A Novel Conditionally Active Biologics Approach to Minimize On-target Off-tumor Effects in Adoptive Immunotherapy

Jianfang Hu[1], Benjamin Lopez[1], Tiffany Lam[1], Anirban Kundu[2], Timothy Mayall[1], Gregory Schreiber[3], Farzad Haerizadeh[1], James Onuffer[1], Gregory Frost[3].

[1]F1 Oncology, Inc, San Diego, CA; [2]Exuma Biotechnology SEZC, Georgetown, Cayman Islands; [3]F1 Oncology, Inc, West Palm Beach, FL

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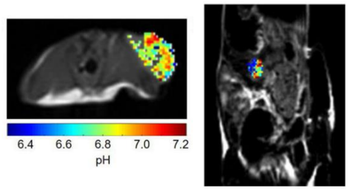
Key Messages

- We described a novel approach to minimize on-Target off-tumor effects in CAR-T cell therapy in solid tumor.
- We developed CAB-CARs by exploiting the unique property of TME that is an acidic extracellular pH environment.
- Our CAB-CAR-T cells display "AND logic gate" properties, requiring both antigen presence and TME conditions for optimized activity.
- Our CAB-CAR technology provides an opportunity to develop safer CAR-T therapeutics for solid tumor.
- By leveraging this technology, we can develop CARs against a wider range of antigens that were previously un-targetable, such as ones present in normal tissues.

Introduction

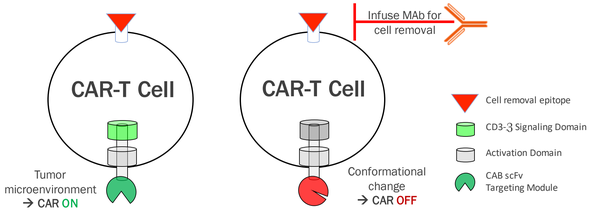
Adoptive immunotherapy using chimeric antigen receptor (CAR) modified T cells has demonstrated promising antitumor effects in hematologic cancers leading to the recent approval of two CAR-T products targeting the B cell receptor associated protein CD19, however the safe development of CAR-T therapies for solid tumor malignancies remains less advanced in part due to the lack of precision in targeting solid tumor antigens. Identification of clinically viable tumor restricted target antigens while avoiding on-target off-tumor toxicities in normal tissue presents a significant challenge in the solid tumor CAR-T therapeutic scenario. Here we describe a novel approach to achieve antigen target specificity for CAR-T therapy in solid tumors by harnessing the unique properties of the tumor microenvironment (TME) itself. The altered glycolytic pathway of the Warburg effect within the TME drives lactic acid production leading to an opportunity to develop novel CARs with functional TME dependent switching ability (Warburg O 1956).

The excess lactic acid produced by cancer cells results in an acidified tumor micro-environment

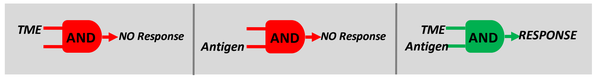


pH maps of flank and orthotopic tumor models (Jones KM et al. 2016)

F1's CAB-CAR-T Core System

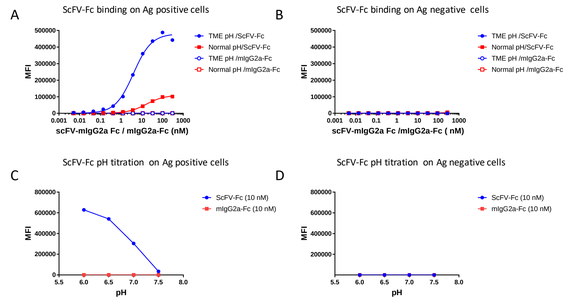


F1's CAB-CAR-T cells display "AND logic gate" properties, requiring both antigen presence AND TME conditions for activity



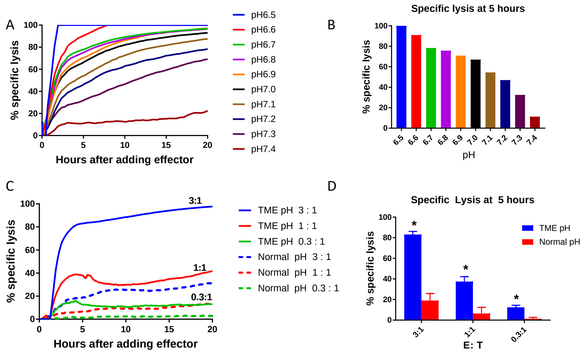
Results

Fig 1: Binding capacity of F1's CAB-ScFV-Fc protein



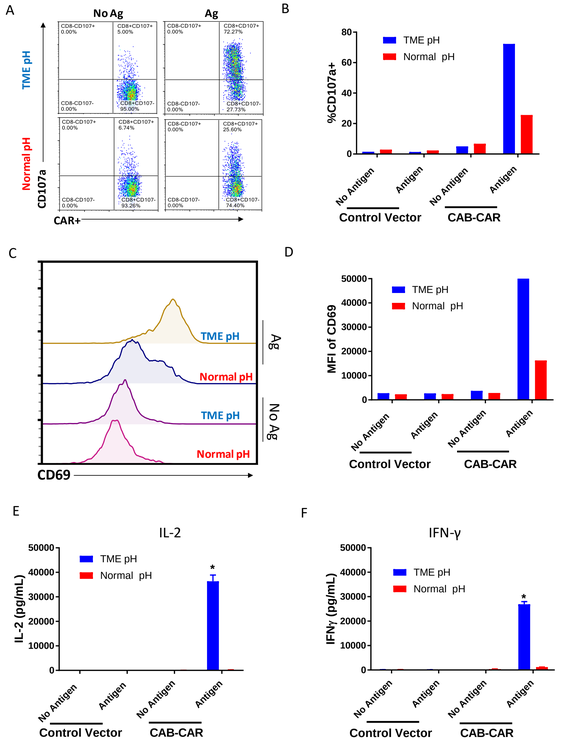
CAB-ScFV-Fc protein requires both antigen presence and TME conditions for optimized binding. (A & B) CAB-ScFV-Fc or mIgG2a-control-Fc protein were cocultured with Antigen-positive or Antigen-negative cells at different concentrations under TME or Normal pH buffer, then fixed, stained with anti-mIgG2a-PE and measured by flow cytometry. (C & D) CAB-ScFV-Fc or mIgG2a-control-Fc protein were cocultured with Antigen-positive or Antigen-negative cells at 10 nM under indicated pH buffer, then fixed, stained with anti-mIgG2a-PE and measured by flow cytometry.

Fig 2: Cytolytic activity of F1's CAB-CAR-T cells



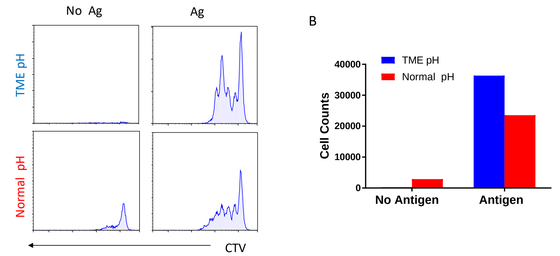
F1's CAB-CAR-T cells killed Antigen-positive cells more efficiently under lower pH conditions. Ag-positive target cells were seeded to an E-plate with indicated pH medium one day prior to the experiment. Effector cells were collected after two day's recovery and added into experimental wells with corresponding medium. Impedance readings were taken by a real time, impedance-based instrument (xCELLigence) and reported as the Cell Index (CI). Percentage of specific cytolysis was calculated by normalized to lysis of control cells. (A&B) Specific lysis at effector to target cell ratios (E/T) of 3:1 under indicated pH medium. (C&D) Specific lysis at different E/T ratios under TME and normal pH medium.(* P<0.05 by t-test) .

Fig3: Activation of F1's CAB-CAR-T cells



F1's CAB-CAR-T cells require both antigen presence and TME conditions for optimized activation. F1's CAB-CAR T or control vector transduced cells were rested for 2 days with human T cell medium in the presence of 100 IU/mL IL-2. After two days recovery, effector cells were collected and cocultured with Antigen-positive or Antigen-negative target cells at CAB-CAR+ effector cell/target cell ratios (E/T) of 3:1 with either TME or normal pH medium. (A&B) Culture was performed in the presence of CD107a antibody. CD107a expression was measured after 5 hour by flow cytometry and quantified on CD8+ CAR+ T cells. (C&D) CD69 expression was measured after 24 hours by flow cytometry and gate on CD3+CAR+ T cells. (E&F) Supernatants were collected after 24 hours and IL-2 and IFNγ secretion were measured by ELISA. (* P<0.05 by t-test) .

Fig 4: Proliferation of F1's CAB-CAR-T cells in response to antigen stimulation



F1's CAB-CAR-T cells require both antigen presence and TME conditions for optimized proliferation. F1's CAB-CAR-T cells were rested for 2 days with human T cell medium in the presence of 100 IU/mL IL-2. Rested CAB-CAR-T cells were labeled with 5 uM of Cell Trace Violet (CTV) then cocultured with Antigen-positive or Antigen-negative cells at 3:1 (E:T) ratio under TME or normal pH condition without IL-2 for 5 days. (A) Following coculture, CTV dilution was measured by flow cytometry (CD3 gated). (B) Live CAR-T Cells recovered from coculture.

Conclusions

Here we demonstrate:

1. scFv-Fc fusion protein of CAR constructs demonstrates TME restricted target antigen engagement.
2. Our CAB-CAR-T cells efficiently lyse antigen positive target cells under low pH condition or TME condition , pH 6.7, and it shows minimal activity at pH 7.4 (or physiological condition or normal tissues) as demonstrated by a cellular impedance based kinetic killing assay.
3. The cytolytic activity of CAB-CAR-T incrementally increases as extracellular pH is decreased.
4. CAB-CAR-T activation, as measured through the early T cell surface activation marker CD69, the degranulation marker CD107a, cytokine release (IL-2 and IFN-Gamma) and proliferation, requires both antigen presence and TME conditions.

Conditionally active chimeric antigen receptor T cells (CAB-CAR-T) harness unique properties of the tumor microenvironment to provide an opportunity to develop safer CAR-T therapeutics for solid tumor by minimizing on-target off-tumor activity.

References

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Acknowledgements

We would like to thank our colleagues at F1 Oncology and Exuma Biotechnology for their help and support. Thanks to our management team for their exceptionally helpful comments.