EVOLVING MULTIPLEXED SHRNA TO GENERATE TAILORED CAR T CELL THERAPY

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Background Manipulating protein expression to generate cells with a specific desired phenotype is one of the central goals of engineered cell therapy. Short Hairpin RNA (shRNA) is a well-established approach to reduce protein expression through the targeted degradation of messenger RNA transcripts. However, the use of shRNA in the Chimeric Antigen Receptor (CAR) T cell therapy has been limited. We have recently shown that single shRNA incorporated into a CAR expression vector can knockdown expression of the target antigen when expressed on the CAR T itself to avoid fratricide or expression of some elements of the T cell receptor (TCR) to generate allogeneic CAR T cells. An attraction of the shRNA approach is to express multiple shRNA from the same vector that can regulate protein expression thereby optimizing CAR T cell phenotype.

Methods Retroviral vectors encoding a CAR targeting a well-studied antigen (generally BCMA) co-expressing a tag for cell enrichment and identification along with shRNA multiplexed were generated. The shRNA multiplexed were inserted within a microRNA (miR) framework to enable expression from a single PolII promoter (the retroviral LTR promoter). Functional assessment of target knockdown target in T cells along with retroviral titers was determined

Results Our products in ongoing clinical development have employed a miR196a2 scaffold enabling the expression of the desired shRNA driven by the same promoter as that used for the CAR and other transgenes. Multiplexing the miR196a2 scaffold to express multiple shRNA (targeting CD247, beta 2 Microglobulin and CD95) was successful in terms of target knockdown but an obvious reduction in retroviral titer was observed. These titer reductions were variable between the duplex and triplex shRNA constructs examined but were uniformly low when considering clinical development. A proprietary scaffold was developed that coupled expression of duplexed and triplexed shRNA while also elevating vector titer by at least 2-3x.

Conclusions Multiplexing shRNA within a single vector format with scaffolds that ensure co-linked expression of the shRNA with therapeutic transgenes is a highly attractive approach to generate CAR T cells with bespoke, desired phenotypes. However, simply multiplexing shRNA using a currently clinical-used scaffold (miR196a2) resulted in reductions in vector titer. Engineering further proprietary scaffolds were produced that maintained shRNA expression but elevated retroviral titer to a level which does not preclude clinical development. These developments now provide the opportunity to develop second generation clinical candidates using shRNA multiplexed technology.

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