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

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BACKGROUND

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 (IgGκ) 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 IgGκ myeloma protein, which may impact initial assessment of complete responses (CRs) by International Myeloma Working Group criteria.

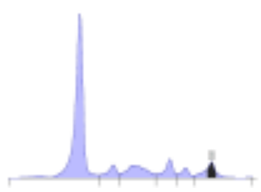


Figure 2: Example of a monoclonal component co-migrating with daratumumab.

The availability of a specific anti-daratumumab antibody has provided the opportunity to overcome this interference and to correctly assess biochemical response. McCudden et al, in collaboration with Janssen, developed the daratumumab interference reflex assay (DIRA), which has been utilized in daratumumab clinical trials.[3] Given the need for a commercially available automated and standardized test, we evaluated the new validated DIRA test, HYDRASHIFT 2/4 daratumumab, that has been developed by Sebia (Lisses, France) and is CE marked and launched in Europe.

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.

PATIENTS AND METHODS

The HYDRASHIFT 2/4 daratumumab assay was prepared by Sebia using the anti-daratumumab antibody that was manufactured under ISO13485 conditions by Janssen and was modified to allow migration of the daratumumab/anti-daratumumab (dara/anti-dara) complex toward the α-globulin fraction on IFE.

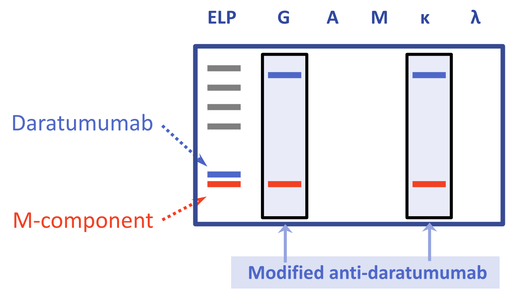


Figure 3: HYDRASHIFT 2/4 daratumumab – principle.

IFE technical procedures, migration, and staining programs were performed according to the manufacturer instructions and run on the standard Sebia HYDRASYS2 platform with the HYDRASHIFT 2/4 daratumumab kit. The kit includes an additional applicator for the anti-daratumumab antibody that is used with the required applicator holder (required accessory).

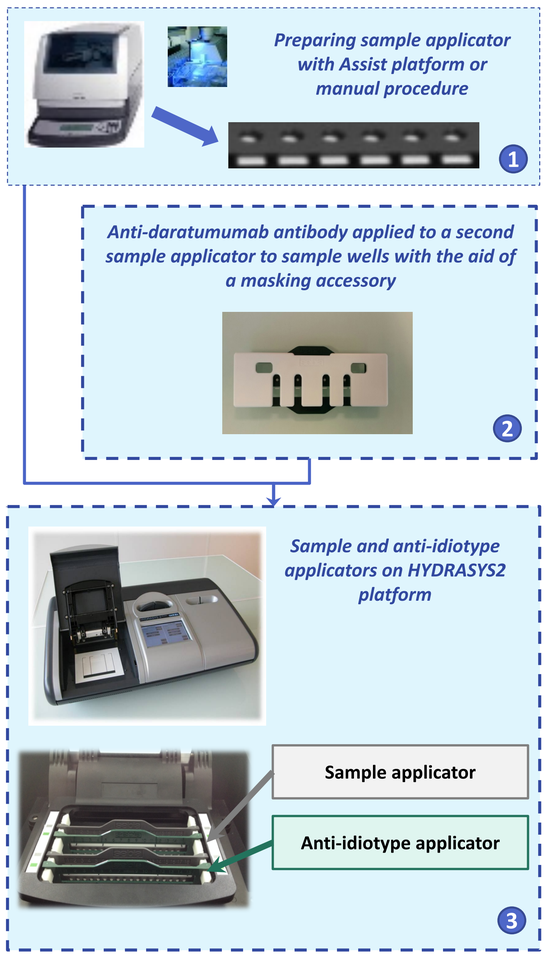


Figure 4: HYDRASHIFT 2/4 daratumumab – technical steps.

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

RESULTS

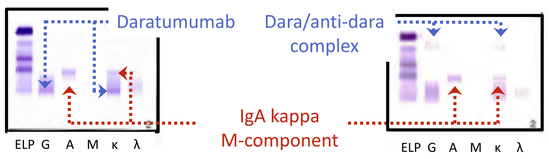
➢ COMPARISON WITH THE ORIGINAL DIRA

The HYDRASHIFT 2/4 daratumumab assay showed 100% concordance 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.

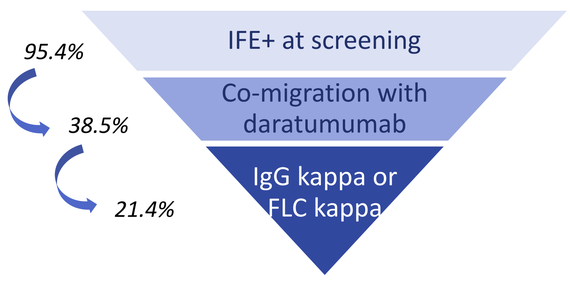


IgA kappa MM at C3D1 evaluation:
Positive DIRA, presence of daratumumab.
Standard immunofixation (left figure) is sufficient to assess response (no shifting required).

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

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

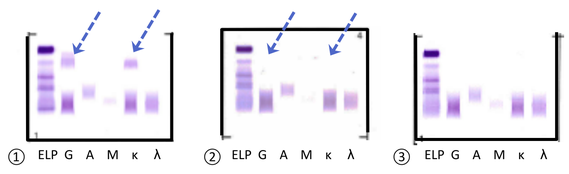


Figure 6: Normal serum spiked with daratumumab at ① 2 g/L, ② 0.2 g/L, and ③ 0.1 g/L.

The complex between daratumumab and anti-daratumumab was difficult to visualize when daratumumab concentrations were <200 mg/L, but daratumumab was shown to be completely removed from the gamma globulin fraction 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).

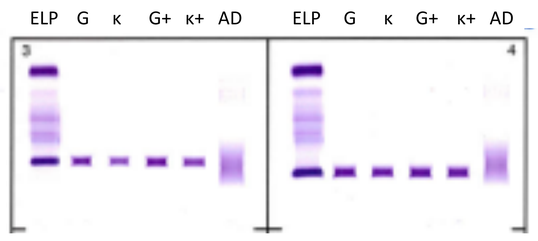


Figure 7: Serum samples (ELP, G, κ) and serum samples spiked with anti-daratumumab (G+, κ+). For each test, a positive control was performed with a normal serum spiked with daratumumab and tested with anti-daratumumab.

➢ 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

- Sensitivity for the daratumumab/anti-daratumumab complex detection at serum concentrations <200 mg/L

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.**

References:

1. Rajkumar SV, et al. Consensus recommendations for the uniform reporting of clinical trials: report of the International Myeloma Workshop Consensus Panel 1. *Blood.* 2011;117(18):4691-4695.
2. Rajkumar SV, Kyle RA. Progress in myeloma – a monoclonal breakthrough. *N Engl J Med*. 2016;375(14):1390-1392.
3. McCudden C, et al. Monitoring multiple myeloma patients treated with daratumumab: teasing out monoclonal antibody interference. *Clin Chem Lab Med*. 2016;54(6):1095-1104.

Conflict of Interest Disclosures:

H. Caillon: Janssen and Sebia (honoraria).

A. Irimia and T. Dejoie: No relevant conflicts of interest to disclose.

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