Comparative assessment of physiochemical and biological attributes of EGFR-targeting bispecific antibodies

Nishant Mohan, Atul Agrawal, Yi Shen, Yukinori Endo, Wen Jin Wu

Division of Biotechnology Review and Research 1, Office of Biotechnology Products, Office of Pharmaceutical Quality, CDER, FDA, Silver Spring, Maryland

Abstract

Bispecific antibodies (BsAb) are an emerging class of therapeutic molecules that have capacity to simultaneously target two antigens and provide a unique opportunity to combine two target functionalities using single antibody-based molecule. In this investigation, novel BsAbs molecules were generated using state-of-the-art recombinant DNA and protein engineering technologies and physiochemical and biological characterization of these BsAb molecules were performed to gain insights into the molecular formatting, assay development and potency testing of BsAbs. Molecular formats selected to generate these BsAb molecules are representative of therapeutic bispecific antibodies under clinical development. Following BsAbs were generated: an anti-EGFR/VEGFR2 BsAb using IgG based symmetrical tetravalent dual variable domain immunoglobulin (DVD-Ig) molecular format; an anti-EGFR/PD-L1 BsAbs using symmetrical tetravalent DVD-Ig format and an asymmetrical bivalent knob-in-hole (KIH) format; anti-EGDR/CD3 BsAb using non-IgG based single chain variable fragment (scFv) format and a symmetrical tetravalent DVD-Ig format; anti-EGFR/VEGF A BsAb using cross-mAb and KIH technology. Physiochemical characterization of these BsAb molecules was performed by SDS-PAGE analysis and binding activity was determined by ELISA, SPR and flow cytometric based assays. Data from binding activity indicate that BsAb molecules bind to their respective antigens similar to its parental mAbs, however, different BsAb formats might have different sensitivity towards antigens when assessed with different binding methods. The biological activity of these BsAbs was measured in triple negative breast cancer (TNBC) and ovarian cancer models using the potency bioassays relevant to mechanism of actions of each BsAb. This lab-based research study provides knowledge, experience, and skills to perform regulatory assessment of bispecific antibodies from product quality perspective.

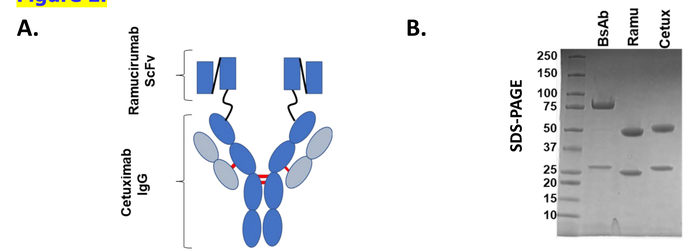
Research publications:

Mohan N, Agrawal A, Shen Y, Winarski KL, Endo Y, Dokmanovic M, Schmiel D, Zheng J, Rotstein DS, Pelosof LC, Wu WJ. Comparative Characterization of Different Molecular Formats of Bispecific Antibodies Targeting EGFR and PD-L1. Pharmaceutics. 2022 Jun 29;14(7):1381. doi: 10.3390/pharmaceutics14071381. PMID: 35890277; PMCID: PMC9325241.

Mohan N, Luo X, Shen Y, Olson Z, Agrawal A, Endo Y, Rotstein DS, Pelosof LC, Wu WJ. A Novel Bispecific Antibody Targeting EGFR and VEGFR2 Is Effective against Triple Negative Breast Cancer via Multiple Mechanisms of Action. Cancers (Basel). 2021 Mar 1;13(5):1027. doi: 10.3390/cancers13051027. PMID: 33804477; PMCID: PMC7957537.

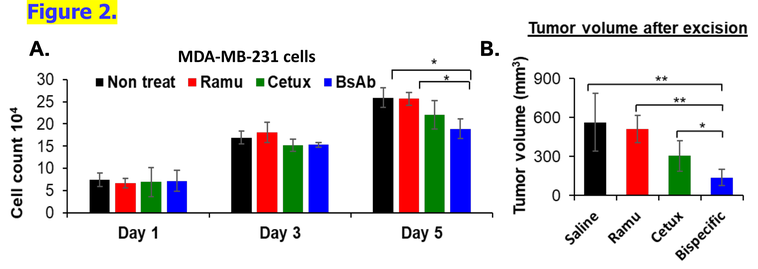
Results and Conclusions

Figure 1.



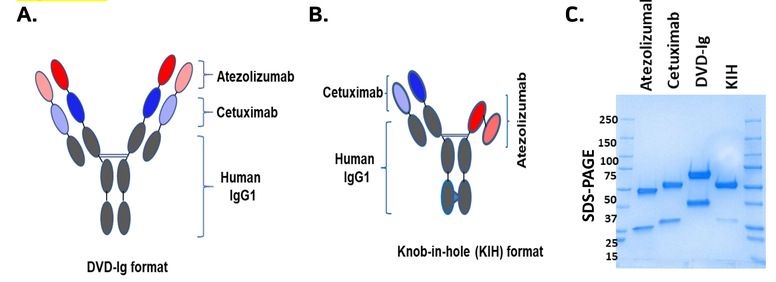
Construction of anti-EGFR/VEGFR2 bispecific antibody: (A) Schematic representation of the structure of anti-EGFR/VEGFR2 BsAb which shows that anti-EGFR/VEGFR2 BsAb is composed of cetuximab IgG linked to the scFv of ramucirumab via a glycine linker. (B) SDS-PAGE analysis showing the heavy and light chains

Figure 2.



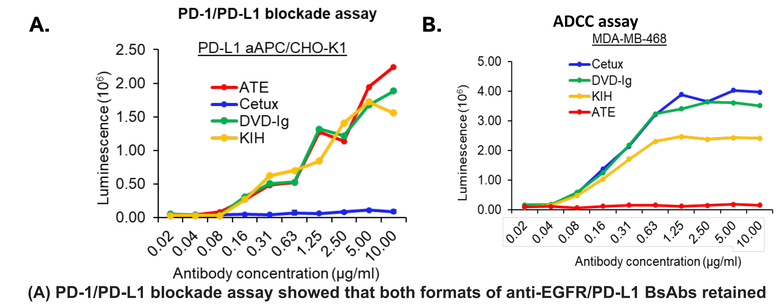
(A) Trypan blue cell proliferation inhibition assay data showed that anti-EGFR/VEGFR2 BsAb significantly inhibited the growth of MDA-MB-231 cells on day 5 as compared to the non-treat and ramucirumab treatment. (B) mice receiving anti-EGFR/VEGFR2 BsAb showed significant inhibition of the tumor growth compared to the saline and individual treated mice

Figure 3.



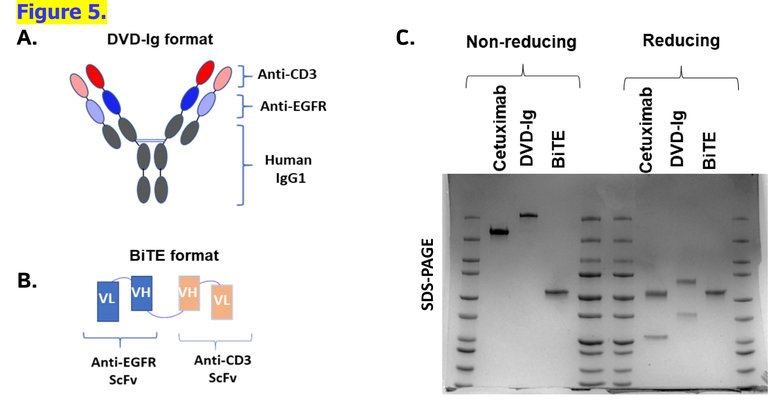
Construction of anti-EGFR/PD-L1 bispecific antibody: (A) Symmetrical DVD-Ig format in which single-chain heavy variable (scFv) fragments of cetuximab (Cetux) and atezolizumab (ATE) were linked to human IgG1 backbone to generate heavy chain. (B) The asymmetrical KIH format consists of two half-heavy chains containing "knob" and "hole" mutations. (C) SDS-PAGE analysis showing the heavy and light chains of DVD-Ig and KIH formats.

Figure 4.



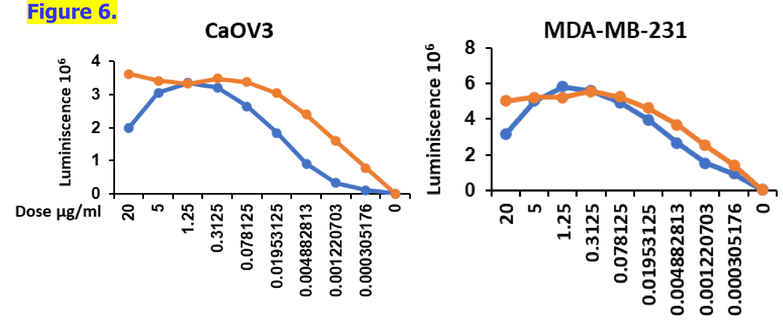
(A) PD-1/PD-L1 blockade assay showed that both formats of anti-EGFR/PD-L1 BsAbs retained their ability to block PD-1/PD-L1 interaction and have a comparable blocking profile to ATE (B) ADCC reporter bioassay showed that dose-dependent ADCC activity induced by the DVD-Ig format was similar to that induced by Cetux in MDA-MB-468 cells.

Figure 5.



Construction of anti-EGFR/CD3 bispecific antibody: (A) Symmetrical DVD-Ig format (B) Non-Fc containing Bispecific T cell engager (BiTE) format in which scFv of anti-EGFR and anti-CD3 mabs are linked via linker. (C) SDS PAGE analysis of BsAbs formats showing heavy and light chains.

Figure 6.



T-cell activation assay data show that both DVD-Ig (blue line) and BiTE format (orange line) of anti-EGFR/CD3 BsAbs can effectively induce T-cell activation in CaOV3 (ovarian cancer) and MDA-MB-231 (breast cancer cells).

Disclaimer

This study reflects the views of the authors and should not be construed to represent FDA's views or policies.