Engineering ICAM-1 Specific CAR T Cells Secreting BiTEs Against Gastric Cancer

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Background

CAR T cell immunotherapy has had much clinical advancement in hematological cancers. However, their efficacy for solid tumors has limited success mainly due to factors such as antigen escape, immune suppression, and T cell exhaustion (Marofi et al., 2021)..
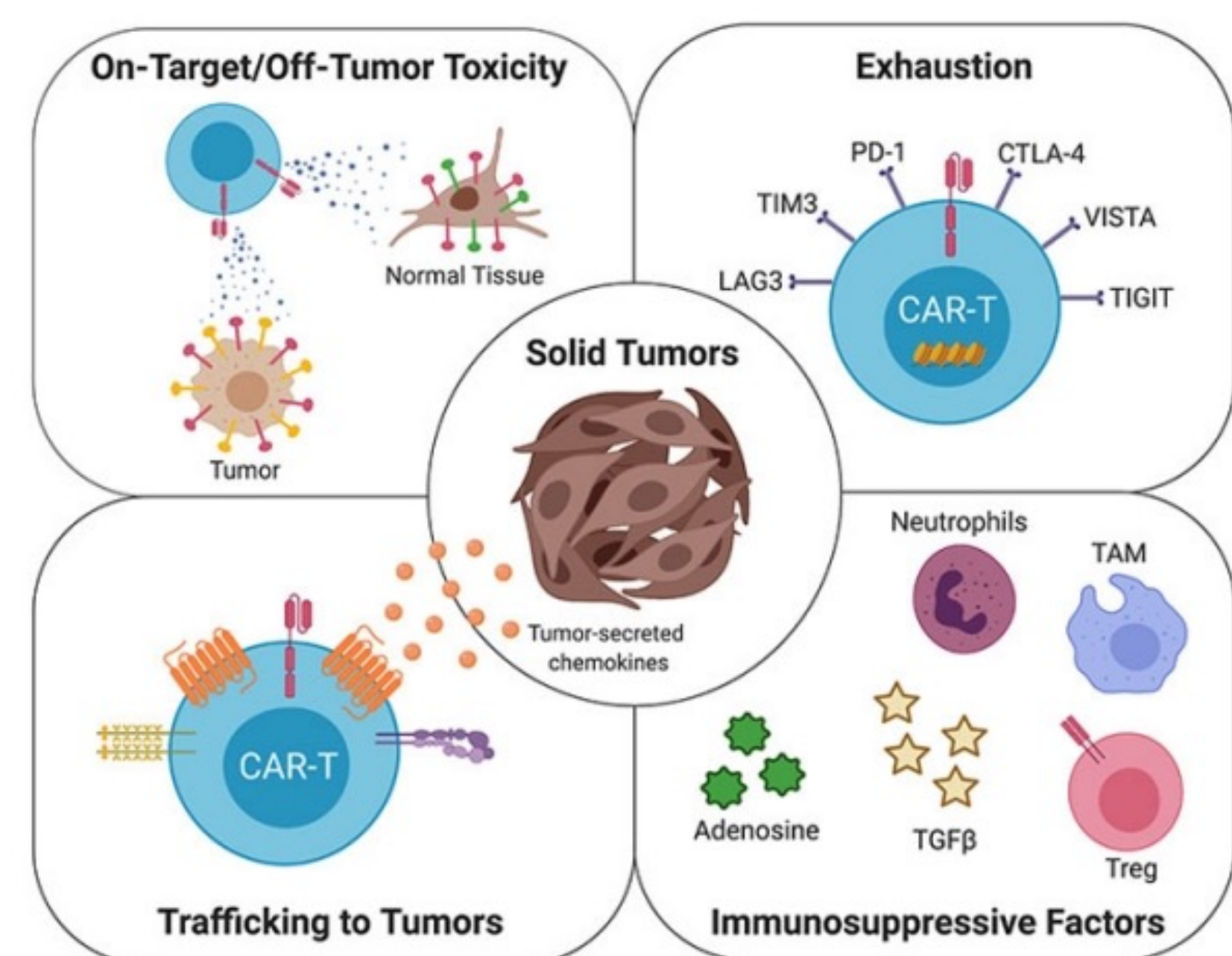

Figure 1: Common Issues in CAR T Cell Immunotherapy (Fonkoua et al., 2022)

Bispecific T-cell engagers (BiTEs) can redirect T cells to specific tumor antigens and activate T cells directly, independent of the major histocompatibility complex (MHC), unlike CAR-T cells (Goebeler, 2020). Here, we aim to develop a CAR and BiTEs specific for ICAM-1, an antigen associated with poor prognosis in gastric cancer patients (Jung et al. 2020).
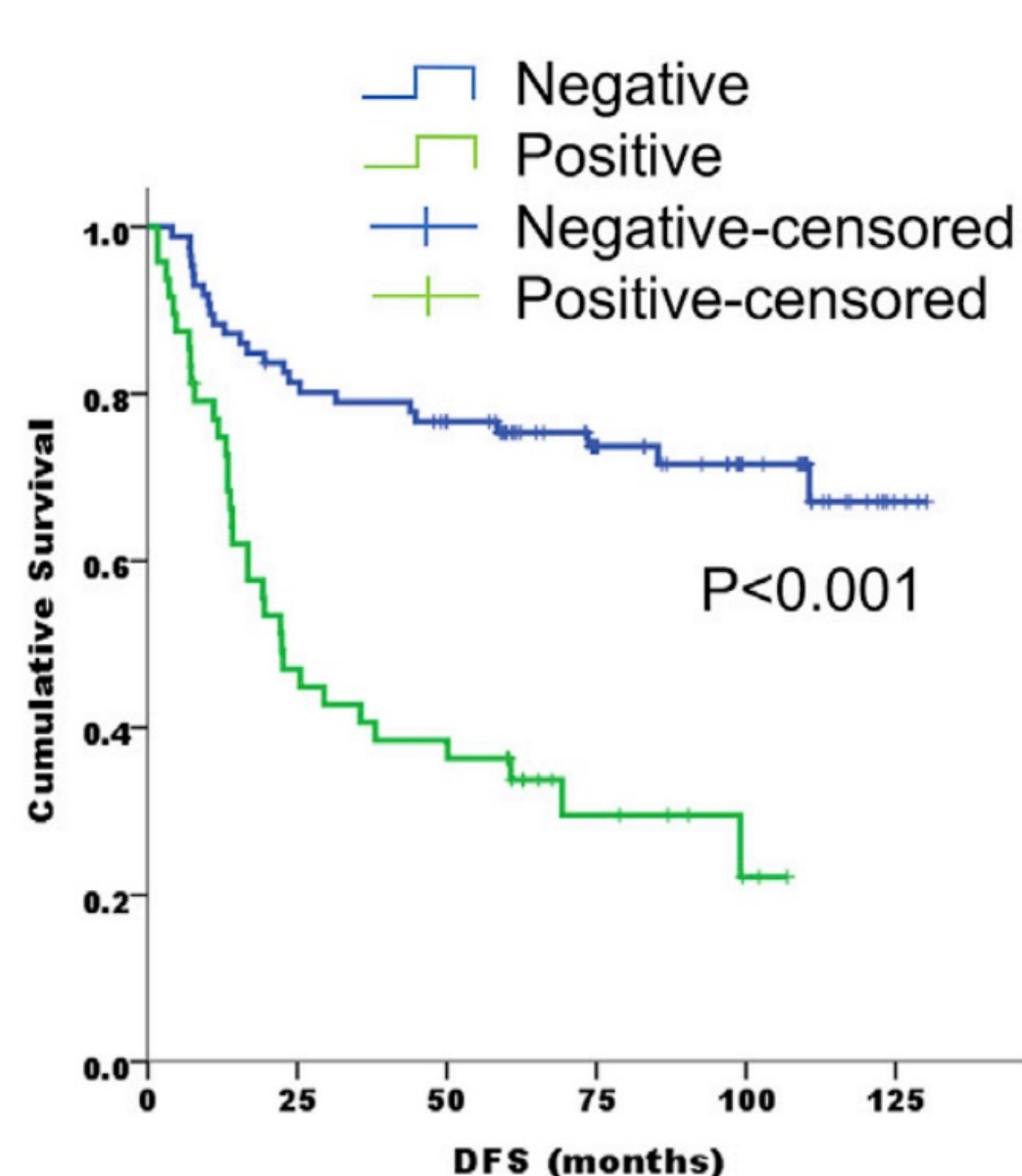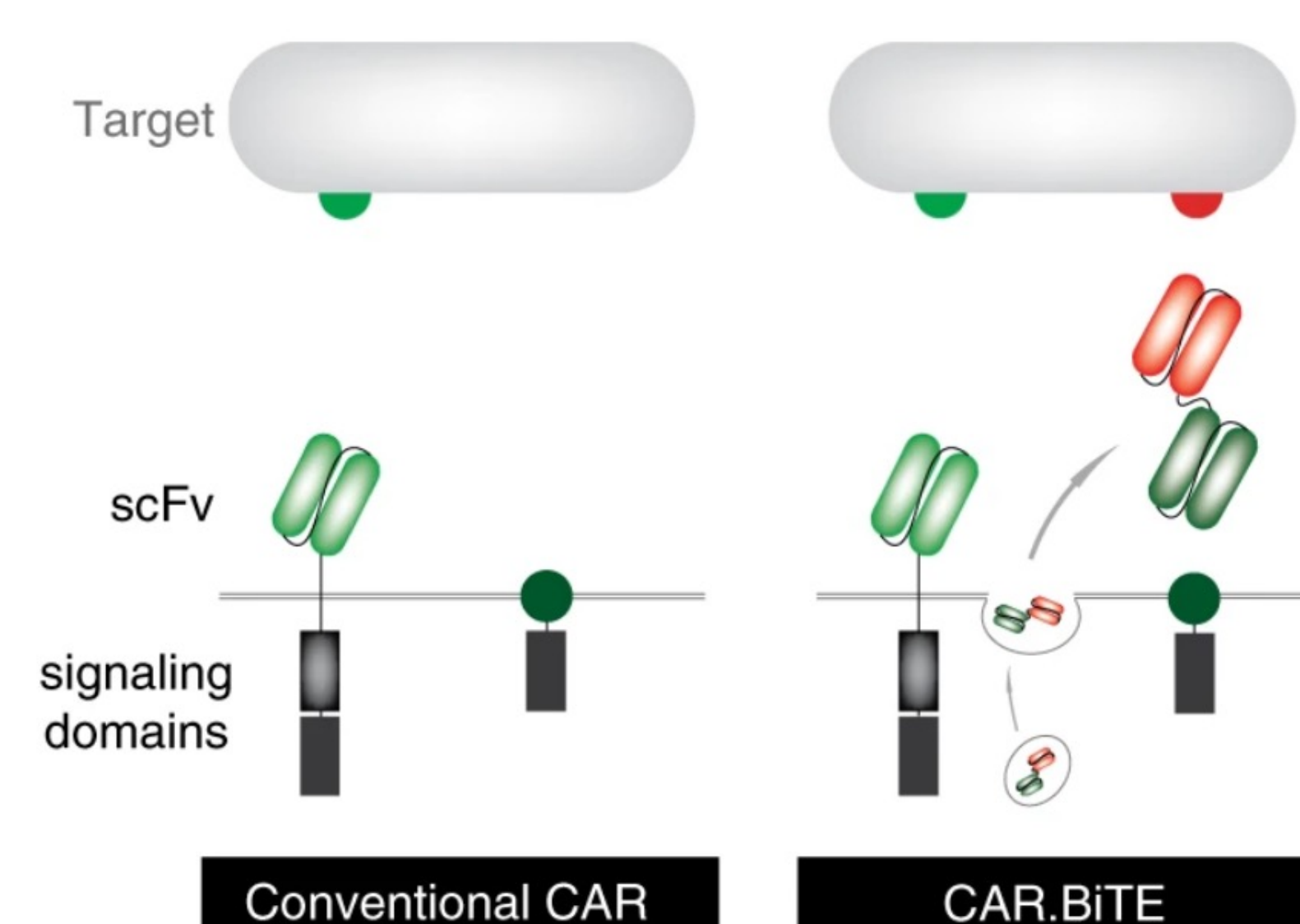

Figure 2: ICAM-1 Expression Is Associated with Poor Prognosis in Gastric Cancer Patients : Kaplan-Meier plots indicating a significant difference in disease-free survival between ICAM-1- and ICAM-1+ gastric cancer patients (n = 134). (Jung et al., 2020)

Figure 3: Mechanisms of Conventional CAR T-Cell Therapy and CAR T cells Secreting BiTEs (Yin et al., 2022)

We hope to use an approach that strategically combines CARs with BiTEs into a single gene-modified T-cell product and demonstrate how this platform can be used to address critical barriers to effective immunotherapy of solid tumors, specifically ICAM-1 positive gastric cancer patients.

Results

Development of ICAM-1 Specific BiTE

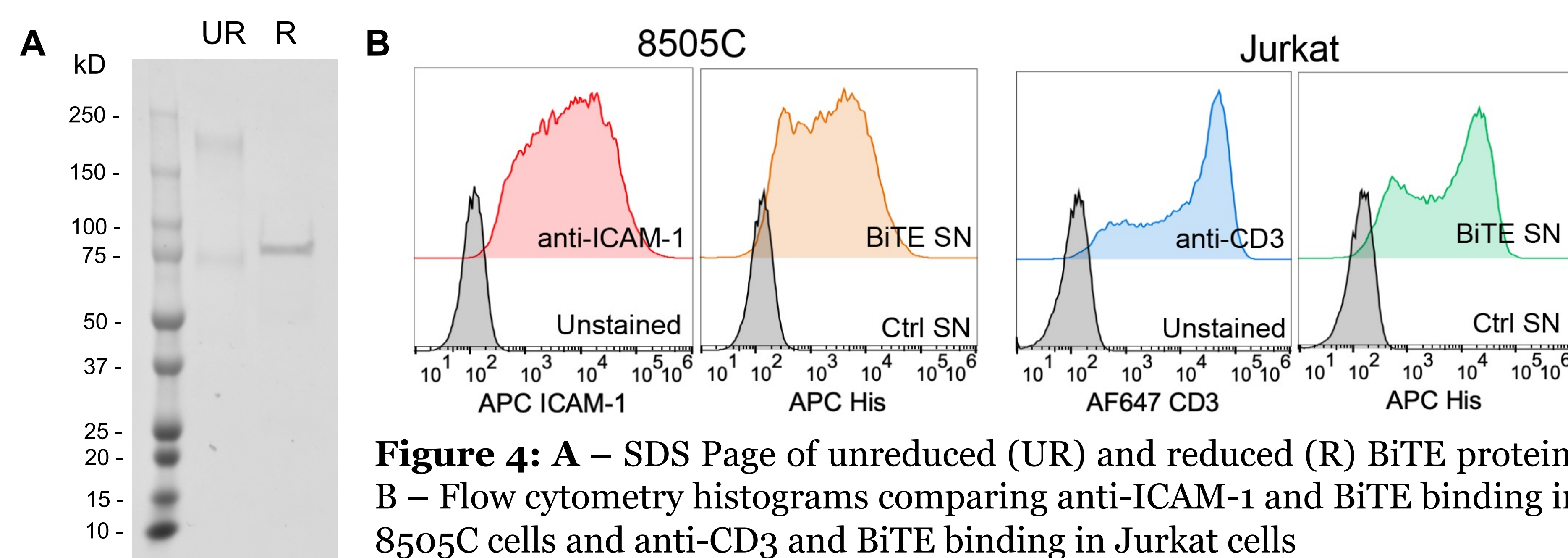**ICAM-1 BiTE Effectively Binds to 8505C & Jurkat T Cells**

Figure 4: A – SDS Page of unreduced (UR) and reduced (R) BiTE protein; B – Flow cytometry histograms comparing anti-ICAM-1 and BiTE binding in 8505C cells and anti-CD3 and BiTE binding in Jurkat cells

BITE Induces Immune Cell Killing in ICAM-1 Expressing Cell Lines

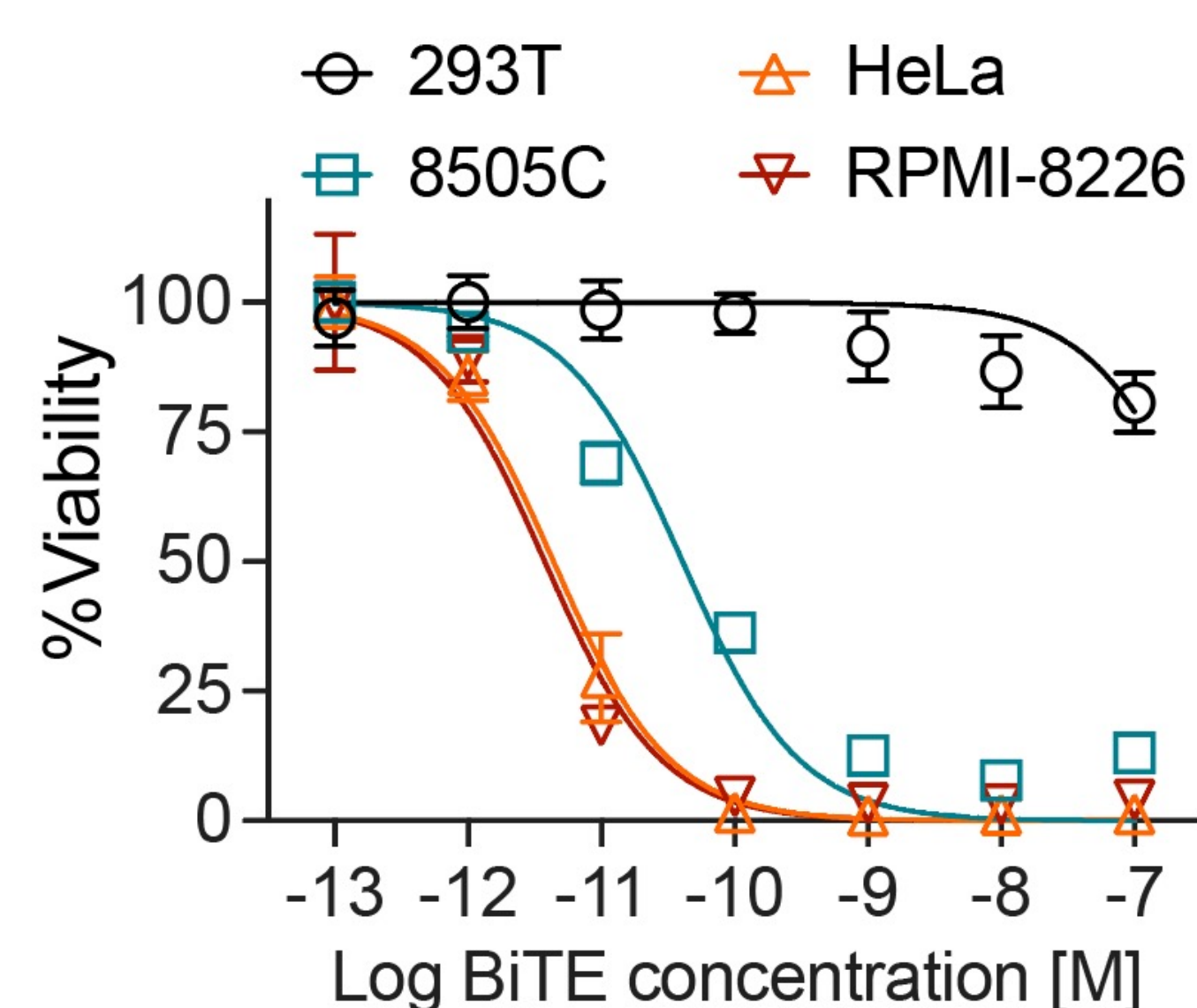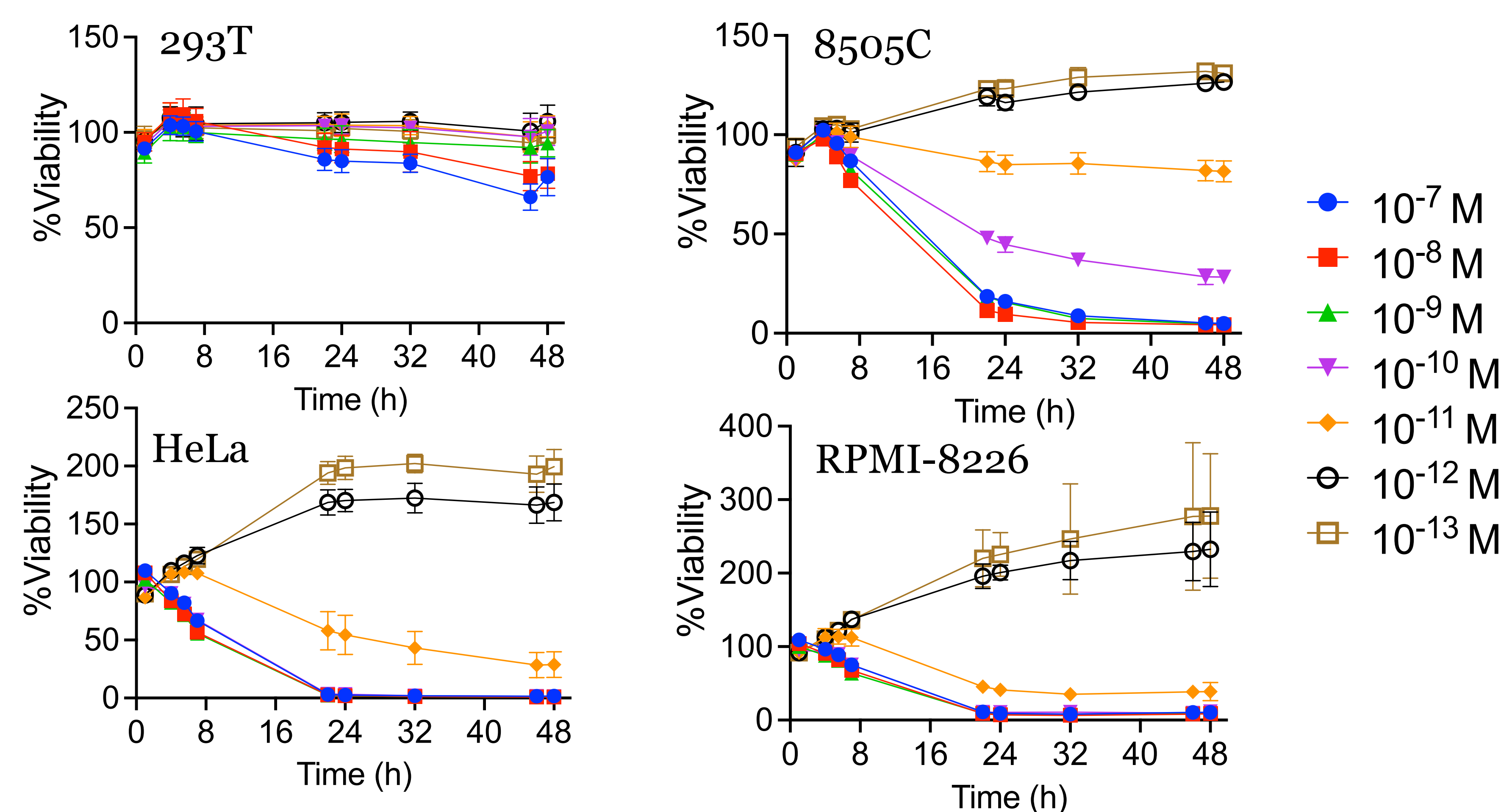| Target cells	ICAM-1 expression	EC_{50}
293T	0	0.41 ± 0.15 μM
8505C	++	41.4 ± 12.0 pM
HeLa	+++	4.5 ± 1.3 pM
RPMI-8226	+++	3.8 ± 0.4 pM

Figure 5: Effector-to-target assay measuring lysis of target cells by ICAM-1 BiTE induced T cells (E:T = 5:1). Live cells was measured by bioluminescence intensity normalized to the level of target cells co-cultured with non-transduced T cells. Data represent mean ± SD of triplicate wells.

Development of CAR.BiTE Complex
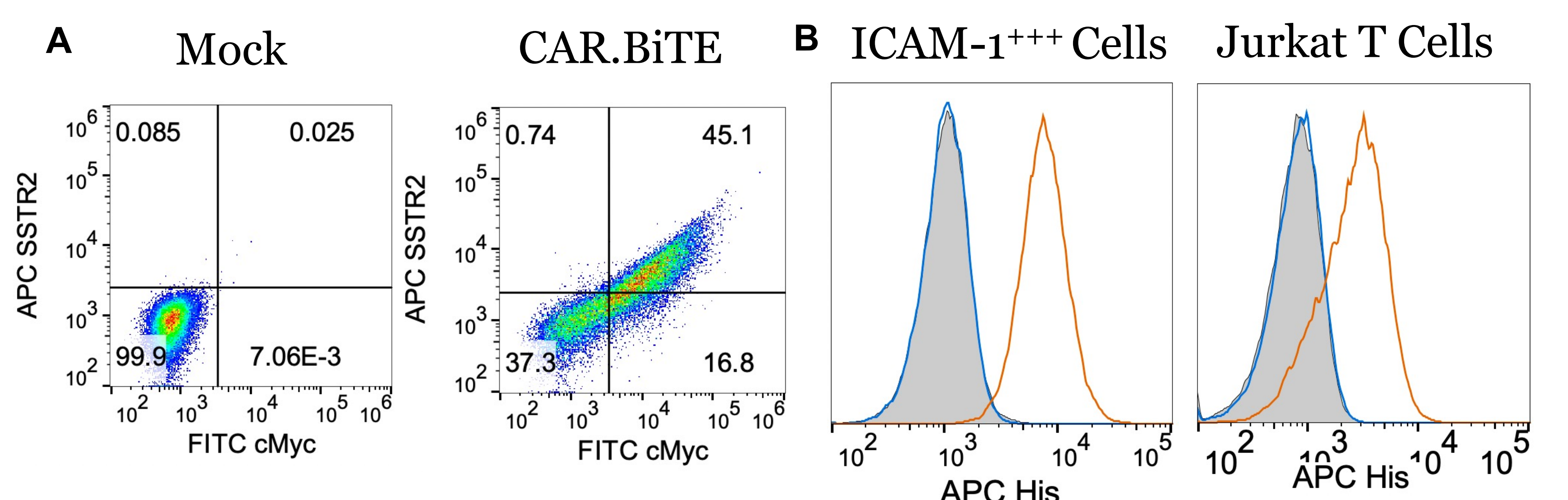
Development of CAR T cells secreting ICAM-1 BiTE

Figure 5: A – Flow cytometry plots showing CAR.BiTE expression determined by anti-cMyc and anti-SSTR2 antibodies; B – Flow cytometry histograms showing CAR.BiTE binding to I-CAM expressing gastric cancer cell line (SNU-638) and to Jurkat T cells

Conclusion

- ICAM-1 BiTE protein complex effectively binds to 8505C ICAM-1++ gastric cancer cells and Jurkat T cells
- ICAM-1 specific BiTEs induces immune killing of gastric cancer cells lines with high ICAM-1 expression
- ICAM-1 CAR.BiTE shows high expression in transduced Jurkat T cells
- ICAM-1 CAR.BiTE effectively binds both ICAM-1++ gastric cancer cells and Jurkat T cells

Future Perspectives
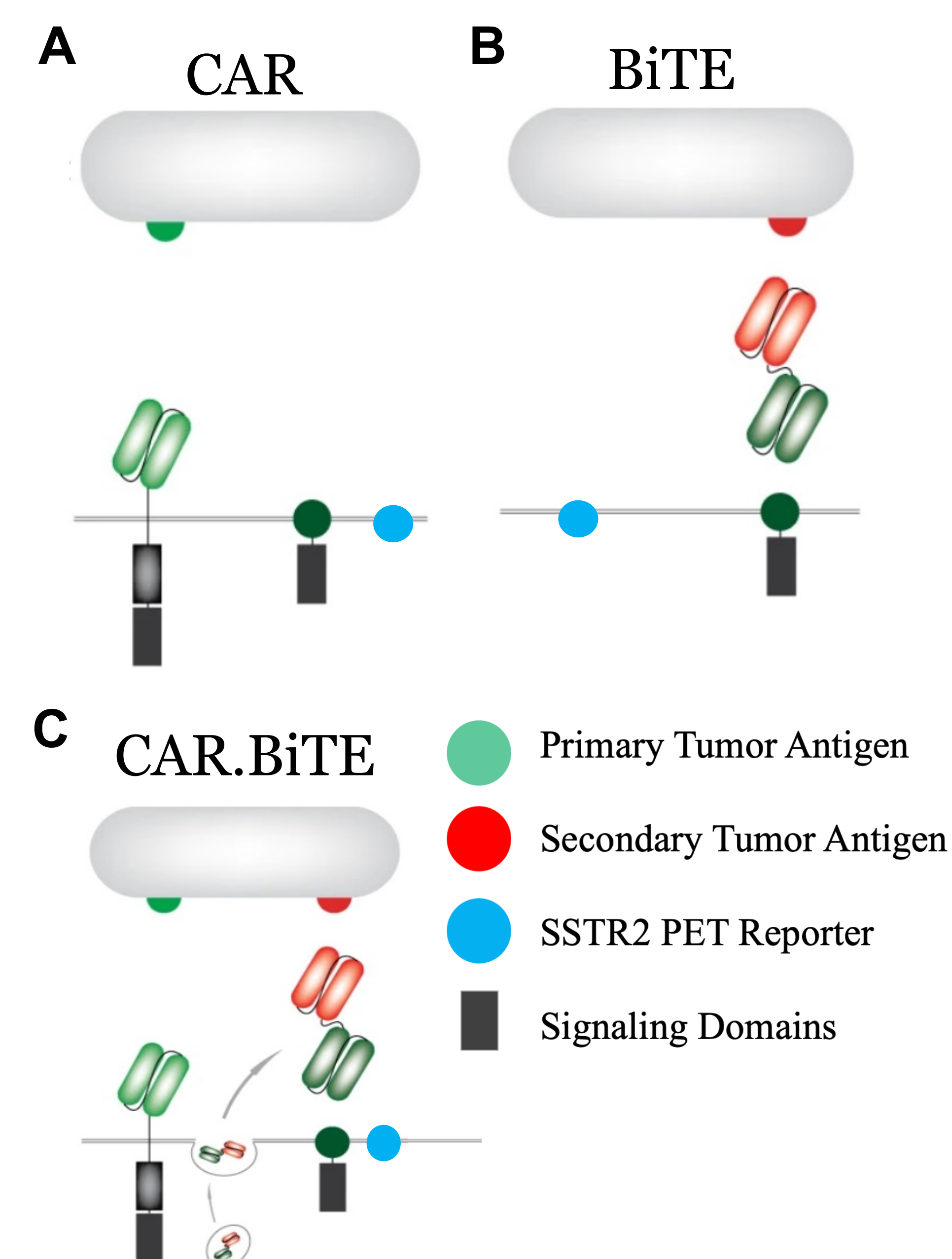
- In-vivo studies to determine the efficacy of the CAR.BiTE complex
- Other combinations of primary and secondary tumor targets

Acknowledgments

More Information: