1 **A novel t(1;9)(p36;p24.1) *JAK2* translocation and review of the literature**

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12 Short Title: *JAK2* Translocations

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26 **1. Abstract**

27 The *JAK2*V617F point mutation has been implicated in the pathogenesis of the vast majority of
28 myeloproliferative neoplasms (MPNs), but translocations involving *JAK2* have increasingly been
29 identified in a subset of patients with *JAK2*V617F-*negative* MPNs. Here we present a case of a patient
30 diagnosed with *JAK2*V617F-*negative* polycythemia vera (PV) that transformed to MPN-blast phase
31 (MPN-BP). Cytogenetic and FISH analysis revealed a novel translocation of t(1;9)(p36;p24.1) and a
32 *PEX14-JAK2* gene fusion, as a result of the translocation, was identified. The t(1;9)(p36;p24.1) has not
33 been previously described and represents a new addition to the list of known translocations involving
34 *JAK2* that have been identified in hematologic malignancies. Although the prognostic and treatment
35 implications of *JAK2* translocations in MPNs is not yet clear, positive outcomes have been described in
36 early case reports of the use of JAK inhibitors in these patients. Further research into the role of *JAK2*
37 translocations in the pathogenesis and outcomes of hematologic malignancies is warranted.

38 **2. Introduction**

39 The Janus Kinase (JAK) proteins are a family of cytoplasmic tyrosine kinases involved in the JAK-STAT
40 signaling pathway and are essential in maintaining normal hematopoiesis. The *JAK2* gene, located on
41 chromosome 9p24, encodes for a receptor predominantly responsive to type I cytokine ligands,
42 including erythropoietin (EPO), thrombopoietin (TPO) and granulocyte-macrophage colony-stimulating
43 factor (GM-CSF). Ligand binding to JAK2 leads to autophosphorylation and activation of signal
44 transducers and activators of transcription (STAT) proteins, which mediate the expression of genes
45 involved in hematopoietic cell production [1].

46

47 Constitutive activation of the JAK-STAT pathway through the acquired point activating mutation on
48 exon 14 (*JAK2*V617F) has been implicated in the pathogenesis of myeloproliferative neoplasms
49 (MPNs). This mutation is present in 98% of polycythemia vera (PV), 40-50% of essential
50 thrombocythemia (ET) and 50-60% of primary myelofibrosis (PMF) [2,3], and is present in 20% of
51 patients with non-classical MPNs [4]. Additionally, a subset of patients with *JAK2*V617F-*negative* MPNs
52 is found to have translocations involving *JAK2* which result in gene fusion products that lead to *JAK2*
53 amplification or constitutive activation of the tyrosine kinase. These translocations are not limited to
54 the MPNs, but have also been identified in *de novo* leukemias of both myeloid and lymphoid lineages
55 (Table 2).

56

57 Here we describe a case of a patient with *JAK2*V617F-negative PV who was found to have a novel *JAK2*
58 translocation, not previously described. We also provide a review of the known *JAK2* translocations
59 associated with PV and other MPNs.

60

61 **Case Report**

62 A 52 year old woman initially presented in 2008 with symptoms of headaches, dizziness, fatigue,
63 shortness of breath and numbness and tingling of the hands and feet and was found to have a
64 hematocrit (HCT) of 61% with normal white blood count (WBC) and platelet (PLT) count. The bone
65 marrow biopsy showed a hypercellular marrow (95%) with increased megakaryocytes in clusters,
66 without reticulin staining. The patient was found to meet World Health Organization's (WHO) diagnostic
67 criteria for *JAK2*V617F mutation negative PV and was initiated on treatment with aspirin and therapeutic
68 phlebotomy, to maintain the Hct <42%.

69

70 A year later, the patient developed leukocytosis with a WBC 30 $x10^9$/L and thrombocytosis with a PLT
71 count of 600,000/L. She was started on hydroxyurea at a dose of 500 mg daily, with subsequent
72 improvement in leukocytosis and thrombocytosis, but developed treatment emergent anemia. Repeat
73 bone marrow biopsy and aspirate at that time showed a hypercellular marrow (100%) with trilineage
74 hematopoiesis, marked granulocytic hyperplasia, increased immature forms, with markedly increased
75 eosinophils (31%) and 7% blasts. Peripheral blood flow cytometry showed a small CD33+ and CD34+
76 myeloblast population (1.4%).

77

78 Several months following initiation of hydroxyurea, the patient developed constitutional symptoms. The
79 WBC was found to be elevated to 72x 10^9/L and peripheral blasts were 21%, consistent with
80 transformation to MPN-blast phase (MPN-BP), a form of secondary acute myeloid leukemia (AML).

81 Decitabine was initiated. After receiving 4 cycles of decitabine, the patient underwent hematopoietic
82 stem cell transplantation (HSCT) in August 2010 with cells from a 10/10 matched unrelated donor, with
83 successful engraftment. Post-transplant bone marrow biopsy, however, showed persistent
84 hypercellularity (80-90%) and persistent myeloblast populations ranging from 1.1% to 8%, consistent
85 with a residual disease. Unfortunately, the patient's post-transplant course was complicated by graft-
86 versus-host disease of the gastrointestinal system and central nervous system, and ultimately the
87 patient succumbed to gram-negative sepsis on day +106 post-transplant.

88

89 3. Materials & Methods

90 Cytogenetic and FISH Analysis

91 Conventional cytogenetic preparations and fluorescent in situ hybridization (FISH) analyses were
92 performed as described previously [5, 6]. To detect the exact region involved in the novel translocation
93 12 FISH probes were used: 9p24.1 (RP11-3H3), 9p24.1 (RP11-28A9)(BACPAC Resources Oakland, CA,
94 USA), 1p36.22 (RP11-483P2), 1p36.22 (RP11-1107P2), RP11-1134M20, RP11-483P2, RP11-1107P2
95 (Empire Genomics, Buffalo, NY, USA), 9q34 (ABL1), 22q11.2 (BCR), 1p36 (p58), 1p36 (CEP108/T7), 1q25,
96 WCP19, telomere 1p, telomere 19p (Abbott Molecular, Abbott Park, IL, USA). FISH chimerism was
97 detected using XX/XY probes (Abbott Molecular). All FISH probes, including BACs, were fluorescently
98 labeled by the manufacturer excluding RP11-3H3 and RP11-28A9, these BACs were fluorescently labeled
99 using the Nick translation kit (Abbott Molecular) following the manufacturer's' procedure [5]. DNA cel
100 isolated from bone marrow was subjected to whole genome sequencing (WGS) and analyzed by
101 standard pipelines at the Sanger Institute, Hinxton, UK [7].

102 Confirmation of a *PEX14-JAK2* genomic DNA fusion was performed using a forward primer in *PEX14* exon
103 8 (5'CCACCAACTGGATCCTGGAGT) and reverse primer in *JAK2* exon 19 (5'AACCCCAGGGCACCTATCCT),
104 on 25ng DNA using the Expand Long-Template LT-PCR system 2 (Roche, Burgess Hill, UK) at an annealing
105 temperature of 64°C and an elongation time of 4 min. Sequencing across the breakpoint was performed
106 using primer *PEX14* exon 9 (5'GTTCCCTCCATCCCCATCAG), using an Applied Biosystems 3130 (Foster City,
107 CA, USA).

108

109 The presence of *PEX14-JAK2* fusion mRNA was confirmed on random hexamer reverse transcribed
110 cDNA, using forward primer *PEX14* exon 8 and a reverse primer in *JAK2* exon 21 (5'
111 TTTTAGATTACGCCGACCAGCA) using the Expand High Fidelity PCR System, an annealing temperature of
112 64°C and an elongation time of 1 min. The product was sequenced in both directions using the same
113 primers.

114

115 **4. Results**

116 Summary of conventional cytogenetic analysis is shown in Table 1. Initial bone marrow cytogenetic
117 analysis, a year after the diagnosis of PV, showed 50% of evaluated metaphase cells to have
118 t(1;9)(p36;p24.1) karyotype. Subsequent metaphase FISH analyses using bacterial artificial chromosome
119 (BAC) FISH probes (RP11-3H3 and RP11-28A9) revealed that the 3' portion of *JAK2* was translocated to
120 1p36 while the 5' portion remained on 9p24.1, indicating a JAK2 structural rearrangement (Figure 1). To
121 investigate the exact breakpoint on chromosome 1, we used a FISH BAC probe, and as shown in Figure 1,
122 BAC FISH probe RP11-4832P2 normally localized on chromosome 1p36 was detected on 9p24.1 (aqua)
123 whereas BAC RP11-1107P2 remained on 1p36. Therefore, the breakpoint on chromosome 1, involved in
124 the *JAK2* translocation, was determined to be within band p36.22 on chromosome 1 [7].

125

126 Five months from the initial analysis, 100% of cells had t(1;9) and 10% developed a subclone consisting
127 of balanced t(7;17)(q22.1;25.3) and trisomy 1q in the form of unbalanced der(15)t(1;15)(q12;q26).
128 Following HSCT, the host cells were never eradicated (Table 1). A year after the initial cytogenetic
129 analysis, the original abnormal host clone and a subclone were present in 100% of cells with additional
130 chromosomal abnormalities consistent with complex subclonal evolution.

131

132 To characterize the t(1;9) in detail, we performed WGS analysis of patient bone marrow DNA to identify
133 the translocation breakpoints. Focusing on the analysis of *JAK2*, we identified two split reads that
134 mapped to *PEX14* exon 9 and *JAK2* intron 18. *PEX14* maps to 1p36.22, and was thus a strong candidate
135 to be fused to *JAK2*. To confirm a *PEX14-JAK2* fusion, we amplified patient and control DNA using
136 primers located in *PEX14* exon 8 and *JAK2* exon 19. A product was obtained from the t(1;9) case only
137 (not shown), which, upon sequencing, confirmed a break within *PEX14* exon 9 and *JAK2* intron 18 (Figure
138 2). Amplification from cDNA also yielded a specific product from the t(1;9) case but not controls (Figure
139 3), sequencing of which showed a fusion between a truncated *PEX14* exon 9 and *JAK2* exon 19 (Figure
140 2). Comparison of the cDNA and genomic sequences indicates that two nucleotides (TA) from *JAK2*
141 intron 8 were retained in the mature mRNA that result in maintenance of the correct reading frame
142 (Figure 3). The TA dinucleotide is immediately followed by GT which must have acted as a splice donor
143 site.

144

145 **Table 1: Results: Summary of Cytogenetic and FISH results**

146 (attached)

147

148 **5. Discussion**

149 The most common *JAK2* abnormality identified in MPNs is the *JAK2*V617F, which is seen in the vast
150 majority of patients with PV, as well as approximately half of the cases of ET and PMF. Subsequent to
151 the 2005 discovery of the acquired somatic point activating mutation by four independent groups,
152 knowledge of the genetic underpinnings of MPNs increased exponentially, along with information on
153 the role that mutations play in diagnosis, prognosis and therapeutic approach [8, 9, 10, 11].

154

155 In stark contrast to this wealth of knowledge about the *JAK2*V617F-positive MPNs, there is a relative
156 dearth of information on how to approach the small subset of patients with MPNs who lack this point
157 mutation, but contain *JAK2* translocations at 9p24.1.

158

159 Translocations at 9p24.1 and the resultant gene fusion products have been identified in a wide spectrum
160 of hematologic malignancies of both myeloid and lymphoid origin, including acute lymphoblastic
161 leukemia (ALL), chronic myelogenous leukemia (CML), chronic eosinophilic lymphoma (CEL), and the
162 *BCR-ABL1* negative MPNs (Table 2). In addition to this, several translocations involving 9p24.1 with
163 subsequent gene fusion products involving *JAK2* have been seen in solid malignancies, such as breast
164 cancer [12] and small cell lung cancer [13].

165

166 It is likely that *JAK2* translocations are more common in hematologic malignancies than previously
167 recognized. In a 2010 publication in *European Journal of Haematology*, Patnaik and colleagues screened
168 over twenty four thousand patient cytogenetic reports and found five patients harboring translocations
169 at 9p24 with gene fusion products involving *JAK2* [3]. All five of the translocations described in this
170 subset of patients had not previously been reported in the literature until this 2010 publication:
171 t(8;9)(q22;p24), t(9;17)(p24;q23), t(4;9)(q25;p24), t(2;9)(p21;p24), and t(8;9)(q13;p24). Four of the five
172 patients carried diagnoses of *JAK2*V617F-positive MPNs (PMF and PV), and the patient with
173 t(8;9)(q13;p24) was diagnosed with diffuse large B-cell lymphoma, without *JAK2*V617F. In each of these
174 translocations, the *JAK2* fusion partner could not be identified.

175

176 Though the above study found *JAK2* translocations only in patients with *JAK2*V617F-positive MPNs,
177 structural rearrangements, including *JAK2* translocations, have been found frequently in chromosomal
178 analysis of samples from patients with *JAK2*V617F-negative MPNs [14].

179

180 The discovery of several of these translocations in *JAK2*V617F-negative MPNs has shed light on theories
181 of the pathogenesis of the diseases and has helped identify unique patterns of disease. A prime example
182 of this is the identification of t(8;9)(p22;p24), resulting in a *PCM1-JAK2* fusion gene product in a variety
183 of hematologic diseases, including many in the spectrum of MPNs, such as atypical chronic myeloid
184 leukemia (aCML), CEL, myelodysplastic syndrome/myeloproliferative neoplasm-unclassified (MDS/MPN-
185 U) and MF. Identifying the common translocation in cases of these disparate disorders has led to the
186 recognition that patients with t(8;9)(p22;p24) are more likely to be male, tend to have prominent
187 erythroid dysplasia and they have high rates of peripheral blood and bone marrow eosinophilia

188 [15]. *PCM1-JAK2* was identified in both myeloid and lymphoid neoplasms, leading to the conclusion that
189 malignancies with acquired t(8;9)(p22;p24) result from disorders of the pluripotent hematopoietic stem
190 cell [16]. Increased recognition of the role of *PCM1-JAK2* in hematologic malignancies has led to a
191 relatively new categorization of "myeloid/lymphoid neoplasms with eosinophilia and rearrangement of
192 *PDGFRA*, *PDGFRB*, or *FGFR1*, or with *PCM1-JAK2"* in the 2016 revision to the WHO classification of
193 myeloid neoplasms and acute leukemia [17].

194

195 Similarly, both myeloid and lymphoid neoplasms with *ETV6-JAK2* fusions have been identified. Animal
196 modeling using this translocation has led to a proposed pathogenic mechanism involving the rearranged
197 genes, which appears to cause constitutive activation of several STATs within the JAK-STAT pathway
198 [18]. A number of cases have also been noted in which *JAK2* fuses with the *BCR* gene on 9p24, most
199 notably associated with Philadelphia chromosome positive CML, resulting in aCML and unclassified
200 MPNs [19, 20].

201

202 Here we present a case of a novel translocation t(1;9)(p36;p24.1) involving *JAK2* and *peroxisomal*
203 *biogenesis factor 14* (*PEX14*) found in a patient with *JAK2*V617F-negative PV, which transformed to an
204 aggressive form of MPN-BP. *PEX14* is a membrane protein involved in protein docking on the
205 peroxisomes, with a role in peroxisome formation and degradation. It also has a unique function as a
206 transcriptional co-repressor and a polypeptide transport modulator. Upregulated expression of *PEX14*
207 has been demonstrated in tissue from carcinomas of the lung, rectum, ovary and esophagus, but the
208 protein's precise role in these malignancies remains unclear and information about its role in
209 hematologic malignancies is absent [21]. Interestingly, like many other cases of *JAK2*V617F-negative,

210 *JAK2*-translocation positive MPNs, this patient's disease was notable for prominent bone marrow
211 eosinophilia of undetermined significance.

212

213 *JAK2* was identified as a fusion gene partner with a gene on chromosome 1 in only one other case of a
214 hematologic malignancy; a *TPM3-JAK2* fusion was found in a case of T-cell acute lymphoblastic leukemia
215 [22].

216

217 Although commonalities between hematologic malignancies with select *JAK2* rearrangements have been
218 identified as result of increased attention to the cytogenetic underpinnings of these diseases, the
219 prognostic significance and therapeutic implications of the translocations are not fully elucidated. As the
220 disease of our patient progressed to MPN-BP, cytogenetic analysis showed a gain of 1q. We recently
221 reported this to be associated with progression of MPNs to MF and AML [23]. *In vitro* studies utilizing
222 cell lines containing *JAK2* rearrangements suggest a promising role of JAK inhibitors in halting malignant
223 cell proliferation [24, 22]. Early case reports of ruxolitinib treatment in patients with MPNs containing
224 *JAK2* translocations (namely, *PCM1-JAK2* and *BCR-JAK2*) also show positive outcomes in cytogenetic
225 response and hematologic remission, although the durability of remission is variable and individual
226 differences in outcomes are not well-studied [25, 26, 27]. As data accumulate about newly identified
227 *JAK2* translocations, further connections can be made between the specific translocations, disease
228 course and prognosis. Further information is needed to understand the translocations' oncogenicity and
229 the potential for novel targeted therapies aimed at targeting the specific JAK2 partners for this minority
230 of MPN patients. In the future, MPNs may be stratified further into distinct entities that take into
231 account specific *JAK2* translocations.

232

233 **Table 2: JAK2 Translocations and Associated Gene Fusion Products**

234 (see attached)

235 **6.1. Statement of Ethics**

236 The authors have no conflicts of interest to declare.

237

238 **6.2. Disclosure Statement**

239 The authors have no conflicts of interest to declare.

240

241 **6.3. Author Contributions**

242 Hannah Levavi and Bridget Marcellino wrote the manuscript. Joseph Tripodi and Diana Gruenstein
243 performed cytogenetic and FISH studies. Amy V Jones and Nicholas C. P. Cross did molecular studies and
244 analyses. John Mascarenhas was involved in clinical care and studies, and Vesna Najfeld conceived and
245 organized the work, and helped in preparing the manuscript.

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8. Figure Legends

Table 1

MPD = myeloproliferative disorder; PV = polycythemia vera; PB = peripheral blood; BM = bone marrow; CSF = cerebrospinal fluid; ND = not done

* status post gender-mismatched allogeneic stem cell transplant (allo-SCT)

† day +58 status post allo-SCT

€ day +58 status post allo-SCT

Table 2

aCML = atypical chronic myeloid leukemia; CML = chronic myeloid leukemia; MPN-U = myeloproliferative neoplasm unspecified; MPN = myeloproliferative neoplasm; MDS = myelodysplastic syndrome; CEL = chronic eosinophilic leukemia; PMF = primary myelofibrosis; PV-AML = polycythemia vera-acute myeloid leukemia; T-ALL = T-cell acute lymphoblastic leukemia; Pre-B ALL = precursor B cell acute lymphoblastic leukemia; MLL-R ALL = MLL rearrangements acute lymphoblastic leukemia

Figure 1

The first row shows a partial karyotype of chromosomes 1 and 9. Metaphase fluorescence in situ Hybridization (FISH) was performed using bacterial artificial chromosome (BAC) FISH probes. RP11-3H3 (labeled in aqua) normally localized to the 5' portion of JAK2 on 9p24.1 was translocated to 1p36 while the telomere of chromosome 1p (labeled in green) translocated to the derivative chromosome 9. The second row shows BAC FISH probe RP11-4832P2 (labeled in aqua) normally localized on chromosome 1p36 was detected on 9p24.1 whereas BAC RP11-1107P2 (labeled in red) remained on 1p36. The chromosomal breakpoint on chromosome 1 was determined to be within band 1p36.22.

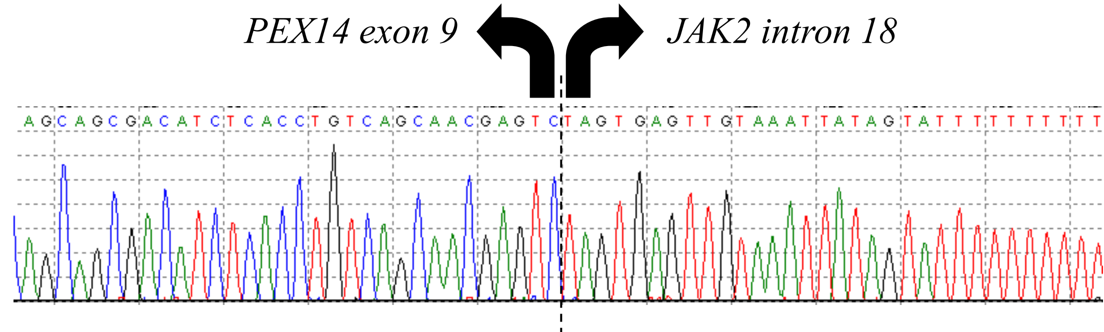
Figure 2

Sequence trace of the PEX14-JAK2 amplicon from genomic DNA (top panel) plus alignment of genomic sequences of PEX14, PEX14-JAK2 and JAK2 in the relevant region (bottom panel). The two nucleotides from JAK2 intron 8 that are retained in the mature mRNA are indicated in italics and the cryptic splice donor site in bold.

Figure 3

Specific amplification of cDNA from the t(1;9) case using primers to PEX14 exon 8 and JAK2 exon 21. Sequence of the mRNA junction with PEX14 sequence in plain type, JAK2 in bold and the two intron-derived nucleotides in italics.

Figure 2



PEX14	TCTCACCTGTCAGCAACGAGTCCACGTCGTCCTCGCCTGGGAAG
PEX14-JAK2	TCTCACCTGTCAGCAACGAGTCTAGTGAGTTGTAAATTATAGTA
JAK2	AACCTAATTTTAGTTTTCCATT*TA***GT**GAGTTGTAAATTATAGTA

Figure 1

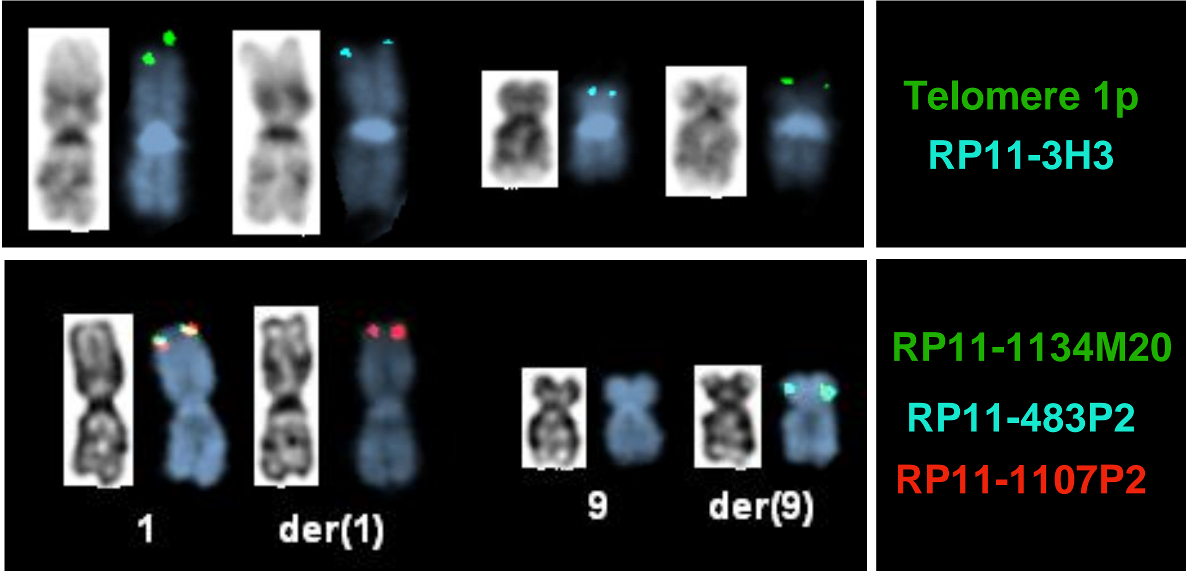
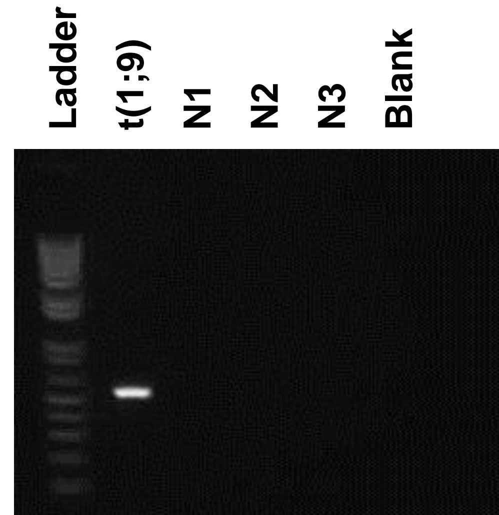


Table 1: Results: Summary of Cytogenetic and FISH Results

Date	Diagnosis	Tissue	Karyotype [number of cells]	I-FISH for Chimerism (% of abnormal cells)
11/24/2009		PB	46,XX,t(1;9)(p36;p24.1)[15]/46,XX[15]	
12/24/2009	MPN accelerated phase 15% blasts	PB	46XX,t(1;9)(p36;p24.1)[8]/46,XX[12]	ND
04/14/2010	PV with 9% peripheral blasts	PB	46XX,t(1;9)(p36;p24.1)[18]/46,idem,t(7;17)(q22.1;q25.3),der(15)t(1;5)(q12;q26)[2] (trisomy 1q)	ND
09/23/2010	MF*	BM	ND	42% host (XX), 48% donor (XY)
10/01/2010	MF-AML[†]	CSF	ND	1.4% host (XX), 98.6% donor (XY)
10/08/2010	MF-AML[€]	PB	ND	56% host (XX), 44% donor (XY)
11/09/2010	AML	BM	46,XX,t(1;9)(p36.22;p24.1),t(7;17)(q21.2;q23),der(15)t(1;15)(q12;q26)[11]/ 46,XX,idem,der(21)t(8;21)(q13;q13)[7] /49,XX,idem,+7,+9,+10[1] / 44,XX,idem,-3,-3,-6,+7,+8[1]	93% host (XX), 7% donor (XY)
11/09/2010		CSF	ND	95% donor (XY), 5% host (XX)

Figure 3

A



B