INTRODUCTION

1.1 Background

Cancer continues to pose a significant global health challenge, with 19.3 million new cases and almost 10 million cancer-related deaths reported in 2020 (Sung et al., 2021). The imperative for advanced treatment methods is evident, and immunotherapy has emerged as a groundbreaking approach, particularly the focus on chimeric antigen receptor (CAR) T cell therapy. This innovative strategy involves genetically engineering donor-derived T cells with synthetic CAR T receptors, enhancing their precision and binding affinity to tumour-associated antigens (TAAs) on the tumour cell surface (Rodrigo et al., 2023). Despite promising results in haematologic malignancies, the application of CAR T technology to solid tumours faces challenges from the tumour microenvironment (TME), hindering CAR T cell activation (Emens et al., 2017; Gajewski et al., 2013).

T cell immune activation is a well-studied system that involves kinase-phosphatase networks. The presence of CD45 molecules in the CAR-antigen interaction region is one of the factors affecting the CAR T cell activation. CD45 inhibits activation sites in Lck, preventing its full activation and inhibiting substrates of Lck and ZAP-70. Xiao et al. (2022) suggested that incorporating larger CD45 variants into CAR T cells could increase CD45 exclusion, potentially enhancing CAR T cell activation. Removing CD45 could eliminate this hindrance, improving CAR T cell activation and effector function. Furthermore, while CAR T cell development has transformed cancer treatment, challenges like T cell exhaustion and unintended effects still reduce their effectiveness. One significant barrier is the upregulation of immune checkpoint proteins, such as PD-1/PD-L1, by tumour cells, leading to the suppression of CAR T cells. The interaction between PD-1 on CAR T cells and PD-L1 on tumour cells initiate signalling events that inhibit T cell proliferation and cytotoxic function (Kalinin et al., 2021; Zhao et al., 2017). To address this, disrupting the PD-1/PD-L1 interaction could potentially prevent CAR T suppression and enhance their function in solid tumour treatment.

Clustered regularly interspaced short palindromic repeats (CRISPR) technology offers a promising avenue to address these challenges in CAR T cell therapy. CRISPR employs sgRNA-guided Cas9 nuclease to target specific genes, inducing double-stranded DNA breaks repaired through non-homologous end joining (NHEJ), introducing genetic mutations (Ehrke-Schulz et al., 2017). Utilising CRISPR/Cas9 to knockout CD45 or PD-1 in CAR T cells aims to enhance their function in solid tumours. Optimising sgRNA design, nucleotide delivery methods, and scalable production protocols are crucial for efficiently producing CD45 or PD-1 negative "enhanced" CAR T cells while addressing off-target issues (Balke-Want et al., 2023).

This proposed study seeks to create and characterise CD45 and PD-1-negative "enhanced" CAR T cells using CRISPR/Cas9 technology. The optimisation of sgRNAs for CD45 and PD-1 knockout aims to minimise toxicity and maximise treatment efficacy. The study's outcomes could pave the way for repeated preclinical and clinical trials, refining single gene knockout procedures using the advanced CRISPR/Cas9 system to define the future landscape of CAR T cell therapy, enhancing efficacy, reducing costs, and increasing availability (Wang et al., 2021; Xu et al., 2018).

1.2 Objective

To employ CRISPR/Cas9 technology to generate and thoroughly characterise T cells with specific knockouts of CD45 and PD-1. The goal is twofold: first, to develop a robust and efficient methodology for introducing targeted genetic modifications in T cells, specifically disrupting either the CD45 and PD-1 gene; and second, to conduct an extensive characterisation of the resulting modified T cells to elucidate their molecular alterations.

1.3 Hypothesis

Optimisation of the CRISPR/Cas9 production protocol can be accomplished by testing a critical parameter, different sgRNAs targeting the same gene, influencing the quantity of live CD45 and PD-1 negative T cells.