

Not all Anti-CD38 Antibodies are Created Equal: The Epitope of Felzartamab is partially DTT-resistant

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Background

Dithiothreitol (DTT) treatment of red blood cells (RBCs) is a common practice to denature CD38 antigens on the cell surface and thereby mitigate interference from anti-CD38 antibodies. This procedure is generally successful with daratumumab and isatuximab, the currently approved anti-CD38 antibodies. Both of these antibodies bind to conformation-sensitive epitopes (Fig. 1).

However, **new anti-CD38 antibodies are in development, some of which may be directed against linear or otherwise DTT-resistant epitopes**. Therefore, we tested anti-CD38 antibody candidates for their ability to bind to DTT-treated CD38 and RBCs.

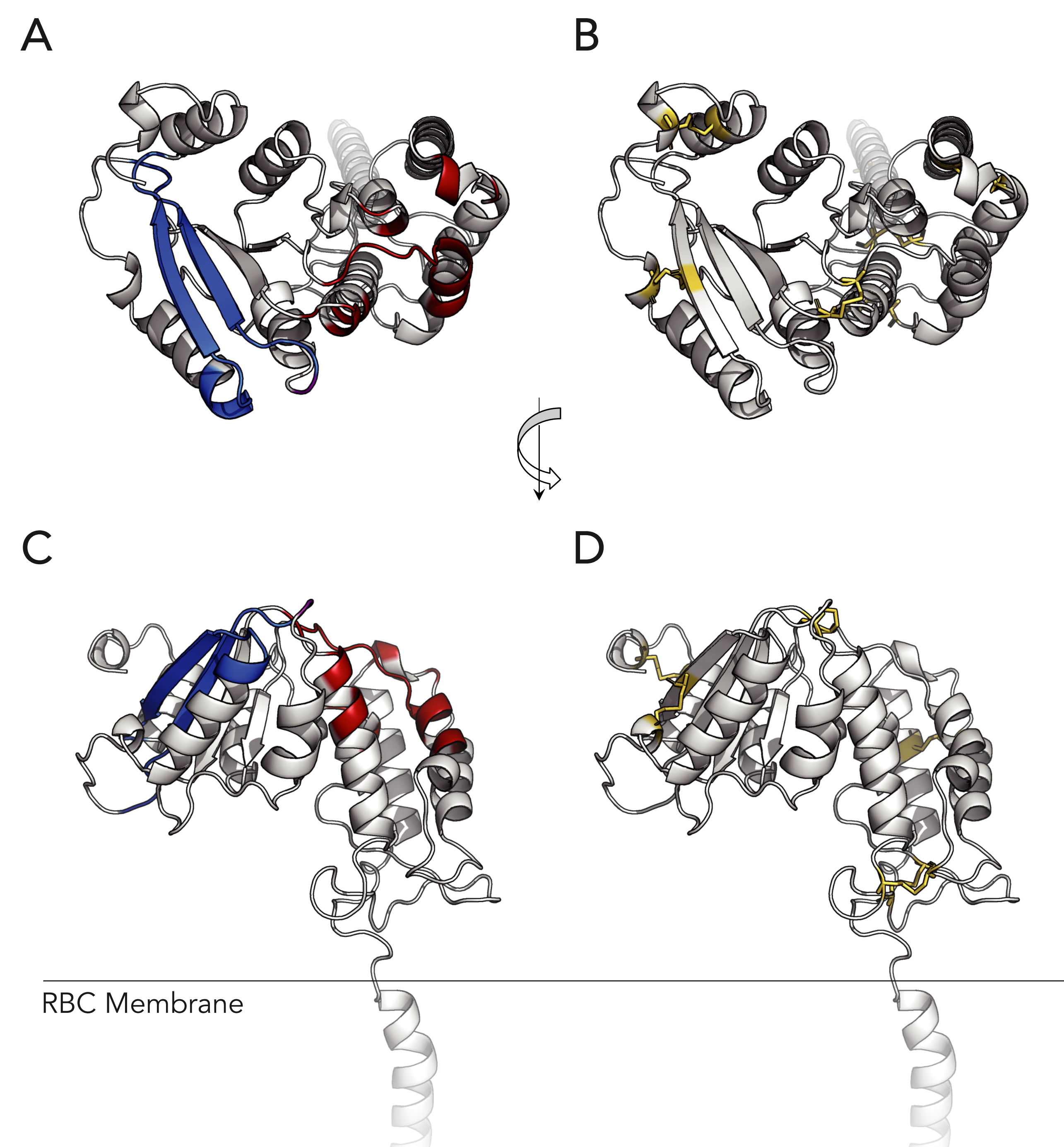


Figure 1. Structural model of CD38 showing the epitopes of daratumumab and isatuximab (A, C) and the position of the disulfide bridges (B, D). CD38 is shown in top (A, B) and side view (C, D). Blue: isatuximab epitope, red: daratumumab epitope, purple: overlap of the epitopes, yellow: disulfide bridges.

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Methods

A series of anti-CD38 antibody candidates (Table 1) were titrated in an ELISA format to assess their ability to bind to either untreated or DTT-treated recombinant CD38. Daratumumab and felzartamab were then tested on a panel of native and DTT-treated RBCs in the indirect antiglobulin test (IAT).

Table 1. Anti-CD38 antibodies used in this study. Given are the free name (and drug candidate number), the clinical status (furthest clinical trial phase, if applicable), the type, and the major indication. MM: relapsed/refractory multiple myeloma and/or newly diagnosed multiple myeloma, SMM: high-risk smoldering multiple myeloma, AMR: antibody-mediated rejection, ITP: primary immune thrombocytopenia, IN: IgA nephropathy.

Anti-CD38 Antibody	Clinical Status	Type	Major Indication
Daratumumab	Approved	IgG1 antibody	MM, SMM
Isatuximab	Approved	IgG1 antibody	MM
Felzartamab (MOR-202)	Phase III	IgG1 antibody	AMR, MM
Mezagitamab (TAK-079)	Phase III	IgG1 antibody	ITP, IN
Lumrotatug (CM-313)	Phase II	IgG1 antibody	MM
Erzotabart (GEN-3014)	Phase II	hexamerization enhanced antibody	MM

Conclusion

Not all epitopes of anti-CD38 antibodies are fully DTT-sensitive. This has important implications for handling of anti-CD38 antibody induced interference in immunohematology, and **raises the question of whether DTT is an adequate solution for interference mitigation**.

Conflict of interest

AZ, CH, and CS are employees of imusyn GmbH & Co. KG.

Download poster at: https://www.imusyn.de/fileadmin/Dokumente/Poster_DGTI2025_DTT_Felzartamab.pdf



Results

All anti-CD38 drugs except felzartamab failed to bind to DTT-denatured CD38 in the ELISA at concentrations of up to 1.25 µg/ml (Fig. 2). When tested in the IAT, **some DTT-treated cells remained positive with felzartamab**, despite being negative with daratumumab (Fig. 3).

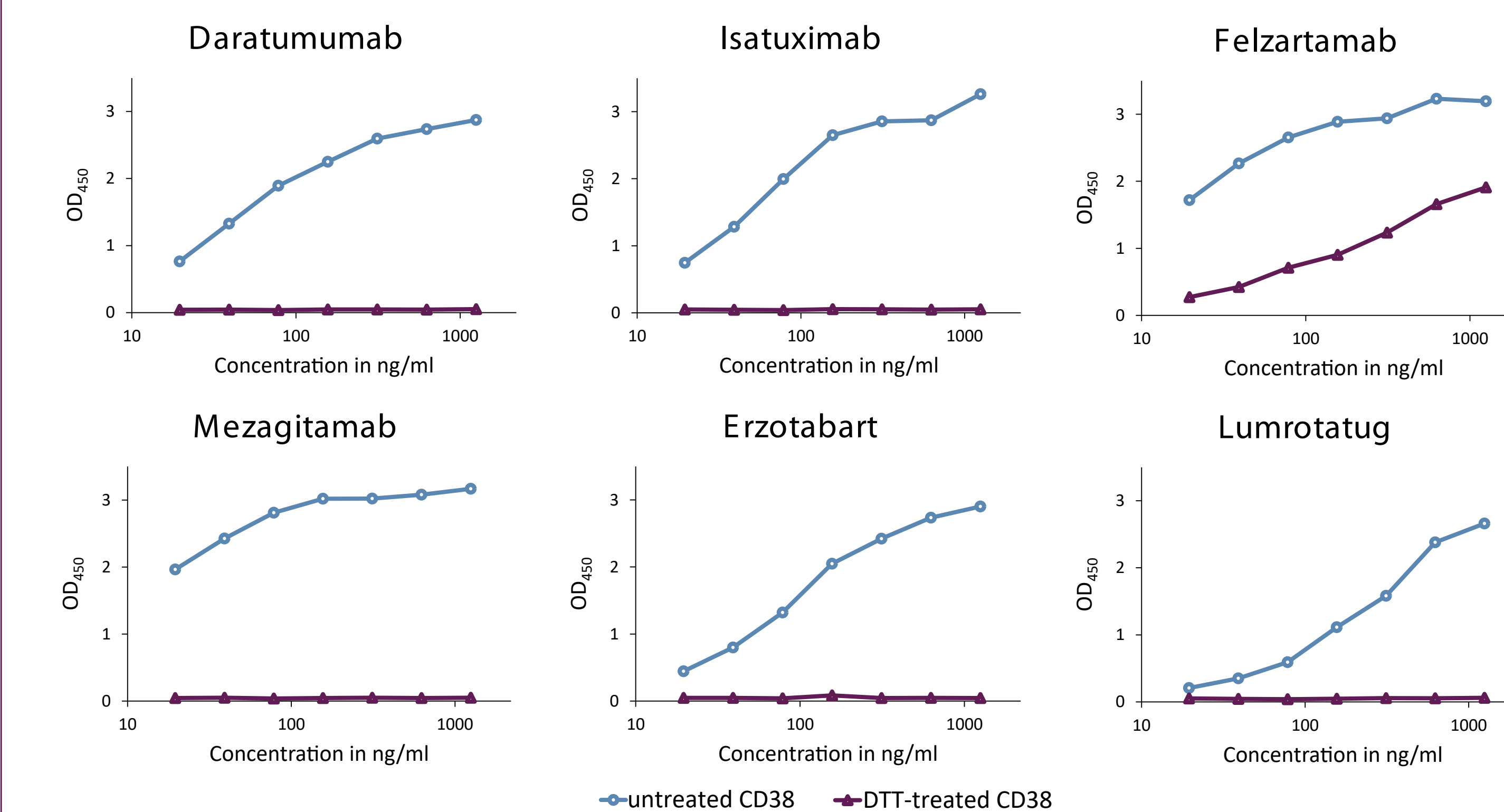


Figure 2. Unlike other anti-CD38 antibodies, felzartamab binds to DTT-treated CD38. Anti-CD38 antibodies were incubated with immobilized CD38 that was either untreated or has been pre-treated with 1 M DTT. Anti-CD38 antibodies were detected with anti-human IgG.

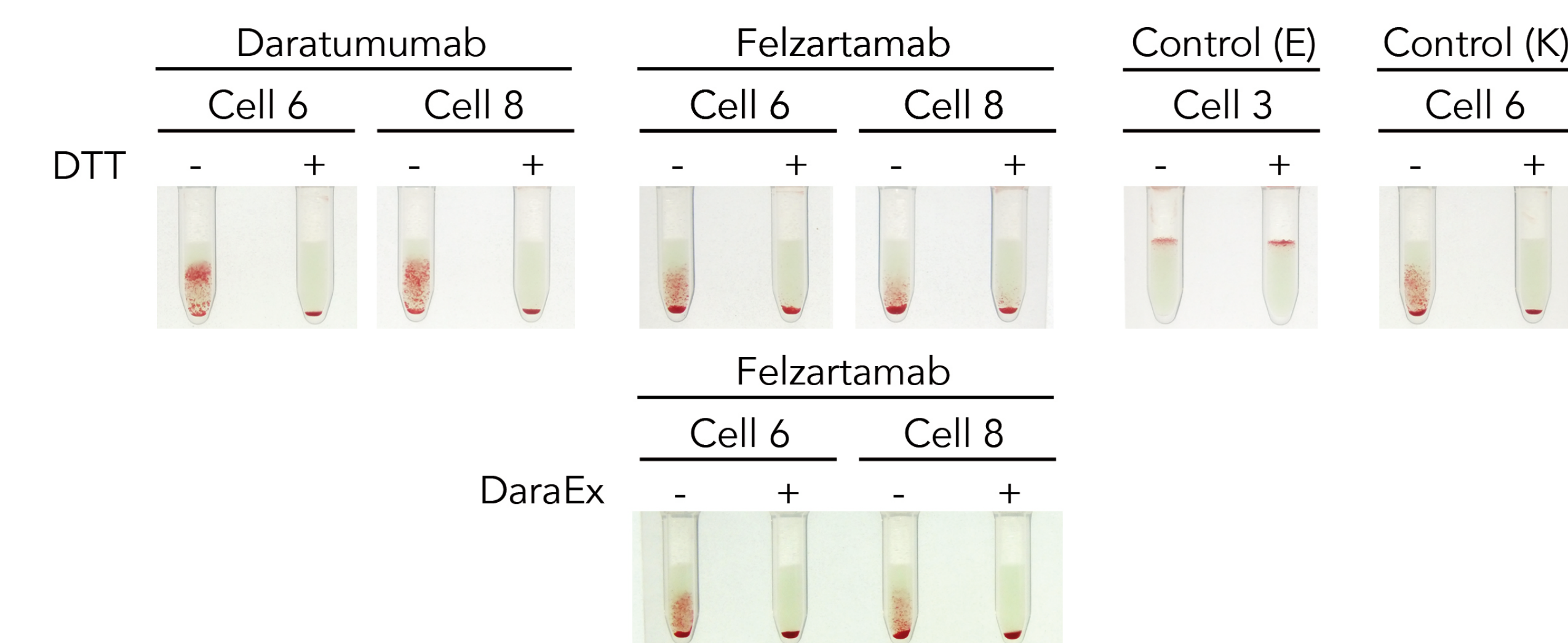


Figure 3. Felzartamab still reacts positively with DTT-treated RBCs. RBCs were treated or not treated with 0.2 M DTT according to Chapuy et al., 2016*, as indicated. IAT was subsequently performed using 1 mg/ml daratumumab or 1 mg/ml felzartamab, as well as anti-E and anti-K as controls. Lower panel: DaraEx renders the felzartamab reaction completely negative.

*Chapuy et al. (2016). Transfusion, 56(12), 2964–2972.