

## IMMUNOTHERAPEUTIC TARGETING OF AML WITH A NOVEL CD123 CAR

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Chimeric antigen receptors (CARs) are engineered receptors transduced into immune effector cells that combine the antigen binding abilities of an antibody with the cytotoxic potential of T cells. CARs are made up of an antigen recognition domain derived from a monoclonal antibody, linked through hinge and transmembrane domains to a costimulatory domain and a CD3 $\zeta$  intracellular signaling domain. The result is a high-specificity receptor targeted against a specific tumour associated antigen (TAA).

Recently, CARs have moved to clinical trials with some dramatic successes, especially in the case of the well-studied CD19 CAR. CD19, however, is only expressed in a certain subset of malignancies. Thus, current work is focused on identifying TAAs on a range of other malignancies that may similarly be targetable by CAR therapy.

CD123 is a TAA that is known to be expressed on AML cells. This antigen plays a role in proliferation and apoptotic resistance, and is associated with poor prognosis in patients. While it is upregulated on AML cells, it demonstrates much lower expression levels on normal cells in the myeloid progenitor subpopulation, thus CD123 is a viable target for a CAR immunotherapy targeting AML.

Novel anti-CD123 antibodies were isolated and sequenced by the Medin lab. The V<sub>L</sub> and V<sub>H</sub> chains were assembled in-frame (in both orientations) into a full CAR sequence. This construct was then subcloned into a lentiviral backbone to facilitate expression in immune effector cells. *In vitro* testing is currently underway to confirm binding specificity and cytotoxic potential of the resulting CAR-expressing effector cells. In our future work, these CD123-directed CAR effector cells will be tested *in vivo* using mouse models of AML. Finally, as we are building a GMP Vector Facility in Toronto to generate lentivectors for clinical trials, these constructs will be fast-tracked into production/pre-clinical testing should experimental results warrant.