Validation of genetic abnormalities involving the *NUP214* gene in T-lineage Acute Lymphoblastic Leukaemia by using MLPA and FISH

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Part 1 - Validating the sensitivity of MLPA at detecting the NUP214-ABL1 fusion

Part 1 - Introduction/Aims

- The characterisation of genetic abnormalities in the bone marrow blasts of patients with T-lineage acute lymphoblastic leukaemia (T-ALL) has led to improvements in their outcome
- The most common abnormality is the gene fusion: *NUP214-ABL1*, which is seen in ~ 6% of cases. It is thought to be a secondary abnormality. [1]
- The aims of the first part of my project was to use multiplex ligation-dependent probe amplification (MLPA) to detect the *NUP214-ABL1* fusion
- This project was of therapeutic relevance as the abnormality can be treated with tyrosine kinase inhibitors such as Imatinib. [2]

Part 1 - Methods

- The ALL-SILL cell line was titrated in amounts of 100%, 75%, 50%, 25%, 20%, 15%, 10%, 5% and 0% against corresponding amounts of normal female DNA.
- The DNA was diluted to 20ng/ul to be an optimum concentration for MLPA.
- The kit used for MLPA was from MRC-Holland and detects the common copy number abnormalities associated with T-ALL, such as the *NUP214-ABL1* fusion.

Part 1 - Results

- MLPA validated monoallelic loss of *ABL1* exons 4 and 12, and monoallelic loss of *NUP214* exon 23
- These were reliably detected at a concentration of 75% but were also visible at 50%.

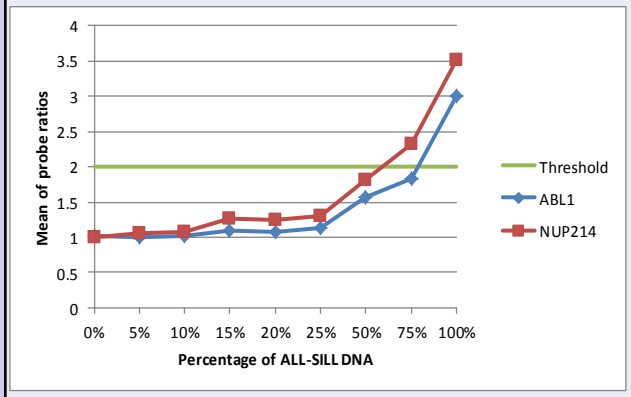

Fig 1. A graph showing the mean of probe ratios against the titrated ALL-SILL DNA, deletions are present at 75% but are detectable at 50%.

Part 2 - Producing an in house FISH probe to detect the *SET-NUP214* rearrangement

Part 2 - Introduction/Aims

- The *SET-NUP214* fusion is also seen in T-ALL, as a consequence of a 2.5mb deletion at 9q34. [3]
- The aim of this part of my project was to produce a FISH probe to detect this abnormality.

Part 2 - Methods

- The clone numbers were RP11-216B9, RP11-550J21, RP11-143H20 and RP11-544A20.
- They were extracted using the nucleobond xtra midi kit and tested on a normal sample first for verification.

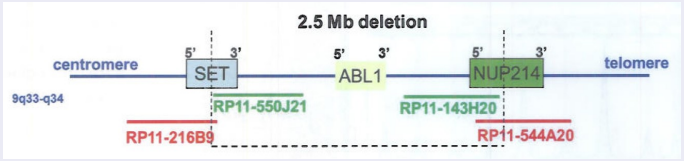

Fig 2. The probes showing that the *SET-NUP214* fusion can be detected by FISH.

Part 2 - Results

- My FISH probes detected the *SET-NUP214* fusion in T-ALL bone marrow samples.
- These results confirms results previously found by MLPA.

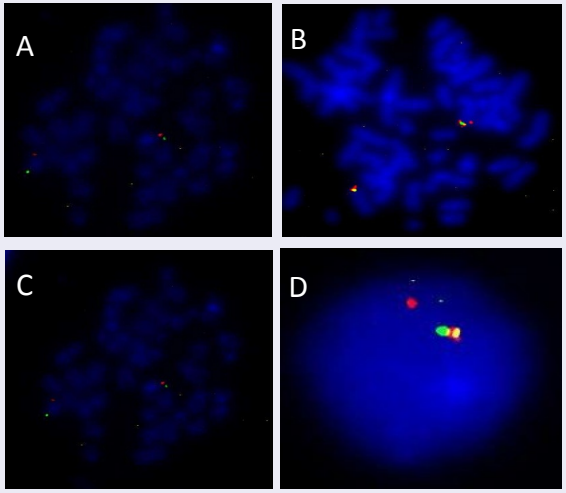

Fig. 3. A shows the normal arrangement of probes RP11-544A12 (Red) and RP11-143H20 (Green). B shows a fusion of these two probes resulting from the deletion at 9q34. C shows the normal arrangement of probes RP11-R21B9 (Red) and RP11-550J21 (Green) with D showing a fusion of these two probes .

[1] Graux, C. Stevens-Kroef, M. and Lafage, M. et al. Heterogeneous patterns of amplification of the *NUP214-ABL1* fusion gene in T-cell acute lymphoblastic leukemia. Leukemia (2009) 23;125-133.[2] Graux, I. Cools, J. and Melotte, C. *et al.* Fusion of *NUP214* to *ABL1* on amplified episomes in T-cell acute lymphoblastic leukemia, Nature Genetics,2004; 36(10):1084-1089. [3] Quentmeier, H. Schneider, B. and Rohrs, S. et al. *SET-NUP214* fusion in acute myeloid leukemia and T-cell acute lymphoblastic leukemia-derived cell lines. Journal of Hematology & Oncology (2009) 2:3