# PIWIL2 modulation in childhood acute lymphoblastic leukaemia


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## 1) Background

Found specifically in the testes, where is has a crucial role in self-renewal of mammalian germline cells, PIWIL2 is a gene which has also been identified in a number of human malignancies. In humans, PIWIL2 expression has been identified in breast, prostate, ovarian, endometrial and gastrointestinal cancers[1].

Although not yet confirmed, it is thought that PIWIL2 may be involved in the self-renewal capacity of cancerous cells, therefore making it a possible therapeutic target. Furthermore, PIWIL2's specificity only to the testes and malignant cells could, in theory, allow the development of treatments with less side effects than therapies available currently.

Work within the NICR has recently confirmed the expression of PIWIL2 in childhood acute lymphoblastic leukaemia (ALL). Modulation of PIWIL2 expression by RNA interference is an interesting avenue for scientific exploration; work already carried out demonstrates that delivery of siRNA by electroporation in the childhood ALL cell line SEM causes a decrease in cell proliferation and increase in apoptosis. These findings support the theory that PIWIL2 has a role in control of cell growth, however there is a lack of research into different childhood ALL cell lines to reinforce the reliability of these conclusions.

## 2) Aims

- To investigate the impact of RNA interference on PIWIL2 expression on two other childhood ALL cell lines, 697 and REH by:
  - Performing serial electroporations on cell lines 697 and REH
  - Completing functional analyses using cell counts to determine proliferation, flow cytometry and quantitative real time PCR
- To provide complementary work to the existing research concerning the SEM cell line in order to clarify, or dispute, conclusions made

## 3) Methods

Methodology was reproduced to reflect that of previous work on the cell line SEM.[2] Childhood ALL cell lines 697 and REH were cultured using RPMI 1640 supplemented with 10% foetal calf serum, in an incubator at 37°C and 5% $CO_2$. Serial electroporation was carried out on alternate days. Previous to each electroporation, cell counts were performed and samples were taken for flow cytometry and RNA extraction.

Electroporation was performed using a rectangle pulse EPI 2500 electroporator with 300-600µl of cells at a concentration of $1x10^7$cells/ml at 350V for 10ms.

Six different siRNA combinations were used. Three were active: siP2_5, siP2_e and a combination of siP2_5 and siP2_e (all at concentration 500nM). The other three acted as controls: siCAPSNi6 (200nM), a positive control which targets a gene present in the cells but not PIWIL2; siAGF1 (500nM), a negative control which targets a fusion gene; and a mock with no siRNA used.

RNA extraction was performed using RNeasy minikits, protein was precipitated with acetone and frozen for later use. RNA concentration was measured using Nanodrop technology. cDNA synthesis was carried out in order to complete quantitative real time PCR.

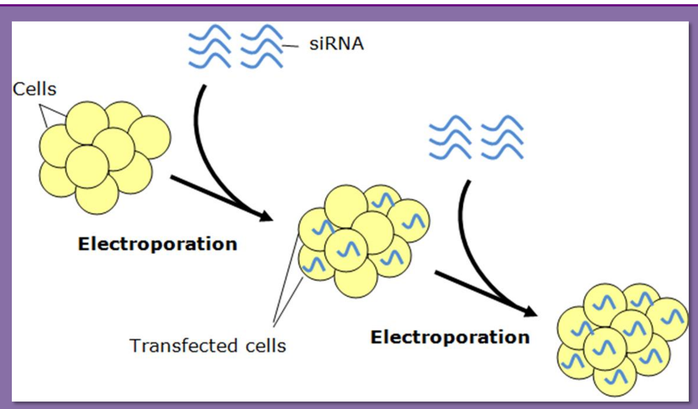


Fig 1:Transfection of cells with siRNA by serial electroporation

## 4) Results

Preliminary work indicated that 697 cells responded better to serial electroporation with siRNA than the REH cell line; day 4 data concerning electroporation in the presence of siP2_e showed 15.5% knockdown in the REH cell line but 30.7% in cell line 697.

On repeating the experiment on 697 cells, day 9 data showed 40.1%, 39.2% and 47.6% PIWIL2 knockdown when using siP2_5, siP2_e and a combination of siP2_5/siP2_e respectively. The latter result is the most encouraging, showing the highest knockdown.

However, the control data on CAPSN knockdown (instigated by siCAPSNi6) is variable, and therefore provides a less reliable reference point than a stable set of results. Also problematic is the variation between knockdown on each day: for example in the siP2_e data, knockdown was greater on day 7 than on day 9, and PIWIL2 mRNA expression was actually greater on day 2 than the mock (see Fig 2 and 3). These results indicate that errors may have been made at some stage in the experimental process, causing inconsistent results.

Unfortunately, the cell proliferation data and cell cycle analysis did not yield significant results.

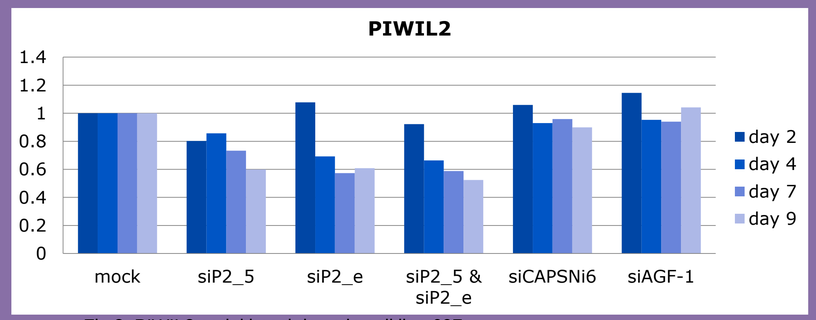


Fig 2: PIWIL2 serial knockdown in cell line 697

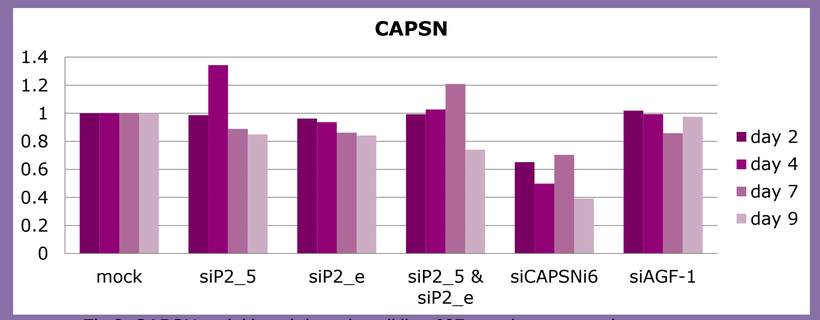


Fig 3: CAPSN serial knockdown in cell line 697, used as a control

## 5) Conclusions

- The most successful PIWIL2 knockdown was achieved after 4 electroporations using a combination of siP2_5 and siP2_e
- Although findings came close to the boundaries indicating a significant knockdown, my results were not conclusive and did not allow reliable judgements to be made
- PIWIL2 still remains an exciting potential therapeutic target in childhood ALL, with future work within the NICR lab aiming to optimise electroporation protocol in 697 and REH cell lines in order to achieve more consistent knockdown

## 6) References


1. Lee J.H. et al (2006) Stem cell protein Piwil2 is widely expressed in tumors and inhibits apoptosis through activation of Stat3/Bcl-$X_L$ pathway. *Human Molecular Genetics*, **15**, 201-211

2. Using methodology from research already performed in the NICR by Simon Bomken et al.