Screening for fusion genes involving PDGFRA 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 (*PDGFRA*) 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 *PDGFRA* 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 *PDGFRA* (n=12) or *PDGFRB* (n=4) fusions, samples with known *PDGFRA* (n=3) or *PDGFRB* (n=1) fusions for *PDGFRA* 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 *PDGFRA* 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 ΔΔCt 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: ΔΔCt sample / ΔΔCt calibrator. The difference between the median 5' expression (assays A - C) and median 3' expression (assays D - F) was used to determine the relative overexpression of the tyrosine kinase domain of *PDGFRA* or *PDGFRB*.

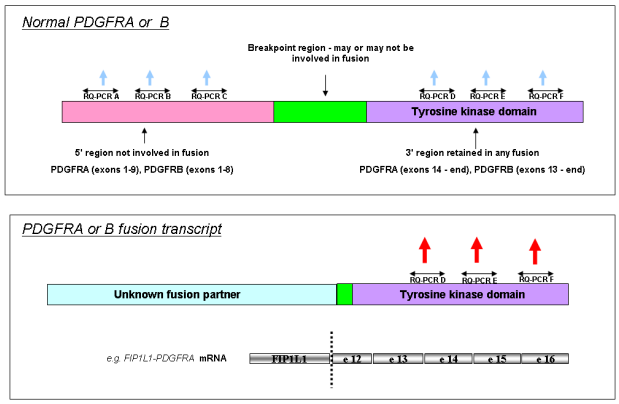

Figure 1: Diagrammatic representation of real time PCR assay design

Results

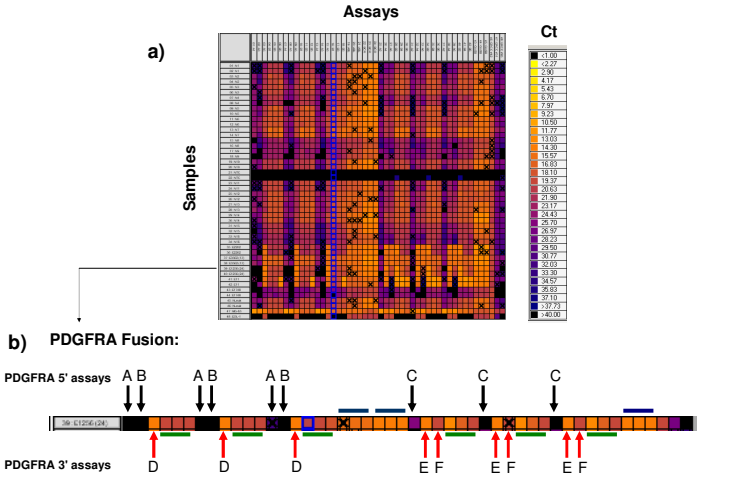

Figure 2: a) Screenshot of the 48.48 Dynamic array. Each column indicates a RT qPCR assay and each row indicates a single cDNA sample. The colour in each square represents the Ct value obtained for a single assay and sample. The representative colours used for the Ct values are shown to the right of the array.
b) Assay data for a known *PDGFRA* fusion cDNA sample. The 5' *PDGFRA* assays (black arrows) show low expression (Ct>30) whereas the 3' assays (red arrows) are highly expressed (Ct< 18). The *PDGFRB* assay wells (green bars) show constant expression across the transcript (*c.*Ct 18). The control gene assays (*ABL, BCR* and *GUSB*) are indicated by the blue bars (*c.*Ct 18).

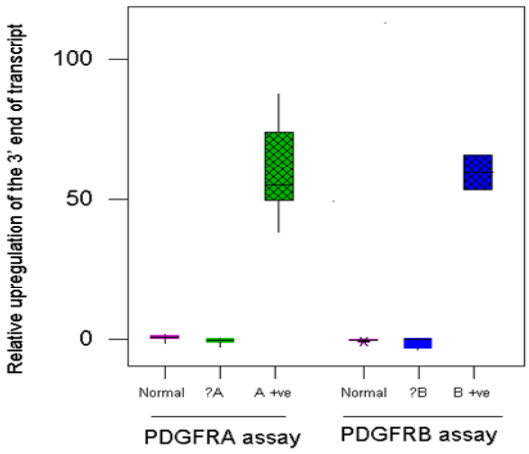

Figure 3: Boxplot showing relative upregulation of 3' end of the *PDGFRA* and *PDGFRB* transcript in normal controls (n=16), samples with putative *PDGFRA* (?A, n=12) or *PDGFRB* (?B, n=4) fusions, samples with known *PDGFRA* (A +ve, n=3) or *PDGFRB* (B+ve, n=1) fusions. Delta-delta Ct analysis relative 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 (*PDGFRA*) for samples with known *PDGFRA* fusions and 59.7(*PDGFRB*) for samples with known *PDGFRB* fusions compared with 0.66 (*PDGFRA; p=0.0005**) and -0.21 (*PDGFRB; 0.03**) in normal controls. *Mann Whitney test

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 *PDGFRA* and *PDGFRA* was 55.3 for samples with known PDGFRA 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 *PDGFRA* & *PDGFRB* in Eos-MPN.

Funding

Department of Health

National Measurement System

For further information please contact Helen White (hew@soton.ac.uk) or visit the NGRL (Wessex) website:

www.ngrl.org.uk/Wessex