Construction of a light-induced Chimeric Antigen Receptor (CAR) expression system

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INTRODUCTION

A great number of new trials use therapies of genetically modified T cells to express Chimeric Antigen Receptors (CARs) for the elimination of tumors. New CARs are being generated using different configurations including domains for T cell stimulation, linkers between domains and the type or number of stimulation domains for T cell activation. Synthetic biological systems have been developed using different approaches to either understand biological mechanisms or grant new functions to cells. We herein use one of the most recent optogenetic synthetic systems developed (linus2) and a brand new one created by our own group to study CAR expression effects on citotoxity and the sensibility to target's expression. To do so, in the linus2 system CAR expression is controlled by an exogenous transcription factor localized in the cytoplasm, and translocates to the nucleus once exposed to light. In our own system, the transcription factor is already in the nucleus, but partitioned in two. Upon light exposure, the two components dimerize in the functional component to promote CAR expression. We will evaluate the effects of one of the most commonly researched anti-CD19 CARs, which has shown remarkable result in patients with B-cell acute lymphoblastic leukemia (B-ALL).

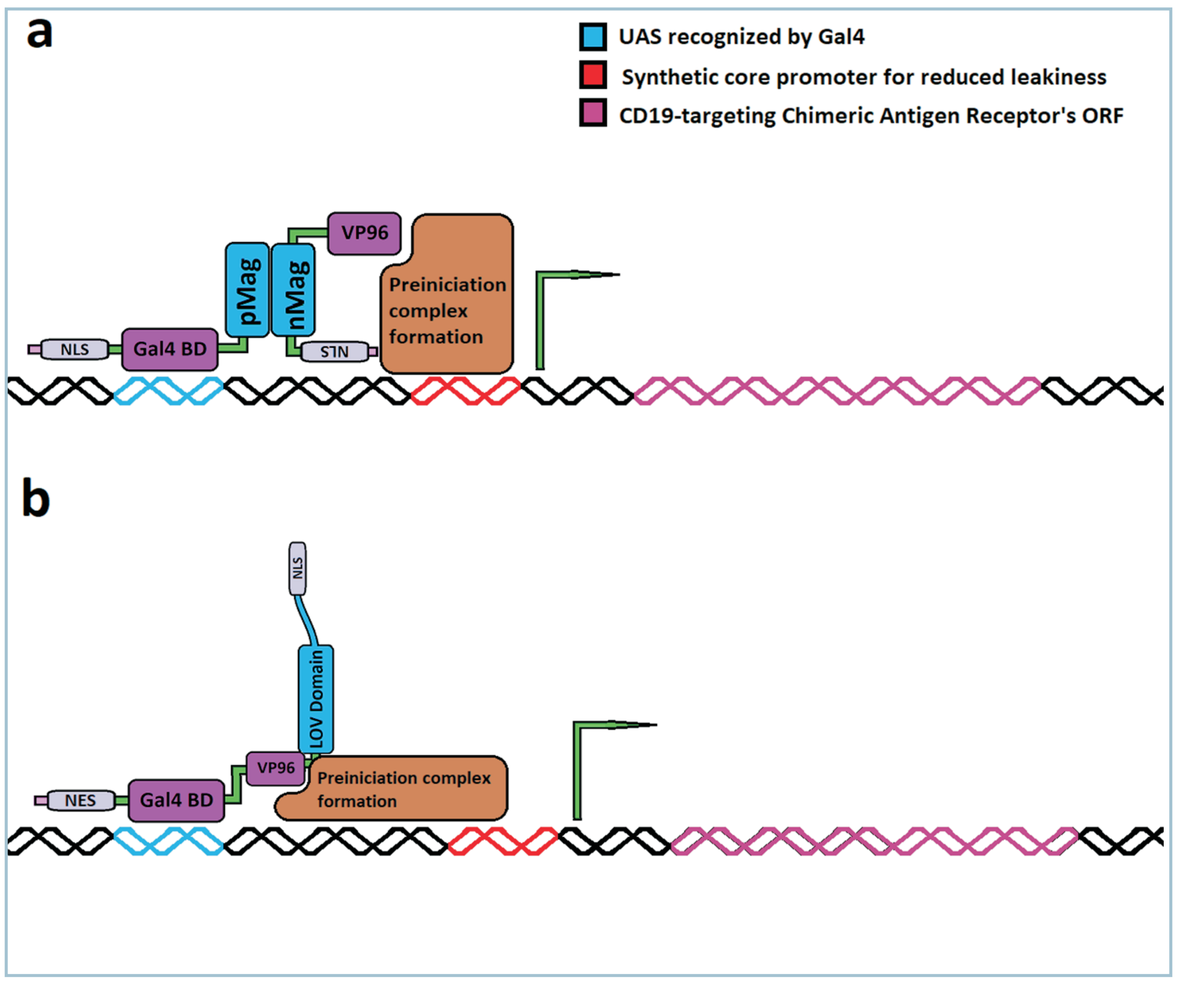

Figure 3: Representation of induction of CAR transgene by (a) pMag/nMag dimerization or (b) nuclear translocation via NLS exposure.

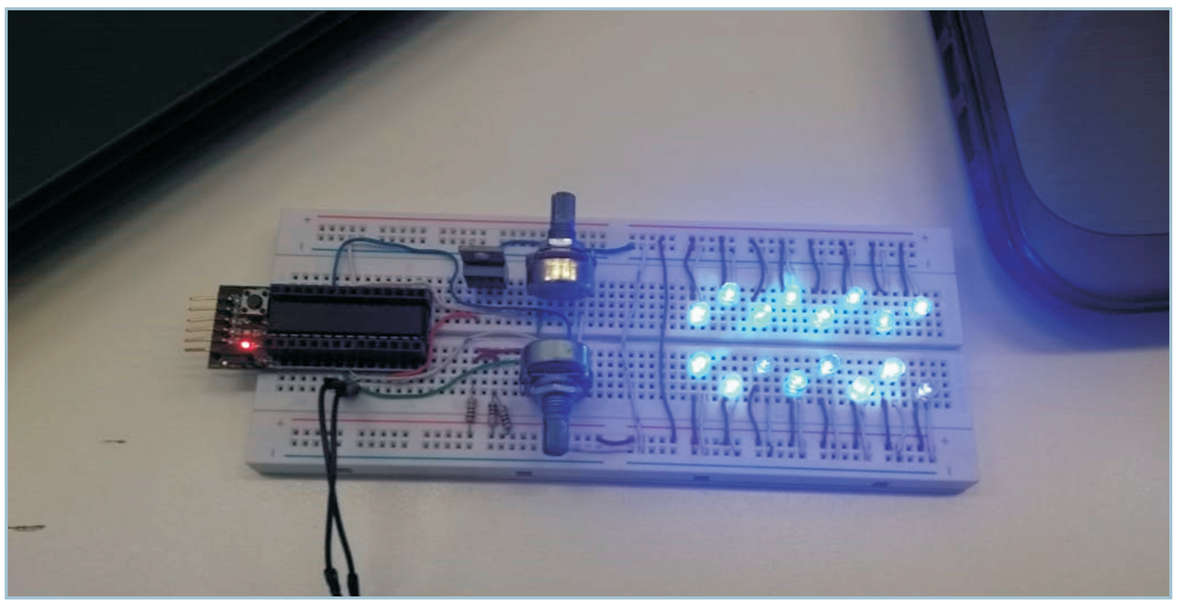

Figure 3: Module built for periodic photostimulation of the systems used. Duty Cycle adjustable by potentiometers on the sideways.

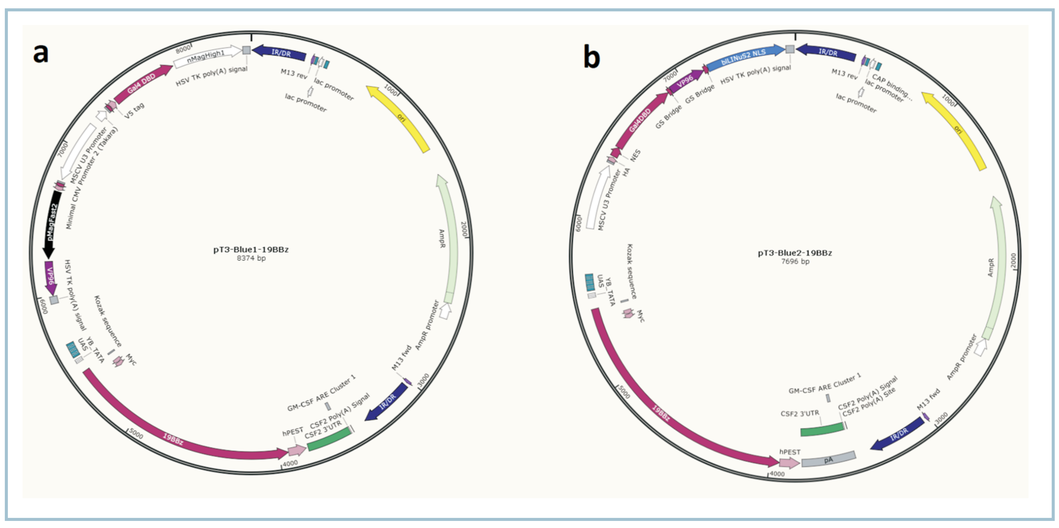

Figure 1: Plasmids pT3-Blue1 (a) and pT3-Blue2 (b) with CAR 19BBz as gene of interest.

METHODS

The CAR coding sequence will be independently cloned under the promoter controlled by each of the synthetic systems. CAR gene and each system will both be cloned together in a pT3 plasmid backbone for integration in the genome with the non-viral vector Sleeping Beauty. For this study, a Jurkat lineage previously modified to express luciferase when activated will be used to measure if different degrees of CAR expression can influence in T cell activation. The degrees of CAR expression will be measured through cytometry using CAR MFI as indicator. CAR MFI will be modulated through the systems, controlling cell's light exposure in regular time periods.

CONCLUSION

We have designed the systems for T cell activation, the equipment for stimulation has been constructed. The sequences coding for the systems to be used were sent for synthesis.

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