Gene Editing
Regulatory and Translation to Clinic

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KEYWORDS

• Genome editing • Zinc finger nucleases • Hematopoietic stem and progenitor cells • CCR5 • Genotoxicity • Safety assessment

KEY POINTS

- This review covers the regulatory and translational preclinical activities needed for a CCR5 zinc finger nuclease genome editing clinical trial modifying hematopoietic stem cells.
- $CD34^+$ HSPC manufacturing and clinical administration considerations are discussed.
- Preclinical evaluations supporting the FIH study include on- and off-target genome editing assessment, in vitro differentiation, in vivo stem cell engraftment, karyotype analysis, 53BP1 assay, soft agar transformation, and an NSG mouse tumorigenicity study.

INTRODUCTION

The clinical application and regulatory strategy of genome editing for ex vivo cell therapy is derived from the intersection of two fields of study: viral vector gene therapy trials (initially retroviral gene therapy[1]), and clinical trials of therapeutics based on ex vivo purification and engraftment of $CD34^+$ hematopoietic stem cells and T cells and tumor cell vaccines.[1–3] This review covers the regulatory and translational preclinical activities needed for a genome editing clinical trial modifying hematopoietic stem and progenitor cells (HSPCs) and the genesis of this current strategy based on previous clinical trials using genome-edited T cells. The SB-728 zinc finger nuclease (ZFN) platform is discussed because this is the most clinically advanced genome editing technology with completed or ongoing clinical studies evaluating the safety and efficacy of ZFN-modified T cells[4] or $CD34^+$ HSPCs in human immunodeficiency virus (HIV)-infected human subjects. The ZFNs used in these studies (SB-728) were designed to target and disrupt the *CCR5* locus, which encodes the CCR5 cell

Disclosure Statement: Dr D. Ando is President of Gene Editing and Gene Therapy Consulting and is a former Sangamo Therapeutics employee. Dr K. Meyer is currently employed by Sangamo Therapeutics.

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surface protein used by HIV-1 to gain entry and infect $CD4^+$ T cells. The first clinical studies in 2009 used adenoviral transduction to deliver the genes encoding the CCR5 ZFNs to autologous T cells (SB-728-T) (NCT01044654@clintrials.gov and NCT00842634@clintrials.gov).[4] In a follow-on study, electroporation was used to deliver CCR5 ZFN mRNA to autologous T cells, resulting in the investigational product SB-728mR-T (NCT02388594@clintrials.gov). As stem cell technology advanced, an Investigational New Drug and clinical protocol were developed where the CCR5 ZFN mRNA was delivered via electroporation to autologous HSPCs (referred to as SB-728mR-HSPC) and a clinical trial was initiated in 2015 (NCT02500849@clintrials.gov). This review describes the preclinical studies conducted to support advancing these investigational genome-editing products into phase 1 clinical studies, with focus on the SB-728mR-HSPC program. An advantage of this autologous stem cell therapy is the ability to modify a subject's own HSPCs and circumvent challenges associated with allogeneic transplantation.

OVERVIEW OF ZINC FINGER NUCLEASES FOR GENOME EDITING

ZFNs consist of a zinc finger DNA binding domain (ZFP) fused to the catalytic domain of the type II Fok1 endonuclease, yielding a designer restriction enzyme capable of cleaving DNA specifically at a unique and predetermined site in the human genome.[5–7] The binding domain contains a tandem array of Cys2-His2 fingers, each recognizing approximately three base pairs of DNA (**Fig. 1**). Each Cys2-His2 zinc finger consists

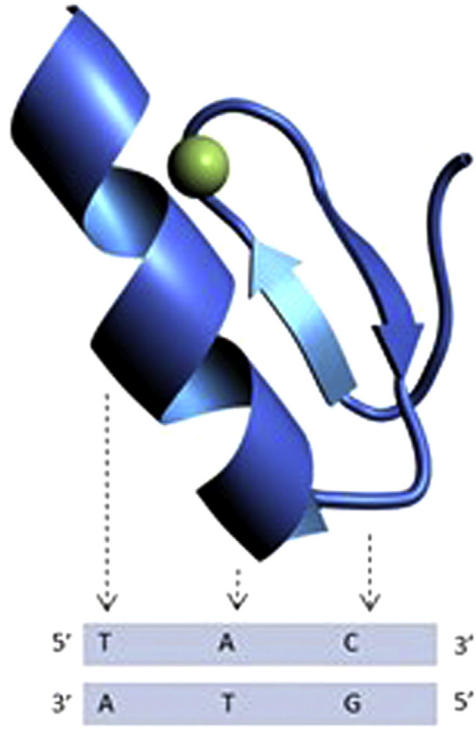

Fig. 1. The zinc finger protein is composed of ~30 residues that fold into a $\beta\beta\alpha$-structure via coordination of a zinc ion, and each finger recognizes approximately three base pairs of DNA. (*Courtesy of* Sangamo Therapeutics, Richmond, CA.)