CID-103, an anti-CD38 monoclonal antibody, demonstrates decreased RBC binding and decreased interference with pre-transfusion test methods

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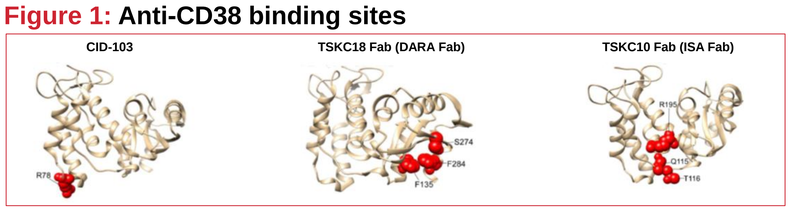
Introduction

- The anti-CD38 monoclonal antibody (MAb) therapies (daratumumab [DARA] and isatuximab [ISA]) are approved for the treatment of multiple myeloma (MM) and are known to interfere with pre-transfusion red blood cell (RBC) antibody detection and cross-matching.[1,2]
- CD38 is a small multi-functional transmembrane glycoprotein, widely expressed on lymphoid and myeloid lineages, but absent from most mature lymphocytes. CD38 is present on many lymphoid tumors, including most MM cells. CD38 is also expressed on RBCs.
- CID-103 is a human IgG1 MAb targeting CD38 that recognizes and targets a different CD38 epitope than DARA and ISA. CID-103 is about to enter a Phase 1 clinical study in patients with relapse/refractory MM.

Figure 1: Anti-CD38 binding sites



- This study was undertaken to evaluate the binding of CID-103 to RBCs and malignant cells, and to determine the drug's impact on pre-transfusion testing to provide guidance for clinical studies.

Objectives

- To investigate the binding of CID-103 on human RBCs and malignant cell lines.
- To characterize the serological reactivity of plasma containing anti-CD38 MAb in routine blood bank assays and test methods known to mitigate interference observed with other anti-CD38 MAbs.
- To provide guidance regarding blood bank testing for the initial CID-103 Phase 1 study.

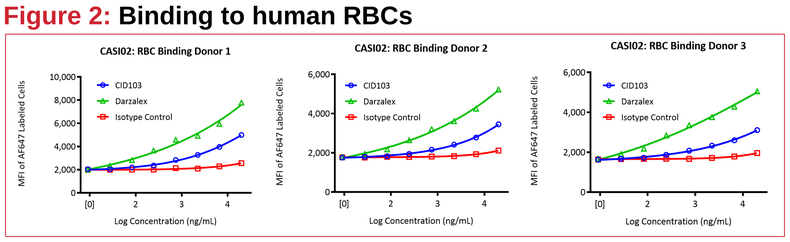
Materials and Methods

- CID-103 binding by flow-cytometry
  - To evaluate CID-103 binding to 3 malignant cell lines compared to DARA and a negative IgG1 isotype control.
  - Second study to evaluate binding to donor RBCs compared to DARA, IgG1 and AF647 conjugated anti-human CD47.
  - Cells were incubated with primary antibodies at 20 µg/mL followed by a 7-point 3-fold dilution series and a control.
  - Alexa Fluor 647 secondary antibody was used to detect binding and Mean Fluorescence Intensity (MFI) and EC_{50} were calculated.
- Pre-transfusion testing
  - Inert group AB plasma was spiked with 1, 10, 100 or 250 µg/mL of CID-103 or DARA.
  - Testing was performed by standard methods
    - In tube using low ionic strength saline (LISS) or polyethylene glycol (PEG) potentiators
    - Using column agglutination technology (CAT, Ortho)
    - On Tango and IH-1000 (BioRad) automated platforms
  - Plasma was tested by indirect antiglobulin test (IAT) with R_1R_1, R_2R_2 and rr RBCs.
  - Test RBCs were untreated or treated with enzymes or 0.2 M DTT.
  - Immucor Gamma-clone, Ortho Bio-Clone, Quotient and Grifols anti-IgG were used in IAT.

Results

Flow cytometry

Figure 2: Binding to human RBCs



Binding to the 3 human RBC donor samples demonstrates that DARA has consistently increased dose-dependent binding compared to CID-103.

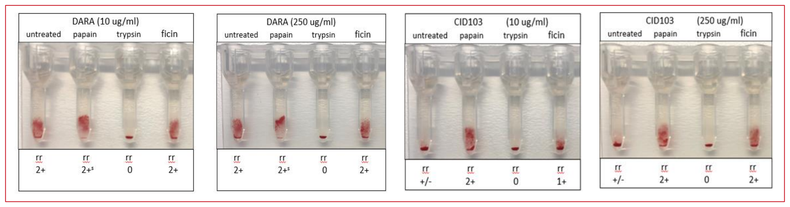
Table 1: Binding to malignant cell lines

TA	Max MFI (Daudi, Raji, Ramos cell lines)
CID-103	3242, 1198, 2106
DARA	2994, 1225, 2009
IgG1	7, 5, 5

Pre-transfusion testing

- **IgG gel card testing**
  - Untreated (1 µg/mL, 10 µg/mL, 100 µg/mL, 250 µg/mL)
    - DARA – robust interference (1+2+ all cells)
    - CID-103 – variable low level of interference (0 – +w)
  - Enzyme treated (10 µg/ml and 250 µg/ml)
    - DARA – non-reactive with DTT- and trypsin-treated cells
    - CID-103 – DTT- and trypsin-treated red cells were non-reactive, while enhanced reactivity was observed with papain and ficin

Figure 3: Gel card testing with enzyme-treated cells



Tube testing with multiple AHGs by PEG and LISS

- DARA – 1+ interference
- CID-103 interference very low, resulting in inconsistent detection only seen under the microscope
- Ortho AHG more likely to be non-reactive

Table 2: PEG enhancement media by indirect antiglobulin testing with multiple different AHG

	CID-103 (250 µg/mL)					DARA (250 µg/mL)					Plasma Only
Test red cells	Ortho	Immucor	Quotient	BioRad	Grifols	Ortho	Immucor	Quotient	BioRad	Grifols	For all AHGs
R_1R_1	micro*	micro*	0	micro*	micro*	1+	1+	1+	1+	1+	0
R_2R_2	0	micro*	micro*	micro*	micro*	1+	1+	1+	1+	1+	0
rr	0	micro*	micro*	0	0	1+	1+	1+	1+	1+	0

*microscopic – requires looking under the microscope to see small agglutinated cells. Many hospital blood banks do not use microscope.

- **Additional testing of CID-103 on automated analyzers**
  - Antibody screen on IH indeterminate-staff evaluation was non-reactive
  - Antibody screen on Tango non-reactive

Summary of Testing Results

Flow cytometry

- The CID-103 MFI and EC_{50} values for Daudi, Raji and Ramos cell lines are higher than DARA.
- CID-103 showed overall MFI and EC_{50} values were lower than DARA for RBC binding.
- Anti-human CD47 demonstrated a high dose-response binding to all 3 RBC donor samples.

Pre-transfusion

- DARA at all concentrations tested gave 1+ – 2+ reactivity by all test methods and AHG reagents.
- No reactivity was observed with CID-103 by automated Tango and IH-1000 platforms. Microscopic-only reactivity was seen in LISS and PEG-IAT with all AHG reagents, except Ortho which was variable. Weak variable reactivity (0 – +w) was observed in gel CAT.

Conclusions

- CID-103 exhibited a saturable concentration-dependent binding to 3 CD38-expressing malignant cell lines, as well as a dose-dependent increase in binding on RBCs.
- CID-103 demonstrated very low binding to RBCs that was not detected by most blood bank test methods independent of the concentration of drug.
- Gel column testing was the most sensitive to interference giving weak variable reactivity, and Ortho AHG was less likely to detect the microscopic binding to RBCs. No interference was seen using automated Tango and IH-1000. CID-103 showed significantly less RBC interference relative to DARA.
- While these observations are encouraging for laboratory testing of patients needing transfusion, confirmation will be undertaken in the CID-103 Phase 1 clinical study.

Guidance for clinical study

- The following guidance will be provided for the clinical study
  - **It is important to notify the transfusion service / blood bank that the patient is being enrolled in the CID-103 trial and to send a sample to the transfusion service / blood bank prior to starting CID-103 therapy.**
  - **Prior to starting therapy**
    1. Perform ABO/Rh and antibody screening.
    2. Obtain an extended red cell genotype or phenotype to facilitate antibody identification and selection of red cell units if future transfusion becomes necessary.
  - **What to expect when testing samples from patients treated with CID-103 anti-CD38 therapy**

    Note: Interference in plasma testing may differ depending on method of testing and duration of CID-103 drug therapy.

    Plasma/serum: No interference to very low level interference
    - No reactivity to very low level reactivity in antiglobulin (IAT) with saline or enhancement solutions including albumin, LISS, PEG and gel.
    - Interference only seen if microscopic reading is performed with manual tube tests.
    - Gel card methods appear to be the most sensitive to detect interference.
    - Ortho AHG appears to be less likely to detect the weak binding of the drug to RBCs.
    - Reactivity is mitigated with DTT- and/or trypsin enzyme-treated red cells.

References

1. *DARZALEX® (daratumumab) injection US Package Insert*
2. *SARCLISA® (isatuximab-irfc) injection US Package Insert*