**Proof-of-Principle: CRISPR-Cas9 Gene Editing for the Treatment of Immune Disorders**

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**RESULTS TO DATE**

- Achieved 60% cleavage efficiency of the SNV, 3 bases away from the C>T.
- Achieved 80% cell viability, C>T editing and HDR DNA repair using a ss-donor template.

**BACKGROUND**

Lymphoma is the 5th most common cancer in Australasia. Incidence rates have doubled over the past 20 years with approximately 6000 new cases diagnosed each year in Australia.

- Tet methylcytosine dioxygenase 2 (TET2) and Moesin (MSN) have a role in both NHL and immunodeficiency, as established in a Proband with a c.511C>T, p.Arg171Trp substitution in MSN, diagnosed with Primary Immunodeficiency Disorder (PID) resulting in T and B cell lymphopenia.
- To date CRISPR-Cas9 gene editing of point mutations in TET2 and MSN in human lymphocytes is yet to be established.

**DISCUSSION**

- Our findings may establish a proof-of-principle for editing disease-causing genes implicated in NHL and PID target genes which may be used for ex-vivo gene therapy as an alternative to standard of care bone marrow transplantation.

**FUTURE DIRECTIONS**

- Perform clonal isolation then replicate the application in donor derived lymphocytes.
- Investigate the functional impact of insufficient MSN and TET2 proteins.

**METHODS**

- Figure 1: Assay designed to correct C>T to replicate the mutation in reverse using CRISPR-Cas9 RNP complex and a ss-donor repair oligonucleotide to encourage homology directed repair (HDR) of the edited strand. Genomic cleavage detection assay and Sanger sequencing was used to detect the edit.

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**Figure 2:** Image of Genomic Cleavage Detection, optimized for MSN gRNA PCR products.

**Figure 3:** Sanger sequencing trace showing the edited DNA strand with a population of C>T nucleotides.