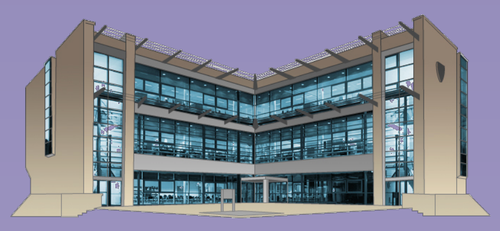
Validation Of Target Genes Of The Leukaemic Fusion Gene MLL/AF4



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Introduction:

Leukaemia is a cancer of the blood. In my studies I have used a cell line (SEM) originating from a 5 year old patient with relapsed acute lymphoblastic leukaemia (ALL). This cell line carries the t(4;11) translocation leading to the formation of the fusion gene MLL/AF4 and the reciprocal fusion AF4/MLL. Leukaemic fusion genes are generated when a part of a chromosome fuses with another chromosome (chromosomal translocation), joining two separate genes. Generation of leukaemic fusion gene is a hallmark of ALL.

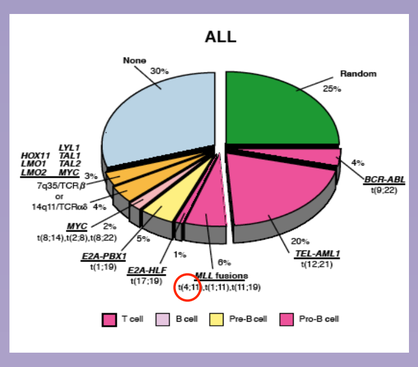


Fig 1: % of different translocations involved in the development of ALL.

Previous research has shown that MLL/AF4 is important for growth and survival of SEM cells. My project aimed to investigate if the expression of AF4/MLL was regulated by MLL/AF4 on silencing MLL/AF4 expression using RNA interference (RNAi).

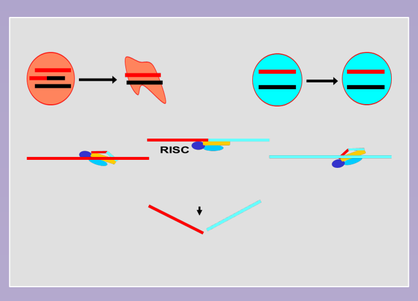
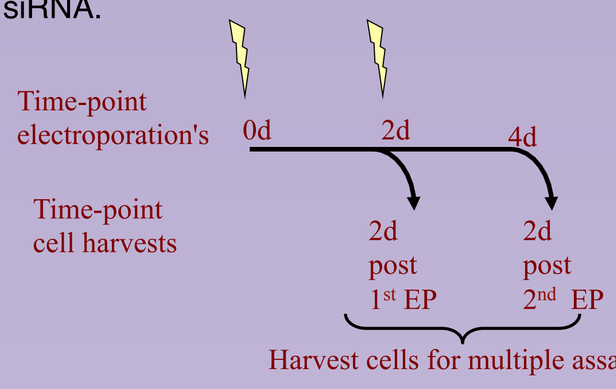


Fig 2: This shows siRNA mediated mRNA degradation specific for fusion genes. This is initiated by formation of RISC complex.

Methods:

- To establish a knockdown, SEM cells were cultured and electroporated on day 0 and day 2 with siMA6 and a negative control (siAGF1), respectively. A third group of SEM cell was electroporated without any siRNA.



- RNA was extracted from each cells after each electroporation and converted to cDNA with the aid of RT-PCR machine.
- A primer was designed for GAPDH (House keeping gene), MLL/AF4, AF4/MLL ex 11 and AF4/MLL. These primers were validated using a dilution series.
- All samples were exposed to each primer in triplicate and analysed in the qRT-PCR machine.
- A ct value from the PCR was used to calculate the power for each sample.
- To investigate if the reduction in AF4/MLL mRNA levels led to a decrease in protein levels, proteins were extracted 48 hours after the second electroporation and analysed using western blotting.

Results:

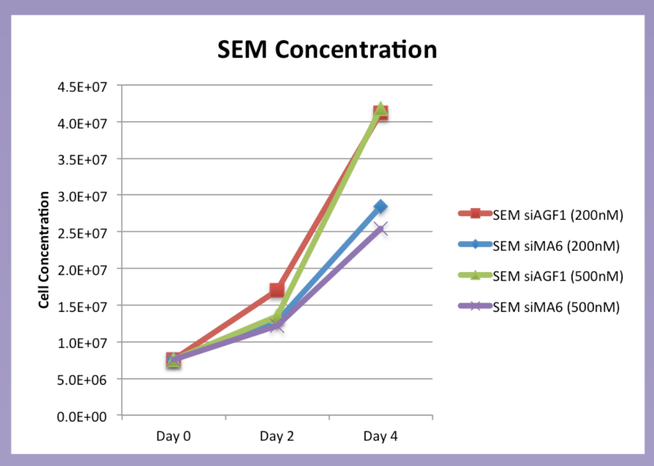


Fig 3: After each electroporation, cells treated with siMA6 grew slower than cells treated with siAGF1(Negative control). There was a further decrease in the concentration of cells treated with a higher concentration of siMA6 after second electroporation.

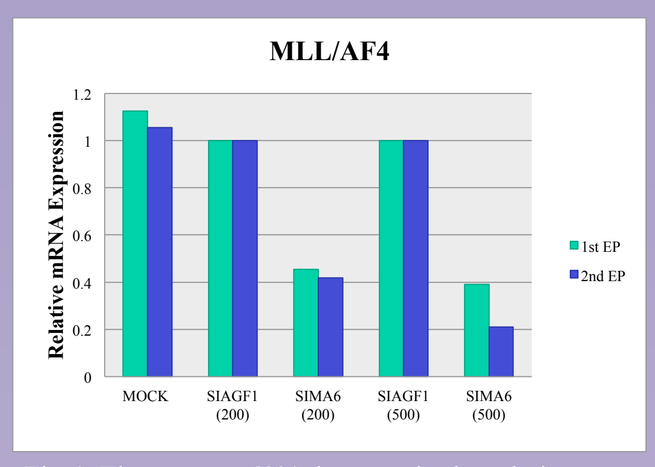


Fig 4: There was a 60% decrease in the relative expression of MLL/AF4 mRNA after 1st electroporation and 80% decrease after 2 rounds of electroporation's in cells treated with 500nM siMA6. No decrease was seen in cells treated with siAGF1(Negative control).

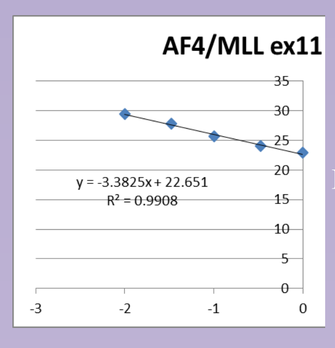


Fig 4.1: 97.5% Primer efficiency

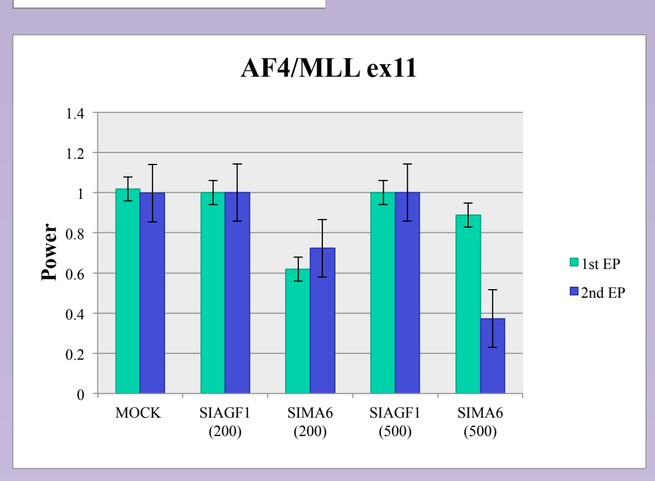


Figure 4.2: In cells treated with 500nM siMA6, there was no significant decrease in the mRNA expression for AF4/MLL ex11 after 1st electroporation but a 65% decrease in expression level was seen after 2nd electroporation.

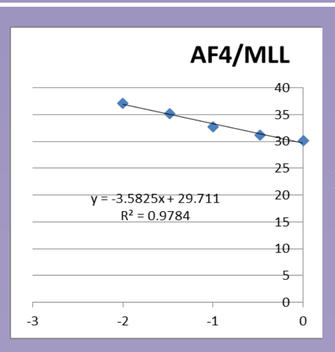
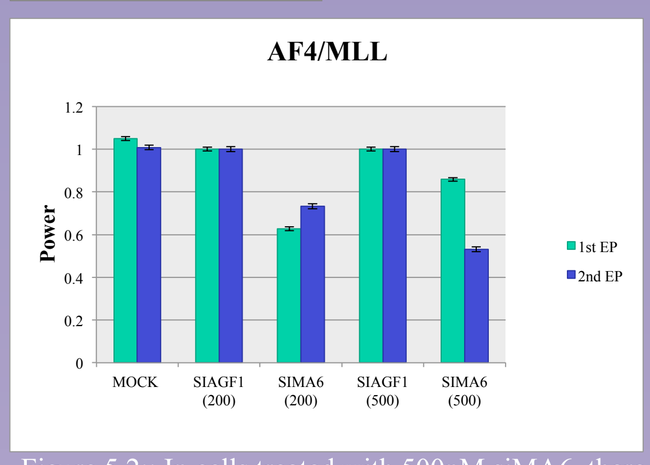


Fig 5.1: 90.2% Primer efficiency



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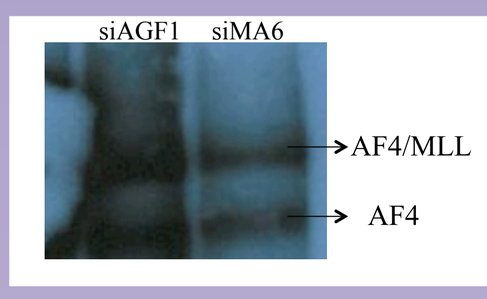

Fig 6: There was no significant decrease in expression of protein observed. The difference in band was due to a lesser amount of protein been loaded for siMA6.

Conclusions:

- My experiments showed that AF4/MLL expression was reduced when we inhibited the expression of MLL/AF4 with RNA interference (RNAi).
- Results from protein analysis showed a minimal decrease in AF4/MLL protein levels. A possible reason can be that a 50% reduction on mRNA level is not sufficient to translate into a significant reduction on protein level.
- Future experiments will be performed analysing the protein expression of AF4/MLL after a third electroporation.

References

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2 M Thomas, A Gebna, H Vomlocher, P Hadwiger, J Greil, O Heidenreich. Targeting MLL-AF4 with short interfering RNAs inhibits clonogenicity and engraftment of t(4;11)-positive human leukemic cells. Blood Journal, 2005; 106: 3559-3566.