

P049 Evaluation of Nucleofection to Deliver PI3K Targeting siRNA into Peripheral Blood- derived T Lymphocytes.
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The aim of this study was to develop a non-viral technique to deliver phosphoinositide 3-kinase (PI3K) targeting siRNA into peripheral blood derived T lymphocytes (PBLs). We focused on siRNA strategies to disrupt individual PI3K catalytic subunits utilizing the Amaxa Nucleofector™ device. This technique, based on electroporation, facilitates the introduction of siRNAs into unstimulated PBLs or PBLs that have been activated and expanded *in vitro*.

The method of activating PBLs can affect the survival rate post nucleofection, we show that compared to CD3/CD28 activation, PHA activation results in the highest survival rate post nucleofection. Furthermore, activated PBLs are more sensitive to 'mock' nucleofection, showing a decrease in chemotactic responsiveness which is not seen within freshly isolated PBLs.

TaqMan analysis confirmed that transfection of a target specific siRNA results in the subsequent knockdown of the mRNA 72 hours post transfection. Cy3 tagged siRNAs were successfully transfected into freshly isolated PBLs and 6 day previously activated PBLs in a dose dependant manner, with 4 μ M siRNA giving the best survival rate while maintaining 100 % transfection efficiency.