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Antigen Masking Indirect Antiglobulin Test: A New Reagent with Improved Protocols

Background

Anti-CD38 antibodies are increasingly used in the clinic. CD38 is a surface molecule expressed on a number of cells and tissues, including erythrocytes. After administration of anti-CD38, interference regularly occurs in indirect antiglobulin tests (IAT).

A strategy for mitigation of this interference is the antigen masking IAT (AMIAT), where the antigens on the erythrocyte are masked by an antibody fragment. The new AMIAT reagent DaraEx plus contains the Fab fragment of an anti-CD38 antibody.

Methods

Incubation protocols were tested for DaraEx plus. We analyzed whether the sample material has an influence on the AMIAT and tested whether worst case concentrations of anti-CD38 antibodies can be effectively inhibited. Finally, 19 combinations of cells and plasma samples of patients treated with Daratumumab that were otherwise negative for irregular antibodies were tested in an AMIAT with DaraEx plus. All tests were performed in Bio-Rad ID System gel cards.

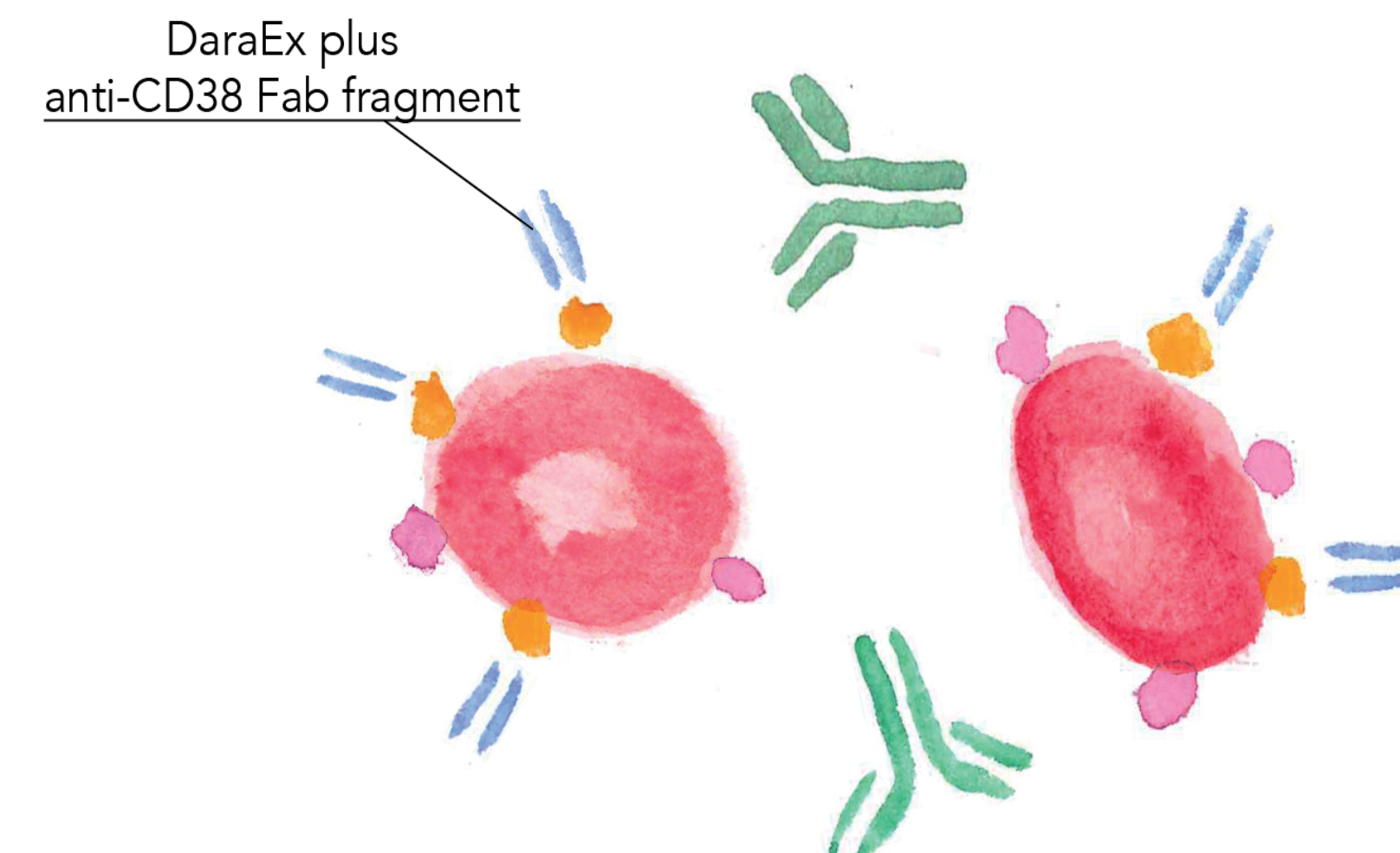
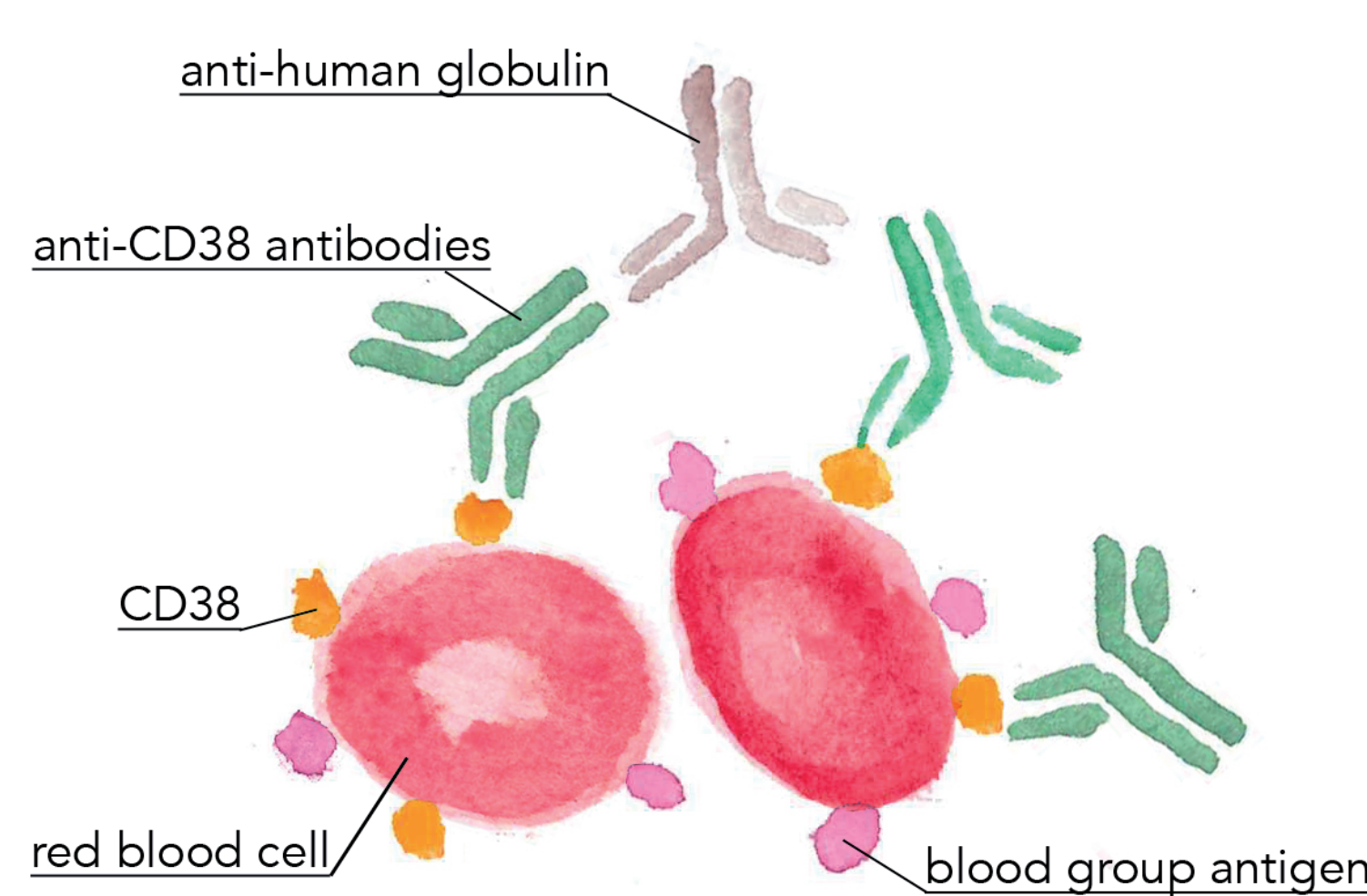


Figure 1. Principle of the Antigen Masking Indirect Antiglobulin Test (AMIAT)

Left: When an anti-CD38 antibody (green) is present in a patient sample, it binds to the CD38 (yellow) on the surface of the red blood cells. The cells are then agglutinated by the anti-human globulin (brown) in the Indirect Antiglobulin Test (IAT). **Right:** In the Antigen Masking Indirect Antiglobulin Test (AMIAT), a specific antigen on the surface of the red blood cells is masked before anti-human globulin is added. In the case of DaraEx plus (blue), which is the Fab fragment of an anti-CD38 antibody, CD38 is masked, thereby preventing anti-CD38 antibodies from binding.

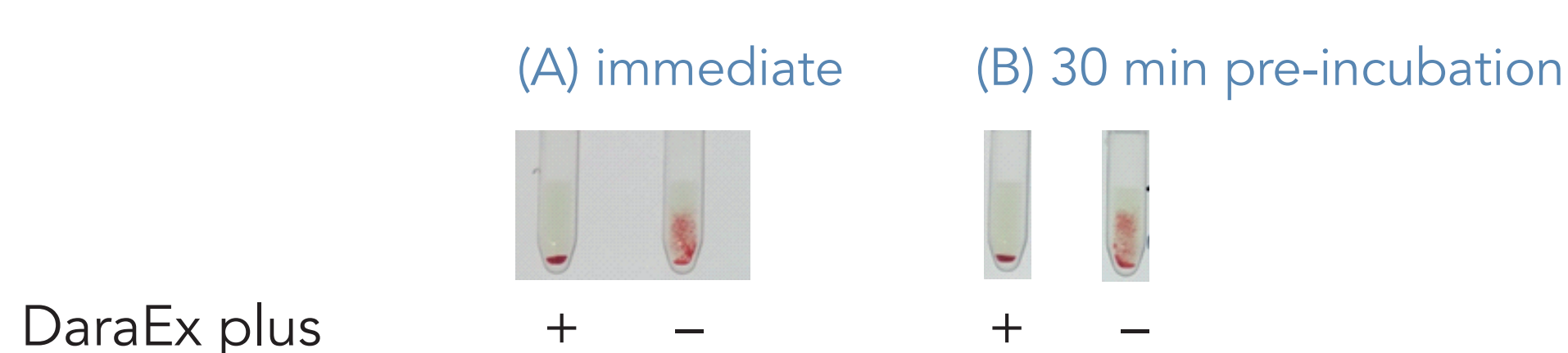


Figure 2. DaraEx plus can be used without pre-incubation

10 µl of DaraEx plus were added to 50 µl of 0.8% cells as indicated. The cells were either (A) used directly in the gel card or (B) incubated for 30 min at 37°C prior the addition of 25 µl 1 mg/ml Daratumumab in PBS to the gel card.

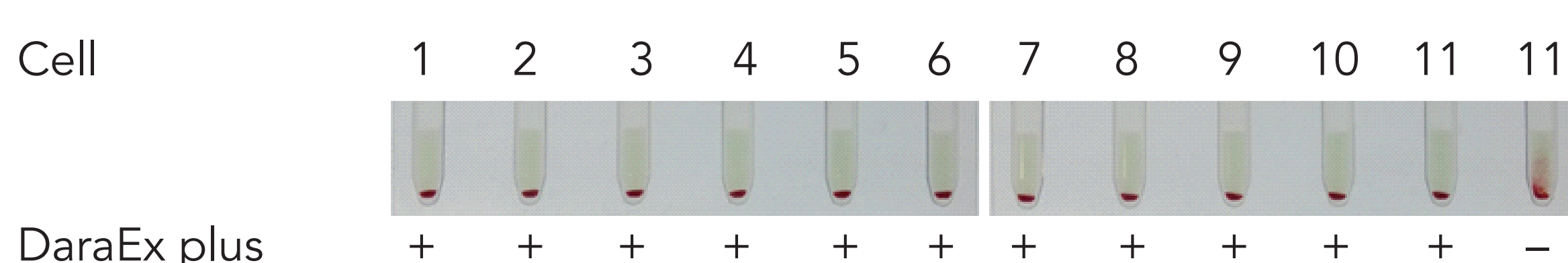


Figure 3. DaraEx plus can inhibit 1 mg/ml Daratumumab

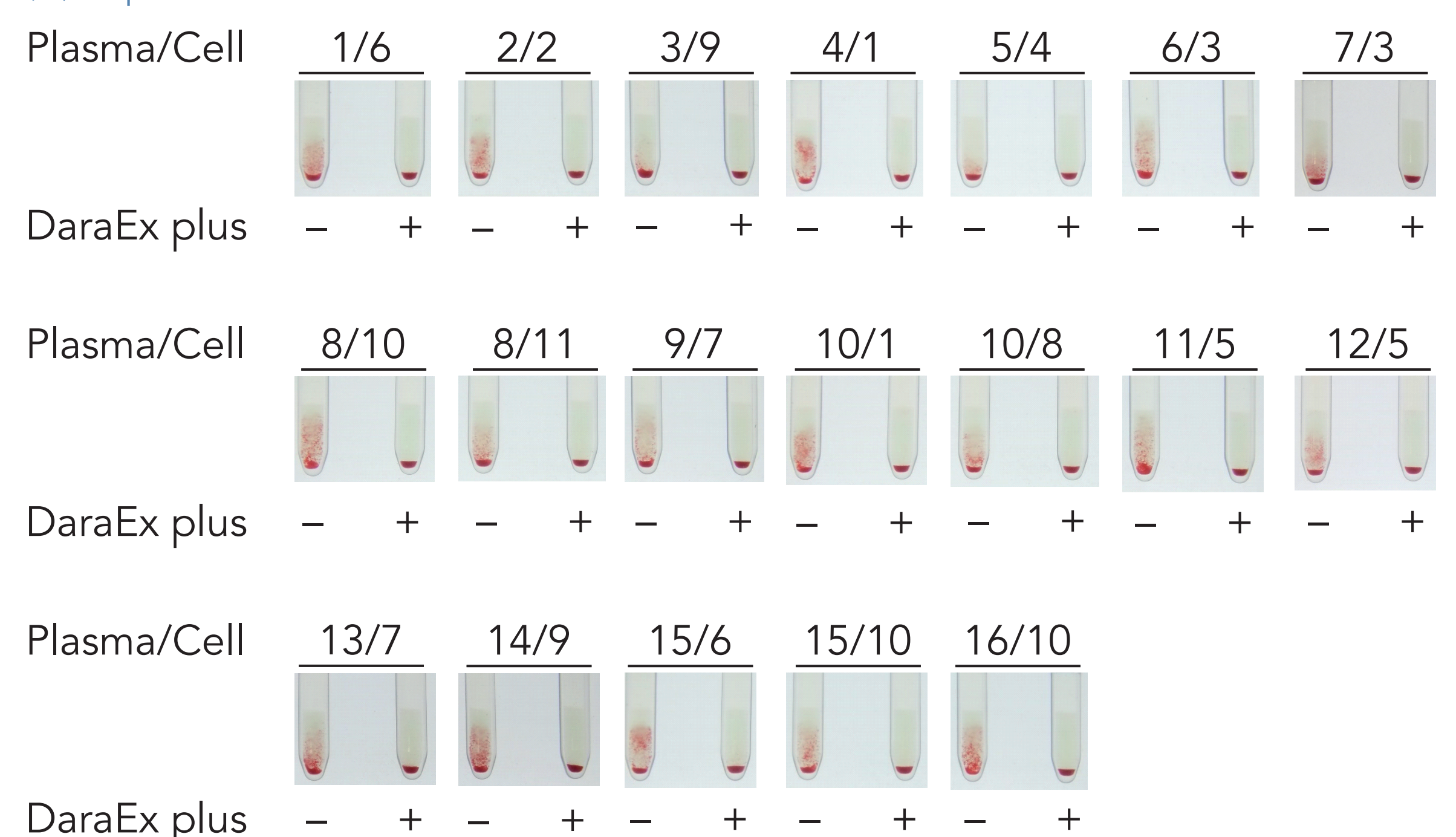
A panel of 11 cells (0.8%) was treated with 10 µl DaraEx plus directly in the gel card. Subsequently, 25 µl of 1 mg/ml Daratumumab in 0.9% NaCl were added to the gel card as indicated.



Figure 4. DaraEx plus works in common sample materials

Citrate, Heparin, and EDTA plasma samples as well as a serum sample were spiked with 0,5 mg/ml Daratumumab. The cells were treated with DaraEx plus or mock treated as indicated.

(A) Express Protocol



(B) Repetition with Alternative Protocol

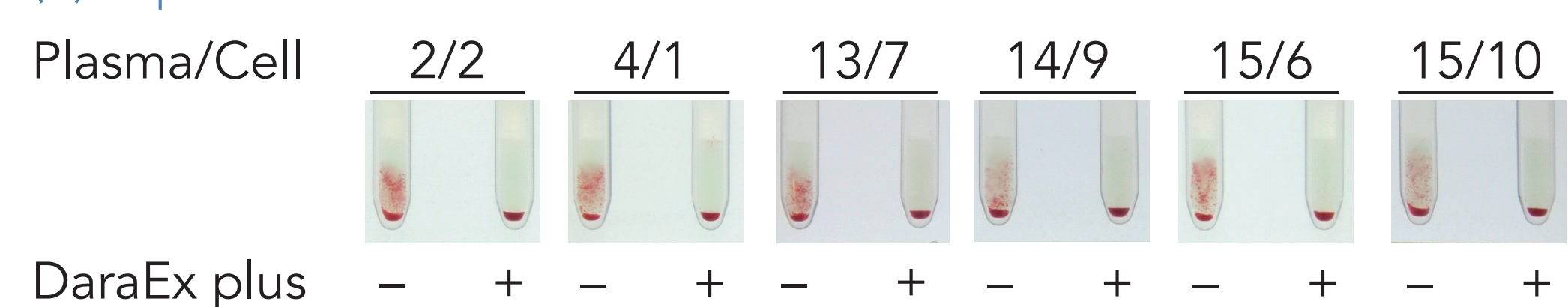


Figure 5. Performance Evaluation

(A) 19 combinations of plasma of patients under Daratumumab, Isatuximab, or Felzartamab treatment and cells (first digit: plasma sample number, second digit: cell number) were treated or not treated with the Express Protocol (10 µl DaraEx plus + 50 µl of 0.8% cells directly in the gel card). (B) All reactions that remained positive after the Express Protocol were repeated using a more stringent Alternative Protocol (25 µl DaraEx plus + 25 µl 1.6% cells directly in the gel card).

Results

We developed a quick ("Express") protocol that inhibited 13 of the 19 sample/cell combinations and a more material intensive ("Alternative") protocol that worked for 5 of the 6 samples that had not been inhibited by the quick protocol. 1 mg/ml Daratumumab could be inhibited and the sample material had no influence on the inhibition efficacy. With DaraEx plus, a pre-incubation step was not necessary.

Conclusion

These data suggest that DaraEx plus treatment is a simple and effective way to mitigate the interference of anti-CD38 antibodies.

Conflict of Interest

CPH, SA, and CS are employees of imusyn GmbH & Co. KG. CS is a designated inventor of the technology presented here.

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