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PROGRESS REPORT

Project / Program Title		Interrogating and manipulating cancer immunotherapy using novel genomic technologies
Name		Dr Joshua Casan
Award Received		2020 RACP NHMRC J J Billings Scholarship
Report Date		15/03/2021 – Year 1
Funding Period	Start Date:	02/03/2020
	Finish Date:	01/03/2023

PROJECT SUMMARY

This project seeks to leverage the recently discovered genetic manipulation technology known as CRISPR-Cas13 to target cancer-causing genetic changes in a new way. Derived from the immune system of bacteria, CRISPR-Cas13 breaks down RNA, which serves as the intermediary messenger between DNA and the proteins it encodes for. Targeting RNA opens up a realm of new possibilities for cancer treatment, by expanding the number of targets and because of the system's exquisite specificity for a particular RNA sequence. We hope to translate this technology into an entirely new form of treatment. Given the project start was delayed by the COVID-19 pandemic, we also re-engineered the system to target the SARs-CoV-2 coronavirus at the beginning of my PhD, demonstrating the capabilities of this technology to impact medicine beyond cancer treatment.

PROJECT AIMS / OBJECTIVES

Project Aims:
1. Demonstrate the capability of CRISPR-Cas13 to silence MLL fusion transcripts
a) Demonstrate silencing of MLL:AF9 fusion transcripts in vitro using CRISPR-Cas13
b) Investigate phenotypic effect of CRISPR-Cas13 mediated MLL fusion silencing in:
    1. THP1 cell line (endogenous MLL:AF9 translocation)
    2. A mouse model of MLL:AF9 driven AML
    3. Investigate capacity and impact of silencing MLL-interacting gene transcripts
    4. Undertake a high throughput CRISP-Cas9/Cas13 screen in an MLL:AF9 AML context
    5. Develop a viable platform for in vivo CRISPR-Cas13 delivery

SIGNIFICANCE AND OUTCOMES

The prospect of RNA targeted therapies is extremely appealing and a Cas13 therapeutic platform offers several conceivable and distinct advantages. Conventional protein-targeting or small molecular drugs are exceptionally challenging to engineer and limited by the conformation and binding properties of the target. Targeting RNA circumvents this hurdle, expanding possible therapeutic targets to high value but as yet ‘undruggable’ proteins such as cMyc or other transcription factors. gRNA design is tractable and increasing use of the platform has revealed numerous factors that influence gRNA binding capacity and silencing efficiency. Particularly in

relation to cancer therapeutics, the capacity to multiplex therapies for combination targeting will be essential to counteract sub-clonal heterogeneity or complex mutational landscapes. Multiplexed targeting is likely to be considerably more feasible with Cas13 in part because of its extremely high specificity. High specificity ensures a negligible risk of off-target effects, and in conjunction with transient nature of RNA silencing, the safety profile of a Cas13 based therapy is likely to compare favorably to a Cas9 system. Additionally, temporary knockdown of a gene could enable strategies that target normal endogenous genes that may be critical vulnerabilities to cancer cells, and which could be tolerated for a brief period by normal cells.
Successful translation into a therapeutic could be of great significance to cancer treatment. Additionally there are myriad potential applications in other areas such as antiviral therapy and molecular diagnostics.

PUBLICATIONS / PRESENTATIONS

Given the PhD commenced immediately prior to the outbreak of COVID19 in Melbourne, then attendant changes to laboratory operations at Peter Mac precluded commencement of the project as originally described.
Special dispensation was provided to me and other members of our group to undertake a project in collaboration with the Peter Doherty Institute to develop a Cas13-based approach for targeting the coronavirus. This has been very successful and has been submitted for publication, currently under revision.
Accordingly, my work targeting AML commenced later in 2020, and I have successfully completed my confirmation at the customary 12 month time-point despite the delayed start.

ACKNOWLEDGEMENTS

The RACP will be acknowledged as a funder for any publication or presentation arising as a result of this project.