Abstract 4780 Engineered ICAM-1-refractory trastuzumab improves its therapeutic activity against HER2-positive cancers in native and antibody-drug conjugate (ADC) format

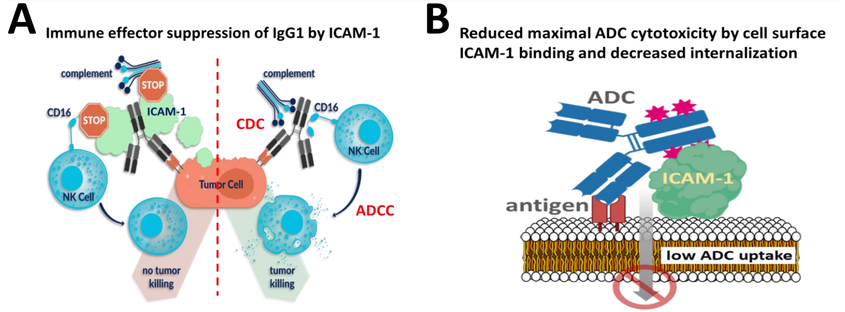
J. Bradford Kline, Luigi Grasso, and Nicholas C. Nicolaides*- Navrogen Inc. Cheyney, PA - nick@navrogen.com

navrogen
Leaders in Humoral Immuno-Oncology

Abstract

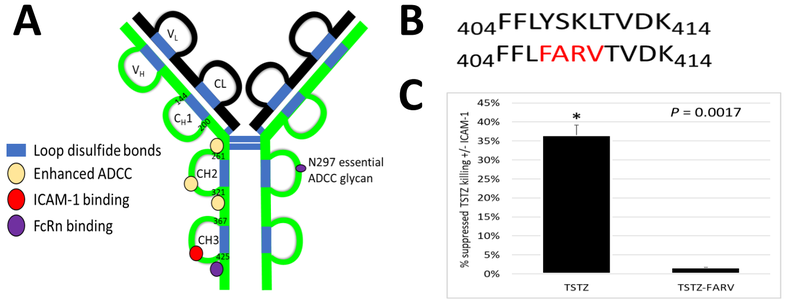
The ICAM-1 protein has recently been found to bind the CH3 domain of IgG1-type antibodies and suppress their immune effector activity and internalization against ICAM-1 overexpressing cancer cells (1). ICAM-1 immune effector suppression of monoclonal antibodies (mAbs) involves reduced binding of IgG1 to CD16a Fc-γ-receptors on NK cells leading to reduced antibody-dependent cellular cytotoxicity (ADCC) as well as binding of the complement initiating C1q protein, leading to reduced complement-dependent cytotoxicity (CDC) via allosteric alteration of the Fc domain. The effect of ICAM-1 on reduced antibody internalization, a requisite for antibody-drug conjugate (ADC) cytotoxicity (2), has shown significant suppression of ADC activity against antigen-positive target cells. We identified a consensus motif (YSKL) within the IgG1 CH3 domain (aa 407-410), when modified, leads to ICAM-1-refractory antibodies thereby restoring maximal immune effector and ADC cytotoxicity on target cells. Here, we present the development of an ICAM-1-refractory trastuzumab and its efficacy against HER2 cancer cells overexpressing ICAM-1 in its native antibody and ADC format. These data demonstrate the importance of how tumor-produced proteins may negatively impact the cytotoxic effects of tumor-targeting antibodies and offers a novel platform to improve upon the efficacy of antibody-based therapeutics, such as trastuzumab, for commercially approved indications (3) in the presence of the immunosuppressive ICAM-1 protein.

Fig. 1 – ICAM-1 binding to IgG1 suppresses maximal immune effector and ADC cytotoxicity



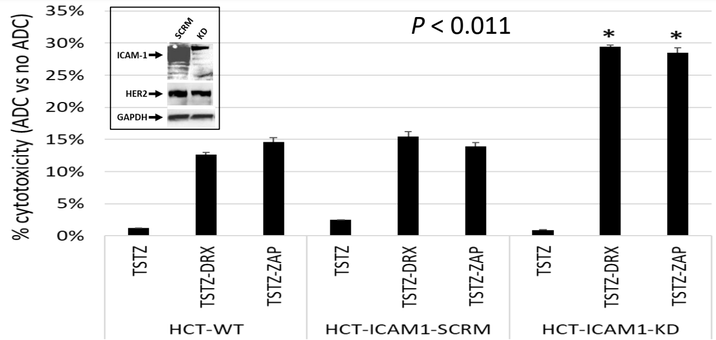
Schematic depicting ICAM-1 immunosuppression on (A) immune effector [ADCC and CDC] activity of native IgG1 and (B) maximal ADC cytotoxicity. Immunosuppression of IgG1 involves ICAM-1 alteration of the Fc domain suppressing IgG1 binding to Fc-γ-receptor (ADCC) on NK cells and C1q protein (CDC). Immunosuppression of ADC involves binding to ICAM-1 and reduced cellular internalization, a requisite for maximal cytotoxicity.

Fig. 2 – Identification of the ICAM-1 binding motif in the IgG1 CH3 domain (aa 407-410) and generation of ICAM-1-refractory native trastuzumab (TSTZ) and TSTZ ADC



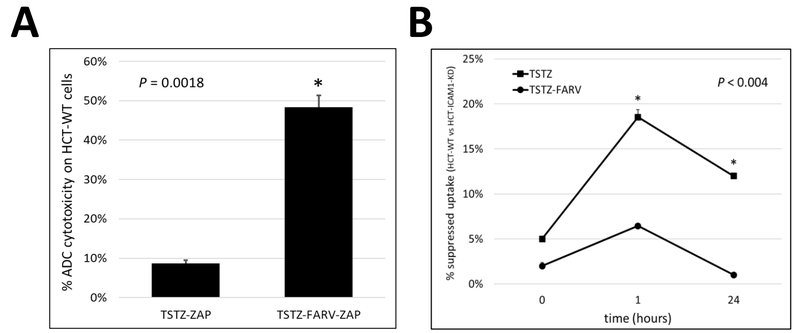
(A) Summary of published motifs in IgG1 that affect immune effector ADCC activity (yellow & N297 glycan) and systemic circulation via FcRn (purple). Mutational mapping identified the ICAM-1 binding site (red) at amino acids 407-410 within the CH3 domain. (B) ICAM-1 motif and alterations (red) that make IgG1 refractory to ICAM-1 binding. (C) ADCC assay of exogenously added ICAM-1 on parental and FARV-modified trastuzumab (TSTZ) shows enhanced ADCC of TSTZ-FARV, confirming ICAM-1 suppressive effect.

Fig. 3 – TSTZ ADCs are more effective in isogenic ICAM-1 null target cells vs parental cells



The ADCs TSTZ-DRX (deruxtecan) and TSTZ-ZAP are significantly enhanced when ICAM-1 is removed using shRNA (HCT-ICAM1-KD) in HER2⁺ HCT116 cells as compared to HCT wild type (HCT-WT) and shRNA ICAM-1 scrambled (HCT-SCRM) lines. Percent cytotoxicity is determined using an optimal concentration of ADC and comparing cell killing vs no ADC treatment. Western blot confirms steady-state level of ICAM-1 and HER2 across cell lines.

Fig. 4 – TSTZ-modified ADC has enhanced target cell internalization and cytotoxicity



(A) Cytotoxicity of TSTZ-ZAP and the ICAM-1-refractory TSTZ-FARV ADCs on HCT-WT cells after 96 hours demonstrates the enhanced activity of FARV-modified IgG1 ADCs on ICAM-1 positive target cells. (B) The enhanced activity of TSTZ-FARV involves ADC internalization rate as determined via the pHrodo Red Internalization Assay (Invitrogen) by comparing uptake of TSTZ and TSTZ-FARV in HCT-WT vs HCT-ICAM1-KD cells at various timepoints.

Summary

- ICAM-1 protein is immunosuppressive to IgG1-based antibodies and ADCs
- ICAM-1 binds to a 4-residue motif within IgG1 CH3 domain at aa 407-410
- Modification of this motif from YSKL → FARV reduces ICAM-1 binding to IgG1
- FARV-modified IgG1s retain ADCC activity in the presence of ICAM-1
- FARV-modified ADCs retain cytotoxicity and internalization rate in ICAM-1⁺ cells
- This motif is conserved in IgG1-based therapeutics and presents an opportunity to generate more potent novel mAbs and ADCs, as well as for lifecycle management of existing agents targeting cancers in which ICAM-1 is expressed

References

1. Kline, J. B., Grasso, L., & Nicolaides, N. C. (2025). ICAM-1 Is Overexpressed by Cancers and Negatively Impacts Antibody-Based Therapies Including Antibody-Drug Conjugates. European Journal of Immunology. https://doi.org/10.1002/EJI.202451611
2. Gupta et al. (2004). EGFR-directed Antibodies Promote HER2 ADC Internalization and Efficacy. Cell Reports Medicine. https://doi.org/10.1016/j.xcrm.2024.101792
3. von Arx, C., et al. (2023). The Evolving Therapeutic Landscape of Trastuzumab-drug Conjugates: Future Perspectives Beyond Her2-positive Breast Cancer. In Cancer Treatment Reviews. https://doi.org/10.1016/j.ctrv.2022.102500