells for both structural and numerical abnormalities.

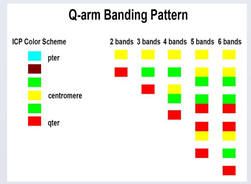
A typical hybridization slide is shown below.

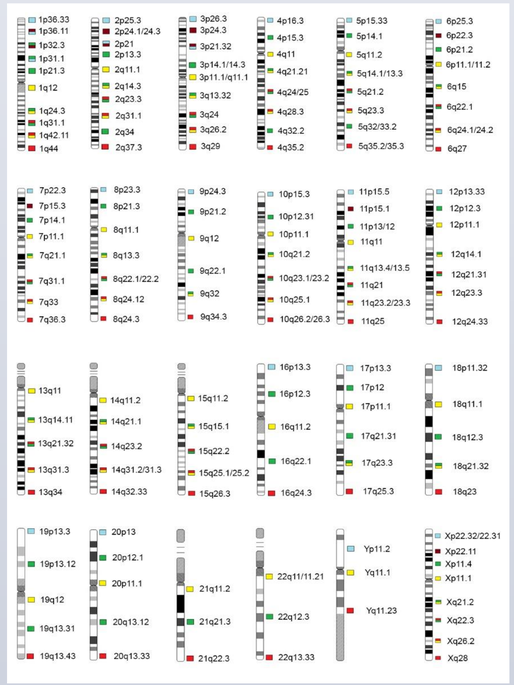
ICP ILLUSTRATIONS

Chr.		Chr.		Chr.	
1	p5/q5	9	p2/q4	17	p2/q4
2	p4/q6	10	p2/q5	18	p1/q4
3	p4/q5	11	p3/q5	19	p2/q3
4	p2/q6	12	p2/q5	20	p2/q3
5	p2/q6	13	p0/q5	21	p0/q3
6	p3/q5	14	p0/q5	22	p0/q3
7	p2/q5	15	p0/q5	X	p3/q5
8	p2/q5	16	p2/q3	Y	p1/q2

Number of bands in each arm of respective chromosomes

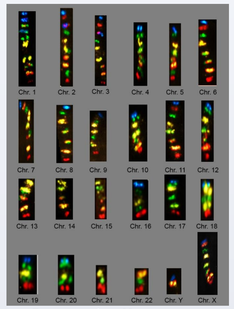




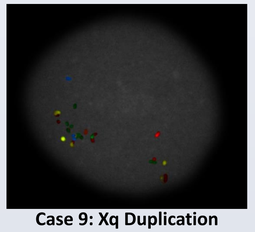


Chromosome Ideograms

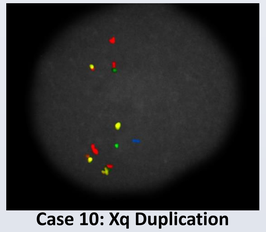
ICP ILLUSTRATIONS (CONT.)



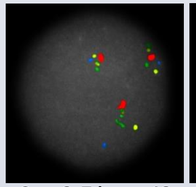
Composite Karyotype



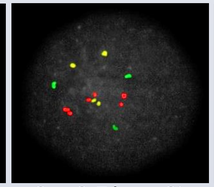
Case 9: Xq Duplication (q21qter)



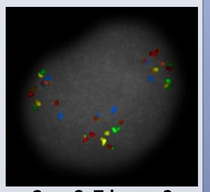
Case 10: Xq Duplication (q21qter)



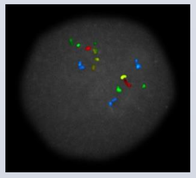
Case 8: Trisomy 18



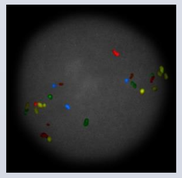
Case 9: Trisomy 21



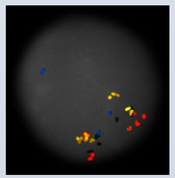
Case 8: Trisomy 3



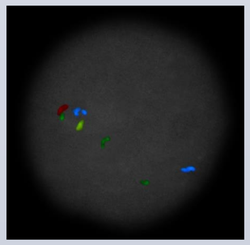
Case 8: Dup 19p



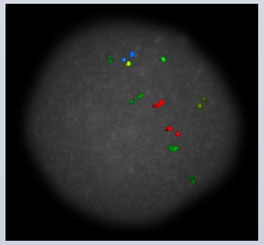
Case 8: Dup 6p



Case 8: Dup 12p



Case 8: Chr. 16q Deletion



Case 10: Chr. 16 Deletion and Duplication

RESULTS

Comparison of ICP Findings with Commonly Used Cytogenetic Methods

Case No.	Cytogenetics/FISH Result	Clonal Abnormalities	ICP Findings
1	Normal	None	Normal
2	Normal	None	Normal
3	Normal	None	Normal
4	Normal	None	Normal
5	Normal	None	Normal
6	Normal	None	Normal
7	Normal	None	Normal
8	53,X,-Y,+del(1)(p13p22),add(2)(p23),+3,del(4)(q31q35),+5,+7, +add(9)(q22),+11,-13,add(15)(q24),+del(15)(q24q26),-16, +add(18)(q23),add(20)(q11.2),add(21)(q22), +mar1, +mar2[cp10]/ 46,XY[10].nuc ish(CDKN2C,CKS1B)x3[99/200],(FGFR3,IGH)x2[199], (D9Z1x3,D15Z4x3~)[105/200],(CCND1x3,IGHx2)[104/200],(RB1x1)[96 /200],(IGHx2,MAFx1)[82/200],(IGHx2,MAFBx1)[101/200], (TP53x3)[10/200]	Trisomy 3, 5, 7, 11	Trisomy 3, 5, 7, 9, 11, 15, 18, Monosomy 13, -Y, Deletion 16q, 20q, 21q, Duplication of 1q and most of 1p, 6p, 12p, 19p
9	56,XY,+2,+3,add(8)(q24.1),+9,+9,+11,+15,del(16)(q22q24),del(17)(p1 1.2p13),+19,+21,+r, +mar[1] /46,XY[19].nuc ish(CDKN2C,CKS1B)x2[200],(FGFR3,IGH)x2[200],(D9Z1x3~5,D15Z4x2 ~3)[3/200],(CCND1x3~4,IGHx2)[24/200],(RB1x2)[200],(IGHx2,MAFx 1)[26/200],(IGH,MAFB)x2[200],(TP53x1)[19/200]	Trisomy 2, 3, 9, 11, 15, 19, 21	Trisomy 2, 3, 5, 9, 11, 15, 19, 21, Duplication of part of Xq
10	44~49,XY,del(1)(p13p22),add(2)(q35),+add(5)(p15),add(6)(q23),+9,+1 1,der(12)t(12;16)(q24.1;q12),t(15;22)(q11.2;q13),- 16,add(20)(q13.1),+21[cp5]/46,XY[14].nuc ish(CDKN2C,CKS1B)x2[200],(FGFR3,IGH)x2[200],(D9Z1x3,D15Z4x2)[4 0/200],(CCND1x3,IGHx2)[38/200],(RB1x2)[200],(IGH,MAF)x2[200],(I GH,MAFB)x2[189],(TP53x2)[200]	Trisomy 9, 11, 21, t(15;22)	Trisomy 5, 9, 11, 19, Duplication of part of Xq, Duplication and deletion of part of 16, t(15;22)

Clonal cytogenetic abnormalities play an important role in the diagnosis and prognosis of multiple myeloma/plasma cell neoplasm. Therefore, it is crucial that clinical laboratories utilize appropriate techniques in identifying these clonal abnormalities. Such efforts include use of CD138 antibody for cell enrichment, use of selected panel of FISH probes since traditional cell culture is unsuccessful in greater than 90% of the cases. The 100% results in this study on 10 un-enriched selected cases of normal and abnormal karyotypes illustrate that ICP is a highly reliable technology.

Besides being concordant with both standard karyotype and regular FISH results, ICP fully characterized and clarified the abnormalities in the karyotypes. The origin of the unidentified material usually referred as "add" in the traditional karyotypes was identified as duplications in all three abnormal cases using ICP as shown in the illustrations. Similarly, what was considered as monosomy and marker by the karyotype approach was easily identified as deletion of part of the chromosome.

Hyperdiploidy, deletions/duplications and specific translocations have prognostic importance in multiple myeloma. Review of the literature indicates that 20% of MM cases have a novel duplication of part of the long arm of chromosome X – dup(X)(q21qter). This part of X chromosome contains Cancer/Testis Antigens (CTAs) belonging to the MAGE family (CTA-X-MAGE). The most commonly cited gene is MAGE C1/CT7 located at Xq26-q27 and overexpression appears to be associated with disease progression. Most importantly, it appears that specific immune response using antibodies against the MAGE C1/CT7 protein can be demonstrated. Thus, this gene is a potential therapeutic target for a subgroup of patients. Yet the widely used current technologies will not detect this important abnormality. ICP detected this aberration in two of the three abnormal cases as shown in the illustrations, demonstrating the higher sensitivity and the utility of the technology.

CONCLUSIONS

Interphase Chromosome Profiling (ICP) is:

- Almost 100% failure-proof.
- More sensitive than currently used techniques – Karyotype and FISH.
- Capable of detecting even low level clonal abnormalities in unenriched samples.

REFERENCES & CONTACT

1) Trask B, Pinkel D (1990): Fluorescence in situ hybridization with DNA probes. Methods Cell Biol 33:388-400.
2) Carvalho FD, Vettore AL, and Colleoni GWB (2012): Cancer/Testis Antigen MAGE-C1/CT7: New Target for Multiple Myeloma Therapy. Clinical Developmental Immunology: Article ID 257695:1-7.
3) Largo C, Saéz B, Alvarez S et al., (2007): Multiple myeloma primary cells show a highly rearranged unbalanced genome with amplifications and homozygous deletions irrespective of the presence of immunoglobulin-related chromosome translocations. Haematologica 92: 795-802.

Dr. Babu is the founder and CEO of InteGen LLC

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