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Efficient shRNA-based knockdown of multiple target genes for cell therapy using a chimeric miRNA cluster platform

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Genome engineering technologies are powerful tools in cell-based immunotherapy to optimize or fine-tune cell functionalities. However, their use for multiple gene edits poses relevant biological and technical challenges. Short hairpin RNA (shRNA)-based cell engineering bypasses these criticalities and represents a valid alternative to CRISPR-based gene editing. Here, we describe a microRNA (miRNA)-based multiplex shRNA platform obtained by combining highly efficient miRNA scaffolds into a chimeric cluster, to deliver up to four shRNA-like sequences. Thanks to its limited size, our cassette could be deployed in a one-step process along with all the CAR components, streamlining the generation of engineered CAR T cells. The plug-and-play design of the shRNA platform allowed us to swap each shRNA-derived guide sequence without affecting the system performance. Appropriately choosing the target sequences, we were able to either achieve a functional KO, or fine-tune the expression levels of the target genes, all without the need for gene editing. Through our strategy we achieved easy, safe, efficient, and tunable modulation of multiple target genes simultaneously. This approach allows for the effective introduction of multiple functionally relevant tweaks in the transcriptome of the engineered cells, which may lead to increased performance in challenging environments, e.g., solid tumors.