



# FISH screening of Receptor- and Cytoplasmic-Tyrosine Kinase genes in BCR-ABL1 negative and JAK2V617F negative chronic myeloproliferative neoplasms (CMPNs)

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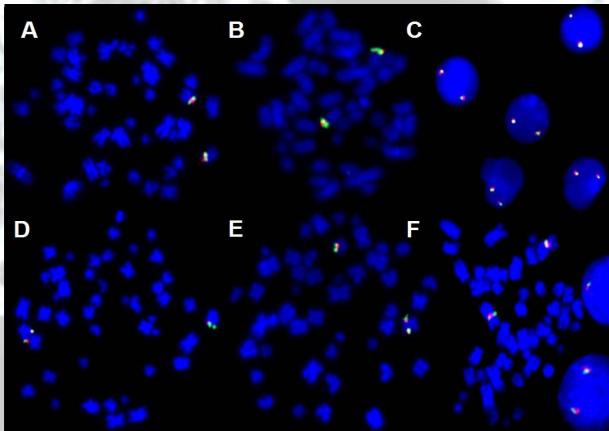
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## Introduction

*BCR-ABL1* negative chronic myeloproliferative neoplasms (CMPNs) are a heterogeneous group of clonal haematological malignancies characterised by abnormal proliferation and survival of one or more myeloid lineage cells. Over the last years, some genetic alterations (translocations, amplifications or point mutations) have been described, most of them activating tyrosine kinases (TKs). However, there is still a significant proportion of patients in which the molecular lesion causing the disease remains to be discovered. The involvement of some TK genes and the success of the targeted anti-TK therapy point to these genes as good candidates for systematic screenings in these diseases.

In this study, we used FISH to search putative cryptic rearrangements of all genes from the families III (*PDGFRA*, *PDGFRB*, *CSF1R*, *KIT* and *FLT3*) and IV (*FGFR1*, *FGFR2*, *FGFR3* and *FGFR4*) of receptor-TKs, as well as all genes from the families Jak (*JAK1*, *JAK2*, *JAK3* and *TYK2*), Abl (*ABL1* and *ABL2*) and Syk (*SYK* and *ZAP70*) of cytoplasmic-TKs. All of them code for proteins with tyrosine kinase activity and some of them have been found mutated in CMPNs and in other tumor types.



FISH analysis with non-commercial flanking probes on nuclei and metaphases when available. All cases show a normal hybridization pattern with two fusion red-green signals, one for each chromosome. **A** *FGFR1* assay. **B** *FGFR2* assay. **C** *FGFR3* assay. **D** *FGFR4* assay. **E** *SYK* assay. **F** *ZAP70* assay.

## Results

Every probe was initially validated on normal samples to verify the correct hybridization and to establish a cut-off value of abnormal signals on interphase normal nuclei.

For *PDGFRA*, we observed an abnormal FISH pattern with gene-flanking probes in an acMPN patient (15081). Whereas the normal hybridization pattern would be two green-red fusion signals, this patient lost the red signal in 85% (170/200) of the nuclei and 8/10 of the metaphases analyzed. This pattern suggested a deletion of the 5' part of the gene. An assay with a commercial FISH probe (QBiogene, Irvine, CA) to check this results, showed a pattern compatible with a *FIP1L1-PDGFR*A type of rearrangement, not detected by conventional RT-PCR.

All other samples showed normal hybridization patterns for all the TK genes and *ETV6*.

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## Materials & methods

We obtained samples from 44 caucasian CMPN patients with normal karyotype (28 males and 16 females), four diagnosed with polycythaemia vera (PV), four with idiopathic myelofibrosis (IMF), 15 with essential thrombocythaemia (ET) and 21 with atypical MPN (acMPN-). *BCR-ABL1* fusion and *V617F/JAK2* mutation were ruled out by conventional analyses (RT-PCR/FISH and ARMS-PCR respectively). Material for all tests was only available for 27 of the 44 patients.

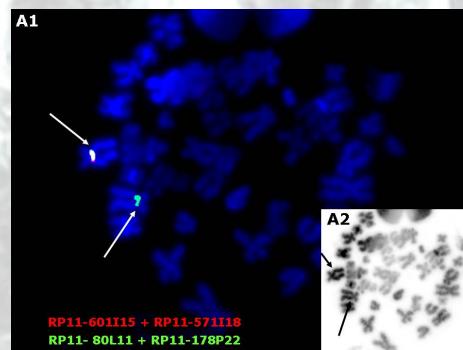
We designed two-colour FISH analyses with flanking probes using BAC clones according to the position showed by UCSC Genome Browser (<http://genome.ucsc.edu>) (Table). Clones were obtained from BACPAC Resources Center at Children's Hospital Oakland Research Institute (Oakland, CA) and Clone Resources at The Wellcome Trust Sanger Institute (Hinxton, UK).

Some of the TK genes selected for the analyses have been found fused with *ETV6* (12p13), so we included an analysis of this gene (with *Vysis LSI ETV6 Dual Color Breakapart Probe* from Abbott Molecular, Abbott Park, IL) in order to check if abnormalities in *ETV6* could unravel new TK fusions.

GENE	5' BACs	3' BACs
<i>PDGFRA</i> 4q12	RP11-60111S + RP11-57111B RP11-80L11 + RP11-178P22	
<i>PDGFRB</i> 5q33	CTB-100B20 + RP11-1104C14 CTB-13H5 + RP11-69G19	
<i>CSF1R</i> 5q33	CTB-100B20 + RP11-1104C14 CTB-46G9 + RP11-136E22	
<i>KIT</i> 4q12	RP11-04L10 + RP11-1141K10 RP11-586A2 + RP11-273B19	
<i>FLT3</i> 13q12.2	RP11-502P18 + RP11-27H10 RP11-438P9 + RP11-153M24	
<i>FGFR1</i> 8p12	RP11-11B9 + RP11-675P6 RP11-265K5 + RP11-90P5	
<i>FGFR2</i> 10q24.12	RP11-255D5 + RP11-78A18 RP11-7P17 + RP11-466C13	
<i>FGFR3</i> 4p16.3	RP11-43P9 + RP11-572017 RP11-263P20 + RP11-115M084	
<i>FGFR4</i> 8q34.2	RP11-606E24 + CTC-340P19 RP11-99N22 + CTC-549A4	
<i>JAK1</i> 1p31.3	RP11-960B3 + RP11-101011 RP11-125L17 + RP11-947H9	
<i>JAK2</i> 9p24.1	RP11-3H3 + RP11-2302 RP11-60G10 + RP11-28A9	
<i>JAK3</i> 19p13.1	RP11-767G23 + RP11-340E23 RP11-79E22 + RP11-63J1	
<i>TYK2</i> 19p13.2	RP11-17734 + RP11-266017 RP11-365L4 + RP11-152C7	
<i>ABL1</i> 9q34.11	RP11-57C19 + RP11-7M2 RP11-544A12 + RP11-643E14	
<i>ABL2</i> 1q52.2	RP11-346D17 + RP11-1054P1 RP11-177A2 + RP11-595C2	
<i>SYK</i> 9q22.2	RP11-102119 + RP11-80J10 RP11-652P2 + RP11-95G21	
<i>ZAP70</i> 2q11.2	RP11-1082A11 + RP11-542D13 RP11-263L6 + RP11-973B20	

**A1.** Metaphase FISH *PDGFRA* analysis with flanking probes. Although normal hybridization pattern would be two red-green fusion signals, we observed one red-green fusion signal from normal chr4 and an extra green signal from der(4) (white arrows). This pattern reveals a deletion of the 5' upstream part of *PDGFRA*.

**A2.** Light microscopy of the same metaphase as in A1



## Conclusions

- FISH analysis of genes from TK families (*PDGFRA*, *PDGFRB*, *CSF1R*, *KIT*, *FLT3*, *FGFR1*, *FGFR2*, *FGFR3*, *FGFR4*, *JAK1*, *JAK2*, *JAK3*, *TYK2*, *ABL1*, *ABL2*, *SYK* and *ZAP70*) did not show any new cryptic chromosomal rearrangement.
- Only in one patient a *FIP1L1-PDGFR*A fusion was detected that had been missed by conventional RT-PCR, confirming that FISH analysis, as other groups have shown, is a good technique to detect the *FIP1L1-PDGFR*A gene fusion when conventional RT-PCR fails due to the variability of breakpoints in *FIP1L1*.
- Rearrangements affecting these genes are very infrequent events in *BCR-ABL1* negative chronic myeloproliferative neoplasms (CMPNs).