A rare case of *IGH/MYC* and *IGH/BCL2* double hit primary plasma cell leukemia

Double hit B-cell lymphomas with concurrent MYC and BCL2 rearrangements are well described and are associated with a rapidly progressive clinical course and dismal prognosis. Cases of double hit myeloma, however, are rare, and there are no reported cases of double hit plasma cell leukemia (PCL). Here, we describe the case of an aggressive plasma cell leukemia characterized by simultaneous IGH/MYC and IGH/BCL2 translocations. Two new cell lines have subsequently been derived from this case.

A 42-year old female was referred to hospital with a one-week history of blurred vision and bone pain on a background of three months of weight loss and lethargy. Physical examination was remarkable for a proptosed left eye and moderate hepatosplenomegaly.

Laboratory evaluation demonstrated moderate anemia (Hb 93 g/L), leukocytosis with plasmacytosis (WCC 23.10x10^9/L, circulating plasma cells 10.4x10^9/L, neutrophils 6.01x10^9/L) and moderate thrombocytopenia (platelets 68x10^9/L).

Serum protein electrophoresis revealed hypogammaglobulinemia. Serum kappa free light chains were elevated at 136.2 mg/L (normal range 3.3-19.4 mg/L), Lambda free light chain 1.0 mg/L (normal range 5.7-26.3 mg/L), ratio 136.20 (normal ratio 0.26-1.65). Monoclonal kappa free light chains were detected on urine protein electrophoresis. β2 microglobulin was 9.1 mg/L. Renal function and serum calcium were normal. Liver function testing demonstrated a predominantly cholestatic picture: GGT 556 U/L (normal <38), ALP 139 U/L (normal 36-106), ALT 76 U/L (normal 9-36). Bilirubin was normal. There was evidence of spontaneous biochemical tumor lysis: markedly elevated LDH 6540 U/L (normal range 125-255 U/L) with hyperuricemia (uric acid 0.61 mmol/L; normal range 0.11-0.36 mmol/L) and hyperphosphatemia (1.37mmol/L; normal range 0.6-1.3 mmol/L).

Computed tomography of the orbits revealed an 18x31x34 mm homogenously enhancing soft tissue mass in the superior left orbit.

Bone marrow biopsy demonstrated complete replacement of normal hematopoietic tissue with immature plasmablasts. Immunophenotypic analysis by flow cytometry utlizing the EuroFlow MM MRD panel[1] confirmed clonal plasma cells (CD38$^+$, CD138$^+$, CD45$^+$, CD56$^-$, CD19$^-$, CD27$^-$, CD117$^-$, CD81$^-$ and Kappa$^+$). Immunohistochemical studies on trephine sections were positive for CD138, Ki67 (60%), c-MYC and BCL2.

Conventional cytogenetics (G-banding) was performed according to standard protocols and reported using the ISCN 2013 nomenclature.[2] Multicolor FISH (M-FISH) and locus-specific probe FISH, using XCyte 24 probes (Metasystems), ON MYC/IGH t(8;14) dual fusion translocation probe (Kreatech) and LSI IGH/BCL2 t(14;18) dual color dual fusion translocation probe (Vysis) were performed according to the manufacturers' instructions and revealed the following complex karyotype (Figure 1):

45,X,-X,der(1;8)(8pter->8q24::18q21->18q23::1p?12->1qter),

der(14)t(8;14)(q24;q32)x2,der(18)t(14;18)(q32;q21)[30].

ishder(1;8)(MYC+,IGH+;IGH+,BCL2+),der(14)t(8;14)(IGH+,MYC+),

der(18)t(14;18)(BCL2+,IGH+)[30].

Overall, the findings were consistent with a diagnosis of primary plasma cell leukemia (pPCL). The patient was

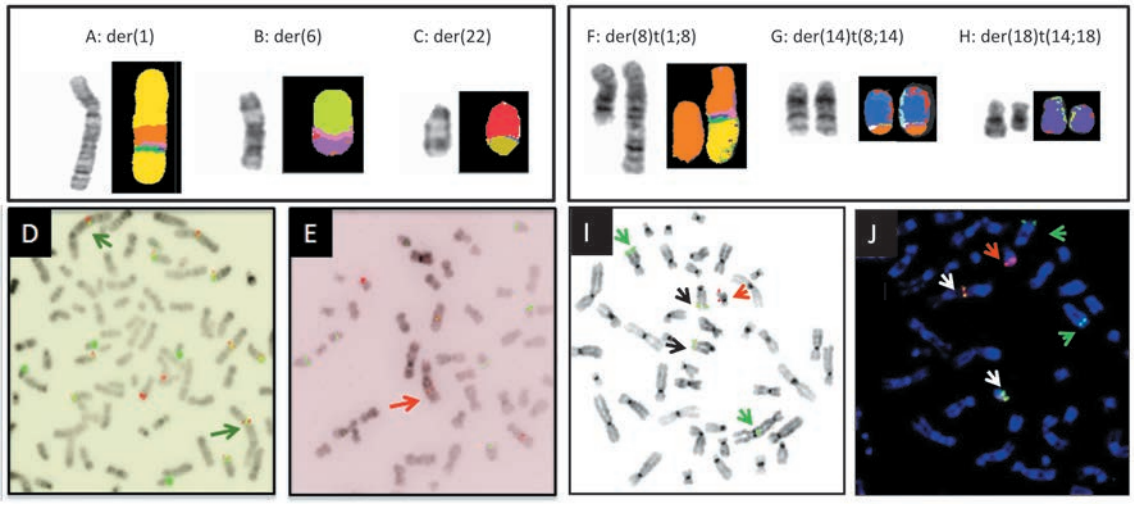

Figure 1. Patient's karyotype. ALF-1 cells are hypotetraploidy with der(1) (A), der(6) (B) and der(22) (C). The green arrows are MYC-IGH fusion signals on the der(1) (D) while the red arrow points to IGH-BCL2 fusion signals on the der(1) (E). ALF-2 cells analyzed were hypodiploid with loss of one X chromosome and unbalanced 1;8 (F), 8;14 (G) and 14;18 translocations (H). (Top) Normal chromosomes 8, 14 and 18 are shown on the left of each chromosome pair with G-banding and M-FISH representations of each derivative chromosome. M-FISH showed that the unbalanced 1;8 translocation also contained chromosome 18 sequences but did not identify any chromosome 14 sequences on the der(1;8) despite the presence of an IGH signal, reflecting the proximity of IGH to the 14q telomere and the lack of sensitivity of M-FISH for the telomeric regions of chromosomes (F). FISH with reverse DAPI banding (I) identified an MYC-IGH fusion signal on the der(1;8) (Bottom, green arrow) and also on both copies of chromosome 14 (black arrows), indicating that there had been a doubling of the derivative 14 of the t(8;14). The top green arrow indicated an MYC signal on the normal chromosome 8 and the red arrow indicated a diminished IGH signal on the der(18)t(14;18) (J). There was also an IGH-BCL2 fusion on the der(1;8) (top white arrow) and a second fusion signal on the derivative chromosome 18 (Bottom, white arrow). The green arrows also indicated IGH signals on the two der(14)t(8;14) and the red arrow indicated a BCL2 signal on one normal chromosome 18.

administered intravenous dexamethasone 40 mg and supportive therapy, which consisted of hydration and rasburicase and allopurinol. However, within 36 h of presentation, rapid clinical deterioration ensued. The patient developed multi-organ failure including acute liver failure causing lactic acidosis (pH 6.9). WCC increased to 123.4x10^9/L, circulating plasma cells 102.82x10^9/L, along with evidence of worsening tumor lysis. Despite maximal inotropic support and hemodiafiltration, acidemia continued to worsen and the patient died within 48 h of initial presentation.

Plasma cell leukemia is rare and the most aggressive variant of multiple myeloma (MM), representing 1%-2% of MM cases at diagnosis.[3] PCL is defined as circulating peripheral blood plasma cells exceeding 2x10^9/L and plasma cells comprising 20% or more of peripheral blood white cells. Prognosis of primary PCL (pPCL) is poor with a median survival of 15 months for patients treated with novel agents.[4] The molecular basis of PCL is less well understood than MM,[5] with reported data based largely on retrospective studies. In contrast to MM, where 60% of cases are characterized by chromosomal gains, more than 80% of patients with PCL have hypodiploid or diploid cells.[5] Chromosome 13 deletion and monosomy are frequent abnormalities. The frequencies of IgH (14q32) translocations by FISH are also high, reported in up to 87% of cases of pPCL, and in a Mayo Clinic study the frequency of t(11;14) in pPCL was 71%.[6] In contrast to the relatively favorable prognosis conferred by the presence of t(11;14) in MM, the high prevalence in pPCL when coupled with high-risk cytogenetics, such as loss of p53, confers a poor prognosis (p53 loss due to mutation or deletion is observed in 56% of pPCL). Only 15% of pPCL have an MYC translocation; Tiedemann *et al.*, found that MYC rearrangements predicted for shorter overall survival.[6]

Double hit B-cell neoplasms are defined by a chromosomal breakpoint affecting MYC/8q24 locus in combination with another recurrent breakpoint, generally a t(14;18)(q32;q21) involving BCL2 (other oncogenes implicated are BCL6 and CCND1). Double hit B-cell lymphomas are well characterized.[7] However, cases of double hit MM are rare, with only 5 cases reported in the literature[8] and none of double hit PCL.

In this present case of double hit pPCL, the sequence of events cannot be established with certainty but results suggested that the t(14;18) occurred prior to the t(8;14). After a reciprocal t(14;18), a second translocation involving 8q24 and *IGH* on the derivative 14, with the breakpoint proximal to the *IGH-MYC* fusion, resulted in the co-location of *MYC*, *IGH* and *BCL2* signals on the der(8) and an *IGH-MYC* fusion on the der(14). There was a subsequent doubling of the der(14), loss of the normal 14 and an extra copy of 1q translocated onto the chromosome 18 sequences on the der(8). The net result was the t(14;18) and two copies of the der(14)t(8;14). The t(14;18) has only been reported in a handful of MM cases and only once with a simultaneous t(8;14).[9]

The rapid demise of this patient reflects the synergistic actions of the MYC and BCL2 oncogenes and is in contrast to 2 other reported cases of double hit MM: a case of IGH/MYC and IGH/BCL2 with plasmablastic morphology surviving eight months from diagnosis,[9] and a case of IGH/MYC and IGH/CCND1 with plasmacytic morphology surviving 18 months from diagnosis.[8]

Two human myeloma cell lines have been propagated from the peripheral blood and bone marrow aspirate collected at time of presentation (ALF-1: derived from peripheral blood; ALF-2: derived from bone marrow). Karyotypic analyses utilizing M-FISH and G-banding show ALF-1 karyotype to be similar to ALF-2 but with additional numerical and structural changes (Figure 1), including a gain of 1q, all abnormalities acquired typically during disease evolution and a feature of progressive MM. In addition to karyotypic differences, these cell lines demonstrate further biological heterogeneity relating to the interaction between the MM cells and the bone marrow microenvironment.

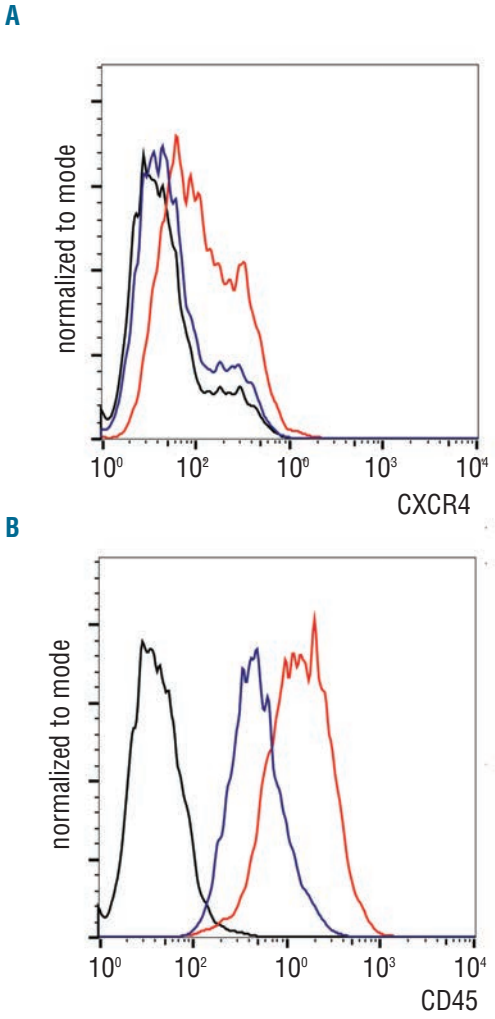

Figure 2. Flow cytometric analysis of the myeloma cell lines: ALF-1 (peripheral blood) and ALF-2 (bone marrow). Cell surface expression of CXCR4 and CD45 were analyzed by flow cytometry using commercially available conjugated antibodies (BD Biosciences). ALF-1 and ALF-2 were cultured from the peripheral blood and bone marrow aspirate, respectively. ALF-1 had lower levels of CXCR4 expression (A), consistent with its key roles in metastasis, tumor cell extravasation, and dissemination from the primary tumor site; and lower levels of CD45 expression (B) compared to ALF-2 which is associated with disease progression and adverse clinical outcome.

Protein expression of CXCR4 by flow cytometry, a critical regulator of MM cell migration and homing to the bone marrow through interaction with its ligand SDF-1a,[10] was reduced in ALF-1 compared with ALF-2 (Figure 2A.). Reduced CXCR4 expression has been shown to be associated with extramedullary disease in both a mouse model of MM[11] and in primary patient samples,[12] as well as predicting for inferior survival in MM patients treated with bortezomib.[11] Interestingly, Singh *et al.*[12] hypothesized that this was likely limited to a subpopulation of neoplastic cells,

which is demonstrated in this case. CD45 expression by flow cytometry was higher in ALF-2 (CD45++, intermediate) compared to ALF-1 (CD45$^+$, dim) (Figure 2B.). Kumar *et al.*[13] report that predominance of CD45- PCs in BM indicates a late stage of disease and portends a less favorable prognosis. In a murine myeloma model, 5T2MM, Asosingh *et al.*[14] demonstrated that at pre-angiogenic phase, MM cells consisted mainly of CD45$^+$ cells. With disease progression, a process of ongoing differentiation of CD45$^+$ MM cells to CD45$^-$ MM cells occurs till the end of the pre-angiogenic stage at which most of the MM cells were CD45-. In this case study, the decline of CD45 and CXCR4 expression in the peripheral blood-derived cell line (ALF-1) compared to the bone marrow-derived cell line (ALF-2) suggests these switches are induced, and are in concert with solid tumorigenesis, indicating the occurrence of metastasis.

This unique case represents an extreme presentation of primary plasma cell leukemia, novel firstly in terms of the simultaneous double hit IGH/MYC and IGH/BCL2 translocations identified. Secondly, the biological heterogeneity of the two propagated cell lines recapitulate earlier published observations/hypotheses regarding medullary *versus* extramedullary disease, highlighting in particular the 'metastatic' phenotype of the circulating plasma cells.

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