OVERCOMING THE INTERFERENCE OF DARATUMUMAB WITH IMMUNOFIXATION ELECTROPHORESIS (IFE) USING AN INDUSTRY-DEVELOPED DIRA TEST: HYDRASHIFT 2/4 DARATUMUMAB

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BACKGROUND

According to International Myeloma Working Group (IMWG) criteria, detection and quantification of monoclonal component (M-spike) by serum protein electrophoresis (SPE) and immunofixation electrophoresis (IFE) are essential for response evaluation in multiple myeloma (MM)[1].

Recent clinical trials of daratumumab, an IgG kappa anti-CD38 monoclonal antibody, have shown impressive results with deep responses[2]. However, daratumumab may be detected on SPE and IFE assays that are used for monitoring disease monoclonal immunoglobulins (M protein).

Figure 1: Normal serum spiked with daratumumab 1 g/L.

This can lead to false-positive SPE and IFE assay results for patients with IgG kappa myeloma protein, which may impact initial assessment of complete responses (CRs) by IMWG criteria. Differentiating therapeutic monoclonal antibodies, such as daratumumab, from endogenous M protein can be challenging when both molecules co-migrate or migrate closely on electrophoresis.

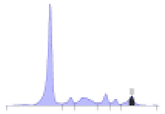

Figure 2: Example of a monoclonal component co-migrating with daratumumab.

The availability of a specific anti-daratumumab antibody has provided the opportunity to overcome this interference and to correctly assess biochemical response. Indeed, McCudden et al, in collaboration with Janssen, developed a technique, the Daratumumab Interference Reflex Assay (DIRA) test, that has been utilized in daratumumab clinical trials[3]. Given the need for a commercially available automated and standardized test, we evaluated the new commercial DIRA kit test that is under development by Sebia (Lisses, France): the Hydrashift 2/4 Daratumumab.

OBJECTIVE

The aim of this study was to evaluate the Hydrashift 2/4 Daratumumab in comparison with our laboratory developed DIRA test for the displacement of daratumumab on IFE.

References:

[1] Rajkumar SV and al. Consensus recommendations for the uniform reporting of clinical trials: report of the International Myeloma. Workshop Consensus Panel 1. Blood 2011;117:4691–5.

[2] Rajkumar SV, Kyle RA. Progress in Myeloma - A Monoclonal Breakthrough. N Engl J Med. 2016 Oct 6;375(14):1390-1392.

[3] McCudden C and al. Monitoring multiple myeloma patients treated with daratumumab: teasing out monoclonal antibody interference. Clin Chem Lab Med. 2016 Jun 1;54(6):1095-104.

Conflict of Interest Disclosures:
H. Caillon, A. Irimia, and T. Dejoie: No relevant conflicts of interest to disclose.
J.S. Simon, A. Axel, A.K. Sasser, and M.J. Scullion: Janssen (employment).
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PATIENTS AND METHODS

The Hydrashift 2/4 Daratumumab assay was prepared by Sebia using the anti-daratumumab antibody produced by Janssen and modified to allow a migration of daratumumab/anti-daratumumab complexes toward the α-globulin fraction on IFE.

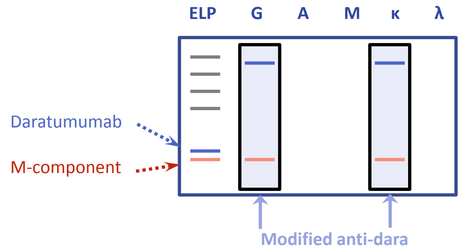


Figure 3: Hydrashift 2/4 Daratumumab – principle.

IFE technical procedures, migration, and staining programs were performed according to the manufacturer instructions, and run on the standard Sebia, Hydrasys platform, with the HYDRAGEL 4 IF kit. In addition to the regular procedure, an additional applicator to apply the anti-daratumumab antibody was used.

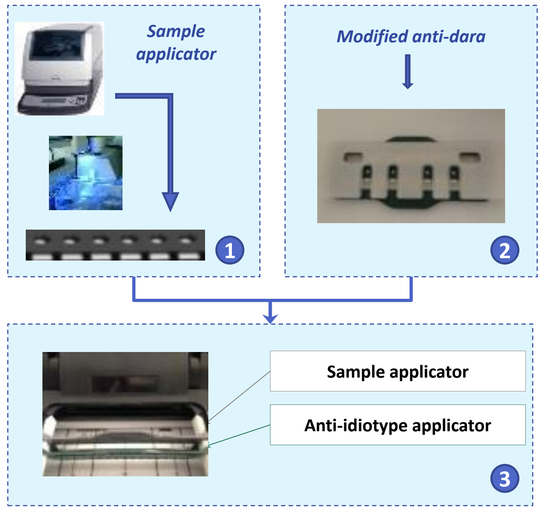


Figure 4: Hydrashift 2/4 Daratumumab – technical steps.

Analytical performances, including sensitivity, specificity, and comparisons with the original DIRA test, were assessed on 99 samples from ongoing daratumumab clinical trials.

RESULTS

➢ COMPARISON WITH THE ORIGINAL DIRA

The Hydrashift 2/4 Daratumumab assay showed excellent concordance (100%) with the laboratory developed test on the 51 samples tested (ie, 28 negative DIRA, 14 positive DIRA and 9 doubtful DIRA).

IgG kappa MM at C3D1 evaluation: Positive DIRA, presence of daratumumab.

IgG kappa MM at C4D28 evaluation: Negative DIRA, presence of daratumumab.

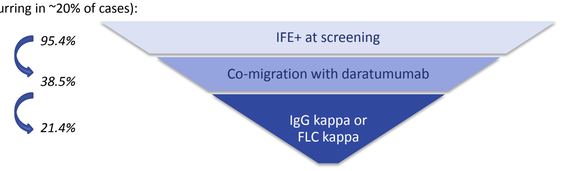
Free light chains kappa MM at C4D28 evaluation: Negative DIRA, presence of daratumumab.

IgA kappa MM at C3D1 evaluation: Positive DIRA, presence of daratumumab. Standard immunofixation (left figure) is sufficient to assess response (no shifting required).

Figure 5: Examples of comparison tests between original DIRA and Hydrashift 2/4 Daratumumab.

➢ WHEN IS DIRA REQUIRED?

Only for patients with an IgG kappa MM or kappa LCMM with a co-migrating M-component (occurring in ~20% of cases):



➢ SENSITIVITY

Daratumumab/anti-daratumumab complexes were detected in the α-globulin fraction with a sensitivity of 200 mg/L.

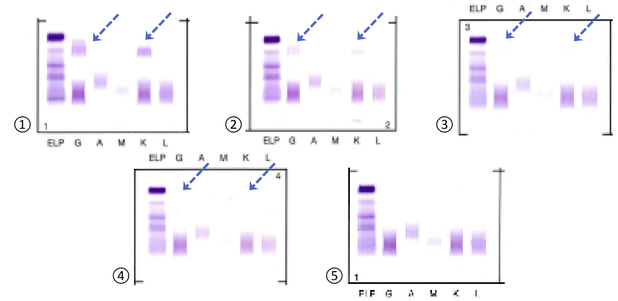


Figure 6: Normal serum spiked with daratumumab at ① 2 g/L, ② 1 g/L, ③ 0.5 g/L, ④ 0.2 g/L, and ⑤ 0.1 g/L.

Daratumumab/anti-daratumumab complex was difficult to visualize when daratumumab concentrations were less than 200 mg/L, but daratumumab was shown to be completely removed from the gamma globulin fraction with no trace left for all concentrations tested.

➢ SPECIFICITY

For 48 samples tested on diagnosis, the anti-daratumumab antibody specifically shifted daratumumab with no effect on the patients' M-spike (100% specificity).

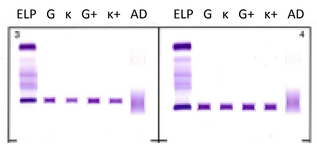


Figure 7: Serum samples (ELP, G, κ) and serums samples spiked with anti-daratumumab (G+, κ+). For each test, a positive control was performed with a normal serum spiked with daratumumab and tested with anti-daratumumab.

➢ HYDRASHIFT 2/4 DARATUMUMAB VERSUS ORIGINAL DIRA:

Advantages
- Standardized and automated test
- Excellent concordance with original DIRA
- Migration of complexes away from the gamma globulin fraction

Limits
- Less sensitivity for daratumumab detection

CONCLUSION

With the growing application of monoclonal antibodies, such as daratumumab, in the treatment of MM, the development of widely available, validated assays to overcome antibody interference will become increasingly important. **The Hydrashift 2/4 Daratumumab test provides the opportunity to standardize and automate the displacement of daratumumab interference and help with the correct interpretation of IFE results for clinical outcome measures.**

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