Functional screening of an anti-B7H6 specific chimeric antigen receptor (CAR)

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**Background**

Efficacious T cells by means of a chimeric antigen receptor (CAR) has recently provided potentially transplantable efficacy in different clinical trials with hematological cancers. CARs generally comprise a single-chain variable fragment antibody (scFv) for tumor antigen recognition coupled to an intracellular CD3ζ signaling domain and, depending on the CAR generation, one or two transducing domains (e.g., CD28 or ICOS). Open engagement of the CAR with its target, a specific antigen stimulation resulting in the tumor cell killing. CAR design aimingpler CR and activating receptors represent a valuable tool for successful tumor elimination. Indeed, each receptor recognizes tumor-bound self-antigens. B7H6-B7H6 expression was associated with tumor progression, poor prognosis and relapse. 

**Methods**

Firstly, all BTM-1 T cell strategy would represent a potentially safe, attractive approach to target a broad range of high risk cancers.

**Results**

btm-1 CAR designs and expression in human T cells

Celyad’s anti-B7H6 CARs were constructed by fusing the scFv, derived from a human or mouse anti-B7H6 antibody, against human CD3ζ and CD28 (CD3ζ:CD28, B7H6 scFv). CARs were then tested in a fixed T cell line model in vivo humanized tumor-bearing xenograft model (B7H6+ tumor-bearing xenografts) serving as a surrogate test of CAR efficacy (Figure 1A).

In order to assess the surface expression of the human anti-B7H6 CAR, transduced human T cells were stained with an anti-C100 monoclonal antibody and analyzed by flow cytometry. As shown in Figure 1B and 1C, 3 generations of CAR T cells were generated after transduction efficiency was comparable to 3rd generation-CARs. BTM-1 is expressed in multiple tumor cell lines.

In order to identify potential candidates for in vivo BTM-1 CAR T cell therapy, a large panel of human cancer cell lines representative of broad and solid tumor of different origins was screened for BTM-1 expression by flow cytometry and analyzed by microarray analysis of expression.

Membrane BTM-1 expression on human tumor cell lines

BTM-1 expression on human primary tumor samples was evaluated by immunohistochemistry using the antibody from which the CAR was constructed and correlated. During this study, 30 neuroendocrine, 25 colorectal and ovarian cancer biopsies were scored for membrane BTM-1 expression according to the IHC method, considering both the intensity and the distribution of the staining. Microarray analysis of membrane BTM-1 expression on human neuroendocrine tumors was performed, highlighting the broad clinical applicability for BTM-1 CAR T cell therapy.

**Conclusions and Perspectives**

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In conclusion, our data suggest that:

- The CAR T cells were able to recognize and eliminate tumor cells expressing the target antigen.
- The CAR T cells displayed tumor-specific cytotoxicity.
- The CAR T cells demonstrated a high degree of specificity.
- The CAR T cells were able to generate a strong immune response.

**References**

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cell activity was measured using a flow cytometry-based assay and analyzed by microarray analysis. As shown in Figure 1B, 3 generations of CAR T cells were generated after transduction efficiency was comparable to 3rd generation-CARs. BTM-1 is expressed in multiple tumor cell lines.

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