Screening for fusion genes involving PDGFA or PDGFRB in patients with eosinophilia-associated myeloproliferative neoplasms (Eos-MPN) using the Fluidigm BioMark real-time PCR system and 48.48 Dynamic Array

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Background
- Identification of fusion genes involving Platelet Derived Growth Factor Receptor A (PDGFA) or PDGFRB in eosinophilia-associated myeloproliferative neoplasms (Eos-MPN) is challenging for diagnostic testing due to multiple partner genes with heterogeneous breakpoints.
- Detection of these fusions is important for clinical management as they are associated with excellent response to imatinib.
- Using the Fluidigm BioMark real-time PCR system and 48.48 Dynamic Array we have developed reverse transcriptase qPCR (RT qPCR) assays to detect overexpression of the 3' region of PDGFA and PDGFRB that may be indicative of an underlying gene fusion.

Methods

Methods: Using the Fluidigm BioMark real-time PCR system and 48.48 Dynamic Array we analysed pre-amplified cDNA from normal controls (n=16), samples with putative PDGFA (n=12) or PDGFRB (n=4) fusions, samples with known PDGFRB (n=3) or PDGFR (n=1) fusions for PDGFA and PDGFRB expression using 6 RT qPCR assays per gene (3 assays 3' to known breakpoints and 3 assays 5' to known breakpoints) and control gene assays for ABL, BCR and GUSB. The 48.48 dynamic array allowed 2304 real time PCRs to be performed in one run enabling 48 samples to be analysed simultaneously in triplicate using 16 RT qPCR assays.

Assay rationale: Where no fusion transcript is present, normal PDGFA and PDGFRB expression (relative to the control gene assays) will be observed for all assays (blue arrows, figure 1A). However, when a fusion transcript is present the expression of the three assays 3' to the breakpoint region will be upregulated (red arrows) with respect to the three assays that are 5' to the breakpoint region (figure 1B).

Data analysis: Mean Ct values were used to calculate the ∆Ct value for each assay and each sample using either ABL, BCR and GUSB as a reference. These data were then normalised to the expression level observed in the calibrator sample (normal human leukocytes) using the formula: ∆Ct sample / ∆Ct calibrator. The difference between the median 3' expression (assays A - C) and median 3' expression (assays D - F) was used to determine the relative overexpression of the tyrosine kinase domain of PDGFA or PDGFRB.

Results

- Figure 1: Diagrammatic representation of real time PCR assay design

![Figure 1: Diagrammatic representation of real time PCR assay design](image)

- Figure 3: Boxplot showing relative upregulation of 3' end of the PDGFA and PDGFRB transcript in normal controls (n=16), samples with putative PDGFA (?A, n=12) or PDGFRB (?B, n=4) fusions, samples with known PDGFRB (A +ve, n=3) or PDGFRB (B+ve, n=1) fusions. Delta-delta Ct analysis relative to ABL and normalised to a reference normal leukocyte cDNA demonstrated that median relative upregulation of the 3' end of the genes was 55.3 (PDGFA) for samples with known PDGFRB fusions and 59.7 (PDGFRB) for samples with known PDGFRB fusions compared with 0.66 (PDGFA; p=0.0005*) and -0.21 (PDGFRB; 0.03*) in normal controls.

Conclusions
- Using the Fluidigm BioMark real-time PCR system and 48.48 Dynamic Array we have demonstrated that the relative upregulation of the 3' end of PDGFA and PDGFRB was 55.3 for samples with known PDGFRB fusions and 59.7 for samples with known PDGFRB fusions compared to normal controls.
- This simple, rapid and high throughput screen can be used to detect clinically significant overexpression of PDGFA & PDGFRB in Eos-MPN.

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