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Aspergillus fumigatus-specific CAR T cells are effective at treating invasive pulmonary aspergillosis

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Invasive pulmonary aspergillosis (IPA), mainly caused by Aspergillus fumigatus (*A. fumigatus*), is a leading cause of mortality in immunocompromised patients. Aspergillus-specific T cells are essential for fungal clearance. Such T cells are infrequent in the endogenous repertoire and cannot be consistently isolated and expanded for adoptive immunotherapy. Therefore, we gene-engineered A. fumigatus-specific chimeric antigen receptor (Af-CAR) T cells and explored their potential in IPA therapy.

We expressed the Af-CAR in T cells using non-viral sleeping beauty transposon system. We verified the CAR expression and specificity by flow cytometry, microscopy, calcium flux, and IFN-? secretion. In co-cultures with *A. fumigatus* hyphae, we analyzed the antifungal activity of Af-CAR T cells alone or together with innate immune cells, we measured cytokine secretion, ROS production, and hyphal damage. Finally, we analyzed Af-CAR T cells therapeutic potential *in vivo*.

T cells expressing the Af-CAR recognized *A. fumigatus* strains and clinical isolates and showed no off-targets in the human lung or cross-reaction with other fungi. $CD8^+$ Af-CAR T cells released perforin and granzyme B and exerted a direct anti-fungal effect against *A. fumigatus* hyphae. Furthermore, $CD4^+$ and $CD8^+$ Af-CAR T cells produced mainly Th1 cytokines that activated macrophages and neutrophils to potentiate the anti-fungal effect. In an in vivo model of IPA in immunodeficient mice, $CD8^+$ Af-CAR T showed a higher potential to reduce fungal burden than $CD4^+$ T cells. They controlled the local inflammation in the lung by recruiting mainly mononuclear cells and reducing the damage induced to the lung. Adoptive transfer of $CD8^+$ Af-CAR T cells in a neutropenic IPA mouse model improved overall survival.

Taken together, our study illustrates the potential of gene-engineered T cells to treat aggressive infectious diseases that are difficult to control with conventional antimicrobial therapy and support the clinical development of Af-CAR T cell therapy to treat IPA.