

Continued Utility of Chromosome Analysis for Plasma Cell Disorders

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INTRODUCTION

Plasma cell separation (PCS) prior to FISH greatly increases detection of chromosome abnormalities commonly seen in plasma cell disorders. Thus, many laboratories have foregone chromosome analysis due to the superior yield of PCS FISH. However, chromosome analysis still holds value for these disorders as it can detect less common, but still clinically relevant anomalies. To evaluate the efficacy of cytogenetic analyses on samples with indications related to plasma cell neoplasms (PCN), a retrospective review was performed on a consecutive series of samples with orders for oncology chromosome analysis, including indication, previous records, and results. Those with indications relating to PCN were further evaluated.

RESULTS

Included in this analysis were 1511 samples with a PCN-related indication; 40 serial samples were received on 16 patients. Normal cytogenetic results were returned on 84.8% of samples, while ~3% had sex chromosome aneuploidy as the sole finding. Data from the remaining abnormal samples were further evaluated, some of which showed unrelated myeloid or lymphoid clonal abnormalities (Table 1).

Total samples	Unique samples	Abnormality
Myeloid		
17	11	del(20q)
3	3	del(5q)
2	2	-7/7q
2	2	complex
2	2	del(9q)
one each		+8, del(5q) with +21, +21, t(12;17)(q24.3;q21), +del(9q)
Acute Lymphoblastic Leukemia		
2	2	one complex pseudodiploid, one complex hypodiploid
Lymphoid		
5	5	+12
1	1	t(14;18)(q32;q21)
1	1	t(11;18)(q21;q21)
1	1	del(6q)

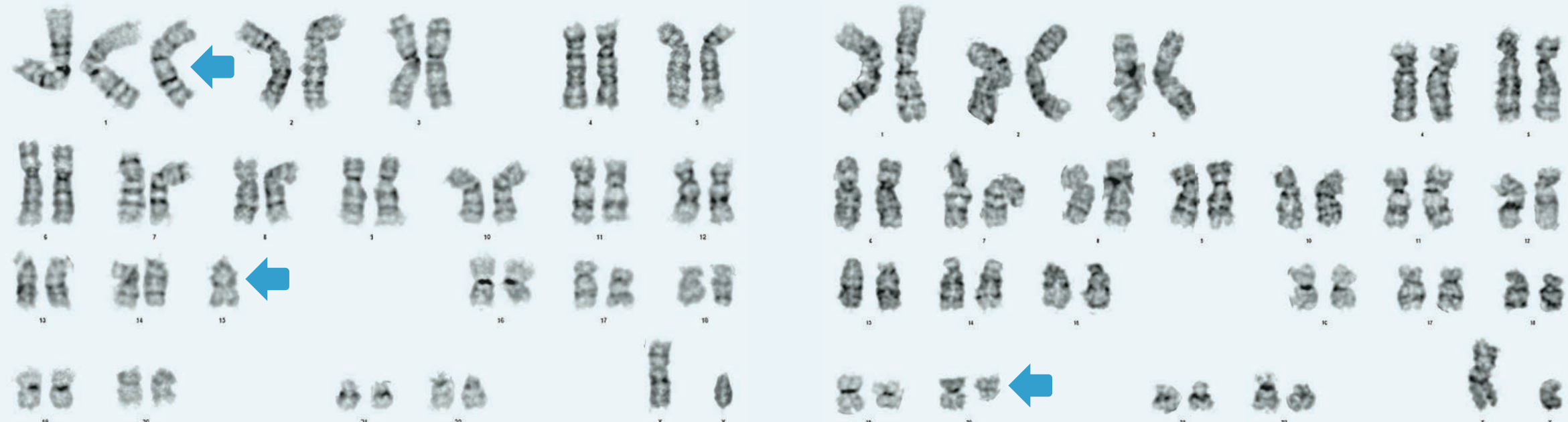
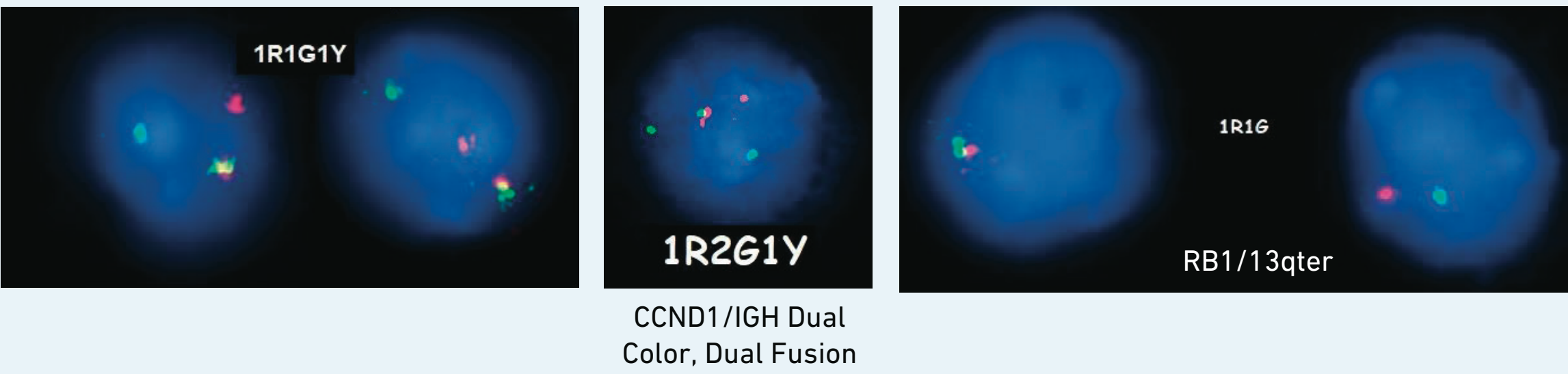
Table 1: Cytogenetic abnormalities observed concurrently with plasma cell disorders

METHODOLOGY

Bone marrow samples with an indication suggestive of a plasma cell neoplasm are processed for plasma cell enrichment by the EasySep™ Human CD138 Positive Selection Kit II (StemCell Technologies) followed by hybridization with a panel of probes targeting common abnormalities in multiple myeloma. Physicians have the option to order an additional panel up front or as a reflex test that targets common IGH rearrangements. The remaining portion of the sample is cultured for chromosome analysis. Typically, a set up includes a 24-hour unstimulated culture and a 120 IL4 stimulated culture with a 72-hour T/L stimulated culture if sufficient sample is available. Harvest, slide dropping and G-banding are all performed with standard cytogenetic techniques. The MDS FISH panel is performed on cells from the 24-hour culture. The Multiple Myeloma FISH panel includes: CDKN2C, CKS1B, D7Z1, CEP9, RB1/13qter, IGH breakapart, SNRPN/15qter, p53/D17Z1. The MDS FISH panel includes: EGR1/D5S630, D7S486/CEP7, KMT2A, p53/D17Z1 and PTPRT/MYBL2.

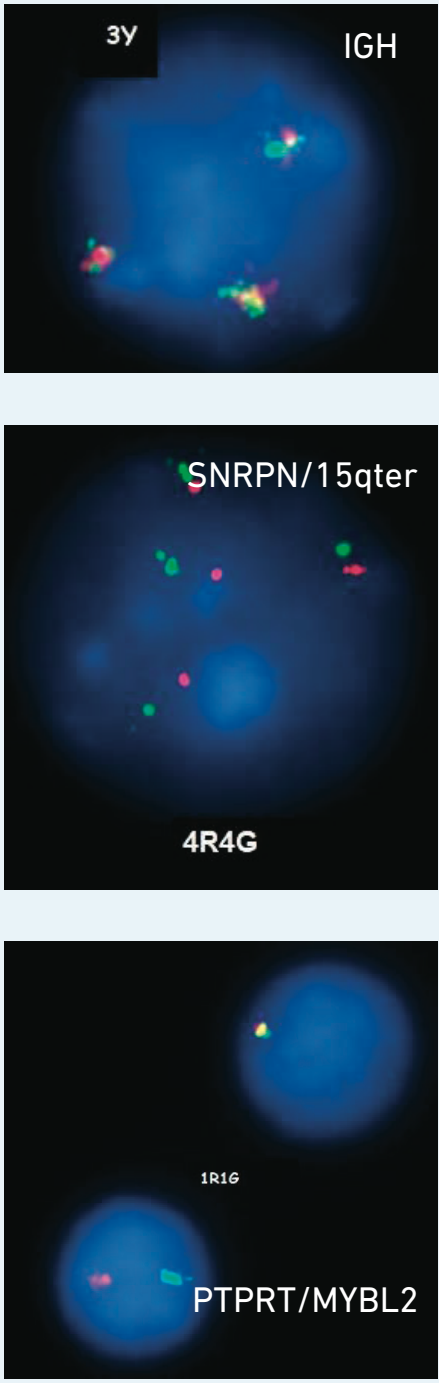
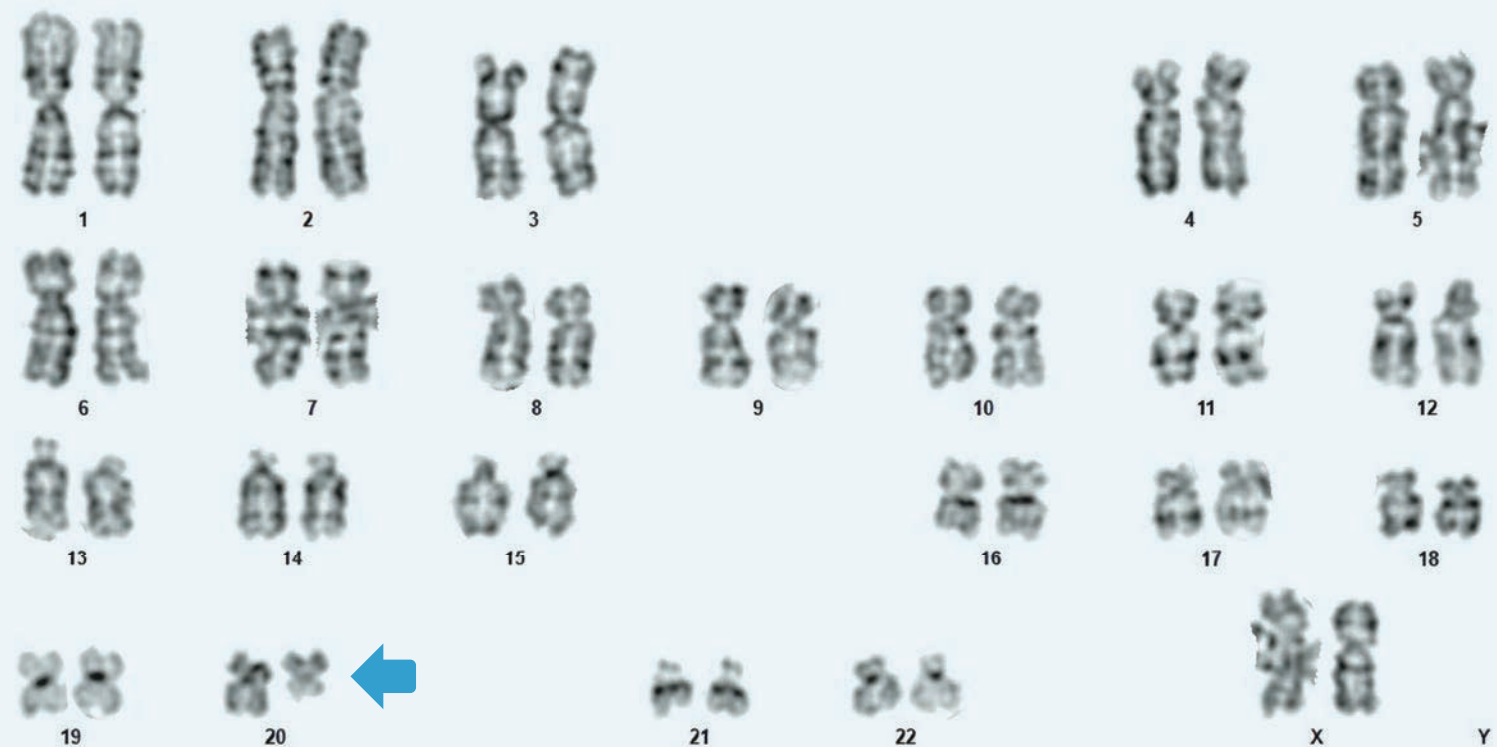
Case 2: Male with new indication of monoclonal gammopathy

- FISH results on PCE: 36% monosomy 13, 49% IGH rearrangement; additional FISH with CCND1/IGH dual color dual fusion showed one fusion, one CCND1 (red) and two IGH (green) signals consistent with an atypical or asymmetric CCND1 rearrangement.
- Chromosome analysis: 46,XY,del(20)(q11.2q13.3)[10]/46,XY,+1,der(1;15)(q10;q10)[3]/46,XY[8]
- The del(20q) cells were found in the 24 hour unstimulated culture; however the +1, der(1;15) cells were found in the 120 IL4 stimulated cultures.
- FISH on plasma cells did not show a gain of CKS1B and thus this clone was not reflective of an abnormal plasma cell clone
- Unbalanced 1q translocations that lead to trisomy 1q are reported in a variety of lymphoid and myeloid neoplasms, including MPN, MDS, AML, and ALL. Specifically, the der(1;15) has been reported as a rare but recurrent finding in hematological malignancies of both lymphoid and myeloid lineage [PMID: 7553585, 18036400, 20513531]. Its impact on prognosis remains unclear.
- Thus this individual had evidence of **at least three neoplastic clones**. The IGH-CCND1 clone identified by FISH, the del(20q) myeloid clone, and the +1, der(1;15) clone of which the origin is unclear.



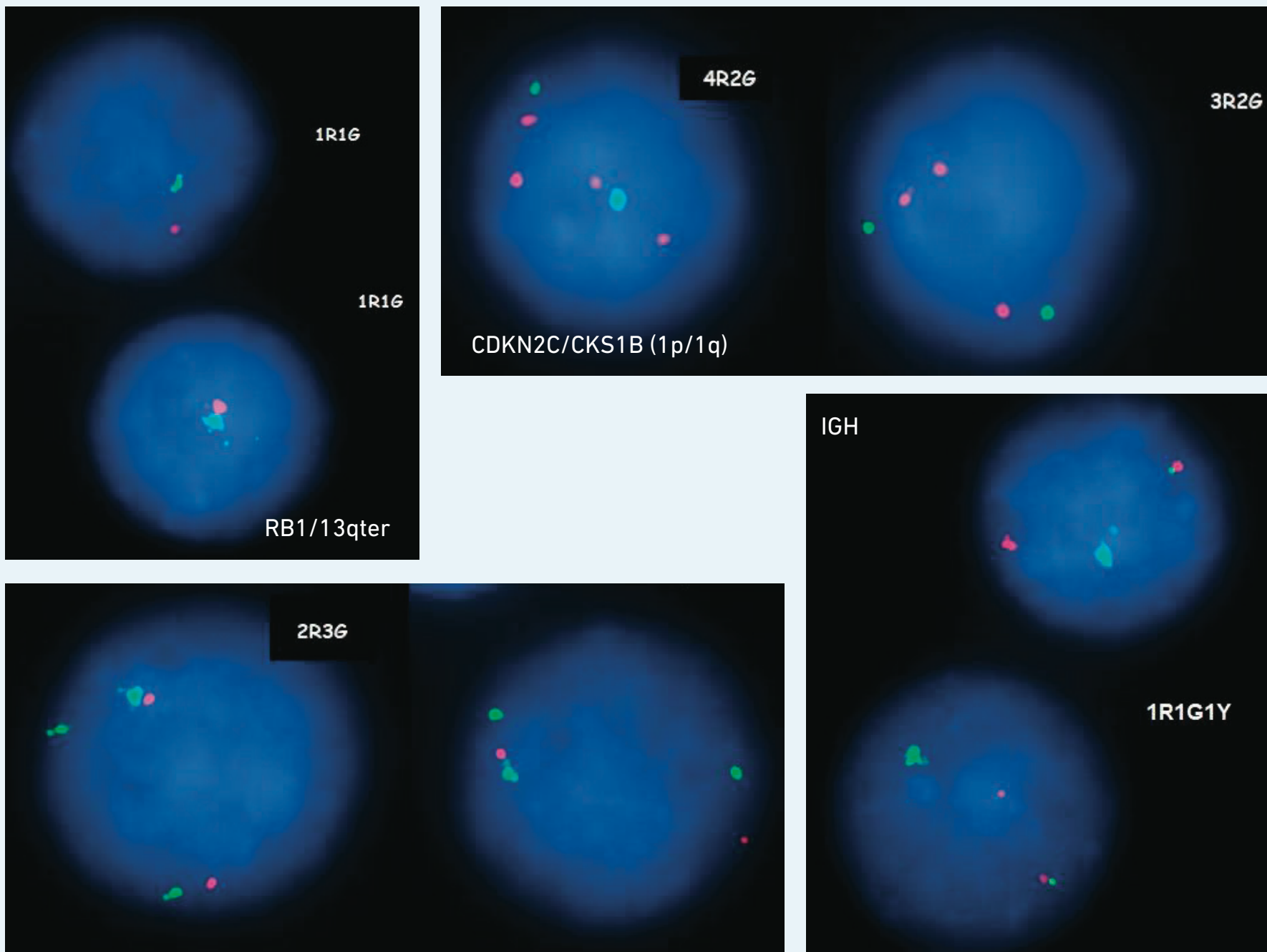
Case 1: Female with dual indications of MDS and plasma cell neoplasm

- MDS FISH results: 80% with 1 red and 1 green signals for PRPTR/MYBL2 probe set; loss of 20q seen in 5 previous samples over 5 year period.
- FISH results on PCE: 12.5% loss of CDKN2C, 72% gain of CEP9, 65% gain of IGH, 98% gain of two copies of 15; 5 previous samples showed gain of 9, 14 and 15. This was the first sample to show loss of 1p.
- Chromosome analysis: 46,XX,del(20)(q11.2q13.3)[16]/46,XX[4]
- Deletion 20q cells seen in both 24 hour unstimulated and 120 IL4 stimulated culture.



Case 3: Female with indication of multiple myeloma

- FISH results on PCE: 6% gain of CKS1B, and 93.5% gain of two copies of CKS1B; 99.5% monosomy 13; 91% IGH rearrangement; 94.5% gain of 15qter without gain of SNRPN
- FISH results on PCE showed FGFR3, CCND1 and MAF were not the translocation partners for IGH
- Chromosome results: 46,X,-X,del(1)(p32p22),+del(3)(p12),add(6)(p21.3),add(7)(p1),t(8;22)(q24.1;11.2),-13,der(14)t(1;14)(q21;p13),der(14)t(14;15)(q32;q24),der(16)t(5;16)(q13;p13.1),+18,+add(19)(q13.4),+21,der(22)t(1;22)(q12;p13)[17]/46,XX[3]
- The abnormal clone was positive for both an IGH and an IGL rearrangement: MYC::IGL and IGH rearranged with an unknown partner. There was a derivative chromosome 14 due to a rearrangement between 14q and 15q; by FISH we know this abnormality resulted in an IGH rearrangement though we could not identify a partner gene for the IGH rearrangement from this analysis.
- Dual immunoglobulin rearrangements are uncommon in MGUS and SMM but have a higher incidence in advanced disease stages and plasma cell leukemia (PMID: 14989251).
- IGK rearrangements are known to be high risk.
- Cases like these would benefit not only from chromosome analysis but optical genome mapping to identify unknown partners.



DISCUSSION

This cohort supports the clinical utility of chromosome analysis for assessment of plasma cell disorders even when FISH is abnormal. Chromosome analysis allows for reporting of additional clinically significant information such as complexity in abnormal clones detected by FISH or constitutional findings that might have implications for the patient or family members. A dual testing approach of FISH and chromosome analysis provides both high diagnostic yield for common PCN-related cytogenetic findings while capturing rarer ones with additional clinical significance. Correlation of the additional data with treatment modalities and outcomes may also contribute to better stratification of patients in the future.