Optimal Strategy for Obtaining Chromosome (CA) and Fluorescence In Situ Hybridization (FISH) Results for Plasma Cell Dyscrasias (PCD) by Using Both Positive and Negative Fractions Of CD138 Enriched Plasma Cells (EPC)

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Abstract

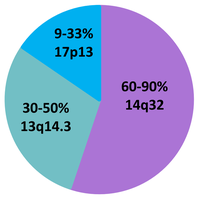
Plasma cell dyscrasias comprise a genetically diverse group of diseases such as monoclonal gammopathy of undetermined significance, plasmacytoma, smoldering myeloma, indolent myeloma, and plasma cell myeloma. FISH is superior to CA in detecting important prognostic genetic abnormalities in PCD; however, its sensitivity is hampered due to paucity of PCs in whole BM and often shows false-negative results when frequency of abnormal cells is below the cut-off values. Studies have shown that the abnormality detection rate in EPCs is greater than unselected plasma cells (UPC), but purification techniques are limiting to only FISH when bone marrow volumes are inadequate. The inability to perform CA may compromise patient care since CA is equally important for detecting non-PC related abnormalities, such as secondary myelodysplastic syndrome, when diagnosis is undefined. To resolve this critical issue and optimize limited quantity received, we designed a pilot study where an immnuno-magnetic CD138 enriched positive selection was used for FISH while the negative selection was used to retrieve other myeloid elements for CA. Parallel FISH studies were performed using UPCs and CD138 EPCs, while karyotyping was achieved using whole BM and discarded myeloid elements. Purity of EPC was confirmed by flow-cytometry (47.3% to 96.9%). Results showed that the abnormality rate of EPC (74%) was doubled compared to UPC (34%) for FISH, and CA displayed 100% (16/16) success rate using the discarded myeloid elements. PCD related chromosome abnormalities were confined to whole BM while non-PCD related abnormalities were found in both whole BM and discarded myeloid elements confirming effective removal of PCs by isolation and efficient utility of the supernatant for CA. Significantly higher frequency of IGH rearrangements (36%) and hyperdiploidy (24%) was observed in the EPC group compared to the UPC group (11% for both). Our results confirm the superior diagnostic potential of selecting PCs and demonstrate the feasibility of using the remaining myeloid elements for CA.

Introduction

- **Plasma cell dyscrasias are hematopoietic neoplasms that are produced as a result of malignant proliferation of a monoclonal population of plasma cells (Pozdnyakova et al, 2009).**
- **Also called Multiple Myeloma (MM), Plasma Cell Myeloma is the most advanced stage of all PCDs.**
- **It is the second most common hematological malignancy in the US comprising approximately 10-15% of all hematopoietic neoplasms (Mailankody et al, 2011; Hartmann et al, 2011; Christensen et al, 2007).**
- **Genomic abnormalities are important prognostic indicators. (Figure 1)**

Genomic Abnormalities

Incidence of Abnormalities Detected by FISH



- **IGH mixed prognosis**
- **RB1 poor prognosis**
- **p53 poor prognosis**

(Slovak et al, 2009)

Figure 1

Detection of Genomic Abnormalities

Detection can be achieved by CA and FISH using whole BM (WBM) samples. CA is restricted due to the slow proliferation rate of plasma cells and limited bone marrow infiltration resulting in 60-70% normal karyotypes and abnormalities may not be detectable until advanced stages.

FISH is superior to CA because it circumvents the need for cell division. It detects abnormalities in earlier stages of disease and can identify more cryptic changes of IGH, RB1 and p53, which is more informative than conventional cytogenetics (Pozdnyakova et al, 2009; Put et al, 2010).

Limitations of FISH using Unselected Plasma Cells (UPC)

Using WBM for FISH has limitations. Non-plasma cells comprise majority of the cell population in BM, so plasma cells may be underrepresented. Even in confirmed cases of PCD, the number of PC ranges from <10% in MGUS to >30% in myeloma. This in turn may show false-negative results when the frequency of abnormal cells is below the laboratory cut-off values (Fonseca et al, 2004; Pozdnyakova et al, 2009).

Limitations of Enriching for PCs (EPC)

Although several studies have shown EPC FISH is superior to UPC, the most significant limitation is sample size. For small samples, most labs prefer FISH on EPCs because of its higher sensitivity while sacrificing CA. The drawback of performing EPC FISH alone is that non-PC related abnormalities remain unidentified which may compromise patient care. Sacrificing CA may also risk accurate diagnosis of therapy-related myeloid malignancies.

Clinical Significance of CA

Secondary AML/MDS has been reported following myeloma for more than 4 decades. Studies show MM patients have an 8-11-fold increased risk of developing AML/MDS. Cytotoxic chemotherapy is known to induce unbalanced translocations involving chromosomes 5 or 7 and del(20q) is a common recurrent aberration in treated myeloma patients resulting from stem cell damage (Mailankody et al, 2008; Papanikolaou et al, 2011).

Materials and Methods

Hypothesis and Study

We hypothesized that EPCs are superior to UPCs by FISH analysis in detecting genomic abnormalities and that the remaining cellular components (RCC) after plasma cell isolation can be used for chromosome analysis to detect non-PC related abnormalities.

Testing the Hypothesis:

Study I: FISH results using UPCs vs. CD138 EPCs
UPC: *57 Samples (January-September 2010)*
EPC: *58 Samples (April-December 2011)*

Study II: Karyotype results using WBM vs. RCC
37 Samples

- **EasySep Human CD138 Selection Kit - *StemCell Technologies* (Figure 2)**
- **GSL10 CytoVision Scanning System – *Leica Microsystems***

Study I (FISH)

To isolate the CD138 enriched plasma cells, whole BM was mixed with Positive Selection Cocktail, then with Magnetic Nanoparticles containing CD138 antibodies. The tube was placed in the magnet and washed with buffer to remove unbound cells. The supernatant comprising the RCC was poured off leaving bound plasma cells inside the tube (StemCell Technologies, 2008).

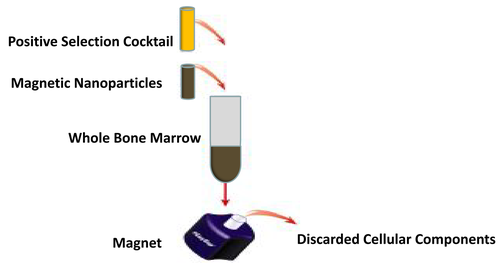


Figure 2

MM FISH Panel

Once isolated, plasma cells are harvested for FISH and hybridized using 5 probes, RB1, IGH/MAF, IGH/FGFR3, ATM and p53. (Figure 3)

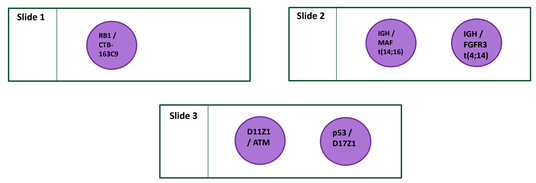


Figure 3

Study II (Karyotyping)

In the second study the negative fraction with RCC was used for CA. The supernatant was poured off but not discarded. It was centrifuged and a 24 hr culture was initiated. (Figure 4)

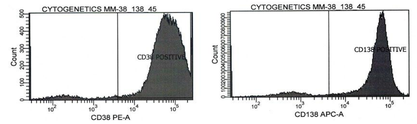
Negative Fraction Containing Discarded Cellular Components (Supernatant)

Figure 4

Results

Flow Cytometry

After isolation, purity of 90.6% CD138+ plasma cells was detected by flow cytometry. (Figure 5)



Tube: 38_138_45

Population	#Events	%Parent	%Total
All Events	30,000	####	100.0
CD38 POSITIVE	27,533	91.8	91.8
CD138 POSITIVE	27,172	90.6	90.6

Figure 5

Study I (FISH)

The top panel shows FISH scores from 2 techs apparently showing hyperdiploidy; 3 signals for ATM and p53 in 19 out of 200 cells. The bottom panel scores from the same patient after isolation of plasma cells demonstrates a significant increase. The frequency of abnormal cells rose by 82.5% . (Figure 6)

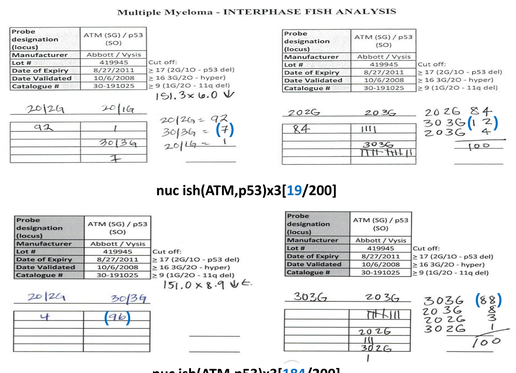


Figure 6

Abnormalities detected increased by ~40%
IGH (14q) abnormalities increased by ~20%
Hyperdiploidy and RB1 (13q) abnormalities increased by ~15% (Figure 7)

UPC vs. EPC FISH cases

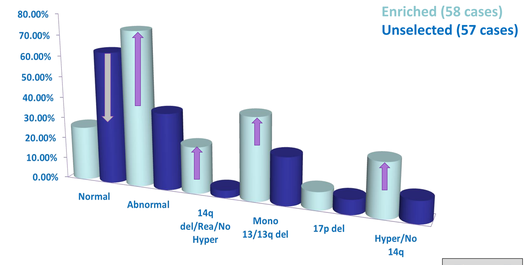


Figure 7

Study II (Karyotyping)

- **CA on the negative fraction was successful in 100% (37) of samples.**
- **Eight cases revealed interesting data with reference to our study using RCC.**
- **Karyotypes with abnormalities of unknown significance and loss of Y chromosome were found in both WBM and RCC. (Table 1, cases 1 & 2)**
- **Karyotypes with abnormalities of myeloid origin and other neoplastic processes were confined to only RCC. (Table 1, cases 3 & 4)**
- **Karyotypes with PCD related aberrations were seen only in the WBM and these aberrations have concordant FISH results on isolated PCs. (Table 1, cases 5, 6, 7 & 8)**

Study II (Karyotyping)

Table 1

Case	Routine Chromosome Analysis Results	WBM CA	RCC CA	EPC FISH Results
1	46,XY,der(9)dup(9)(p13)ins(9) (p11p13)[14]/ 45,idem,-Y [6]	Yes	Yes	Normal
2	45,X,-Y[3]/46,XY[17]	Yes	Yes	Normal
3	52,XX,+5,+add(5)(q31),+7,+del(7)(q22),+19,+19, -20,+22[2]/46,XX [19]	ND	Yes	Normal
4	46,XX,del(13)(q14q22)[10]/46,XX[10]*	ND	Yes	Monosomy 13 (99%)
5	46,XY,t(11;14)(q13.1;q32)[1]/46,XY[19]	Yes	No	IGH rearrangement (69.5%)
6	47~48,XY,dup(1)(q12q42),+add(3)(p11), add(6)(q23),+9,+11,-13,-15,add(17)(p11.2),-18, add(22)(p11.2),+mar1,+mar2,+mar3,+mar4[cp3]/ 46,XY[17]	Yes	No	Trisomy 11 (77.5%) 13q deletion (69.5%) p53 deletion (84%)
7	57~58,X,X,add(1)(p32),+add(1)(p32),+5, +add(6)(q15),+7,-8,-10,add(10)(q22),+11,+15,+19, +21,+21,+21,+mar1,+mar2,+mar3,+mar4[cp3]/ 46,XX[19]	Yes	No	Trisomy 11 (78%)
8	45,X,-Y[9]/45,X,-Y,del(13)(q14q22)[2]/ 46,XY,del(13)(q14q22)[2]/46,XY[9]**	Yes [del(13q),-Y]	Yes (-Y only)	13q deletion (73%)

*** Since the result of the MM EPC FISH study is ABNORMAL and showed a monosomy 13/13q deletion, additional FISH studies with the same RB1,CTB-63C9 probe were performed on the metaphases to determine whether the 13q deletion seen on chromosomes is the same clone as seen in the plasma cells. The results of these studies showed that the deletion observed on chromosomes is different to the one observed on plasma cells and as such may have originated in non-plasma cells. A 13q deletion is a nonspecific abnormality that can occur in either lymphoid or myeloid disorders.**

**** The monosomy 13/13q deletion found only in the WBM CA and confirmed by MM EPC FISH demonstrates effective removal of plasma cells from the RCC leaving only the –Y abnormality found in the RCC CA. Loss of Y is a nonspecific abnormality with unknown clinical significance.**

Conclusions

- **Data from Study I confirms that EPC FISH increases the detection rate of genomic abnormalities.**
- **Inadequate sample size is limiting and the inability to perform CA may compromise patient care since it is equally important for detecting non-PC related abnormalities in diagnostic and post-treatment samples.**
- **Results from Study II support the superior diagnostic potential of selecting PCs and demonstrate the feasibility of using the remaining cellular components (RCC) for CA.**
- **Retrieving the RCC from the negative fraction proves to be an innovative strategy for performing CA.**
- **An algorithm was developed to assist in the initiation process when CA and FISH are requested. Tests performed are based on sample volume as detailed in Figure 8. Any volume less than 1 ml is reduced to CA only to avoid compromising testing.**

Algorithm for Initiation

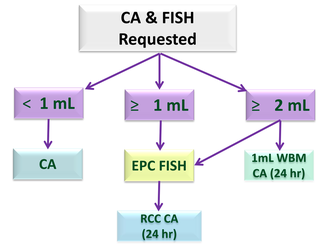


Figure 8

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