PEARLS OF LABORATORY MEDICINE

Daratumumab Interference in Pre-Transfusion Testing

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Daratumumab as treatment for Multiple Myeloma

Multiple myeloma (MM) is a hematologic malignancy affecting antibody producing plasma cells.

One of the targeted antibody based therapies is Daratumumab (DARA), a human IgG1\textsubscript{K} monoclonal antibody that targets the CD38 glycoprotein located on the cellular surface of the neoplastic plasma cells.

It is currently approved by FDA for MM patients who have received only one prior therapy. This approval by the FDA came as the result of two simultaneous clinical trials demonstrating similar and “substantial improvements” in progression free survival despite different accompanying drug regimens, proving that DARA was the primary agent responsible for the improved clinical endpoints.
CD 38 expression and Daratumumab interference

- CD38 besides being expressed on plasma cells is also expressed on multiple other hematologic cell types including red blood cells (RBCs), medullary thymocytes, activated B and T cells, natural killer cells, monocytes, granulocytes, and others.
- This expression led to interference of Daratumumab in the assays that use secondary anti-human IgG antibody for amplification of signal, such as AHG phase of red cell antibody screen and others.
Case presentation and initial antibody screen

60 year old female patient, undergoing treatment for multiple myeloma was found to have low hemoglobin and needed transfusion. A routine red cell antibody screen test was submitted to hospital’s transfusion laboratory and results are shown below.

| Cell | D | C | c | E | e | V | C^e | K | k | Kp^a | Kp^b | Js^a | Js^b | Fy^a | Fy^b | Jk^a | Jk^b | Le^a | Le^b | P1 | M | N | S | s | Lu^a | Lu^b | Xg^a | IS | AHG | CC |
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| II   | + | 0 | + | + | 0 | 0 | 0 | 0 | + | 0 | + | 0 | + | 0 | + | 0 | + | 0 | + | 0 | 0 | + | 0 | - | w+ | na |
| III  | 0 | 0 | + | 0 | + | 0 | 0 | 0 | + | 0 | + | 0 | + | 0 | 0 | + | 0 | + | 0 | 0 | + | 0 | - | w+ | na |

Patient's cells

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## Antibody screen, extended panel

| Cell | D | C | c | E | e | V | C⁺ | K | k | Kp⁺ | Kp⁻ | Js⁺ | Js⁻ | Fy⁺ | Fy⁻ | Jk⁺ | Jk⁻ | Le⁺ | Le⁻ | P₁ | M | N | S | s | Lu⁺ | Lu⁻ | Xg⁺ | IS | AHG | CC |
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| II   | + | + | 0 | 0 | + | 0 | + | 0 | + | 0   | +   | 0   | +   | 0   | +   | 0   | +   | 0   | +   | 0   | +   | 0   | +   | 0   | +   | -   | W⁺ |     |
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| 3    | 0 | + | + | 0 | + | 0 | 0 | 0 | + | 0   | +   | 0   | +   | 0   | +   | 0   | +   | 0   | +   | 0   | +   | 0   | +   | 0   | +   | -   | W⁺ |     |
| 4    | 0 | 0 | + | + | + | 0 | 0 | 0 | + | 0   | +   | 0   | +   | 0   | +   | 0   | +   | 0   | +   | 0   | +   | 0   | +   | 0   | +   | -   | W⁺ |     |
| 5    | 0 | 0 | + | 0 | + | 0 | 0 | + | 0   | +   | 0   | +   | 0   | +   | 0   | +   | 0   | +   | 0   | +   | 0   | +   | 0   | +   | -   | W⁺ |     |
| 6    | 0 | 0 | + | 0 | + | 0 | 0 | + | 0   | +   | 0   | +   | 0   | +   | 0   | +   | 0   | +   | 0   | +   | 0   | +   | 0   | +   | -   | W⁺ |     |
| 7    | + | + | 0 | 0 | + | 0 | 0 | 0 | + | 0   | +   | 0   | +   | 0   | +   | 0   | +   | 0   | +   | 0   | +   | 0   | +   | 0   | +   | -   | W⁺ |     |
| 8    | + | + | 0 | 0 | + | 0 | 0 | 0 | + | 0   | +   | 0   | +   | 0   | +   | 0   | +   | 0   | +   | 0   | +   | 0   | +   | 0   | +   | -   | W⁺ |     |
| 9    | + | 0 | + | + | 0 | 0 | 0 | + | 0   | +   | 0   | +   | 0   | +   | 0   | +   | 0   | +   | 0   | +   | 0   | +   | 0   | +   | -   | W⁺ |     |
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Patient's cells: - - 2+
Patterns of antibody screen associated with pan reactivity

- Panreactivity
  - Auto-control positive
    - Warm autoantibody
    - Cold autoantibody
    - Interference from monoclonal antibodies
  - Auto-control negative
    - Antibody to high incidence antigen
    - Interference from monoclonal antibodies
Immunomodulation as possible mechanism of negative autocontrol results

- Immunomodulation is selective removal of antigen from RBC membrane by spleen monocytes, when they are passing through the spleen
- It was shown in the models explaining RhIG and anti Kell immunoglobulin prophylaxis
Interpretation of antibody screen results

Negative autocontrol effectively rules out warm and cold autoantibodies.

The differentiation between antibody toward high incidence antigen and Daratumumab or other monoclonal therapeutic antibody interference requires review of medication administration records and/or special technics in transfusion laboratory.
Special techniques that help in distinguishing Daratumumab interference

DTT (Dithiothreitol) treatment.
- Destroys CD38 – target for Daratumumab
- Destroys Kell blood group system antigens

Other techniques, like using enzymes, (papain) can be useful in distinguishing high incidence antigens
# Antibody screen, after DTT treatment

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**Patient's cells**

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Practical approach

Before Daratumumab treatment.
  • Type patient for Kell antigens

After Daratumumab treatment.
  • Use DTT treatment to screen for RBC antibodies
  • Provide Kell negative blood
Assays affected

Red cell antibody screening
Anti Neutrophil Antibody testing
Anti platelet antibody testing
Platelet crossmatch
And others that use secondary anti human antibodies
Other strategies to overcome interference

- soluble CD38 antigen
- anti-idiotype antibody
- F(ab’)2 fragments of DARA
- blocking mouse anti CD38 antibody
- Cord blood testing

All are expensive and/or commercially not available
Conclusions

- Daratumumab is a humanized antibody for treatment of multiple Myeloma that can cause interference due to not specific binding.
- The most affected assay is red cell antibody screen, but other assays that use secondary anti human antibody can be affected as well.
- The accepted approach to overcome interference is pre-treatment of the cells with DTT that destroys CD38. Other approaches has been tried, but not currently commercially available or accepted.
References

9. Baig, N., Dudek B., Falbo DK et al. false positive anti neutrophil antibody test due to daratumumab interference can be overcome using non human blocking antibodies. Transfusion. 2019; submitted for publication
Disclosures/Potential Conflicts of Interest

Upon Pearl submission, the presenter completed the Clinical Chemistry disclosure form. Disclosures and/or potential conflicts of interest:

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