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Assessing Clinical Response in Multiple Myeloma (MM) Patients Treated With Monoclonal Antibodies (mAbs): Validation of a Daratumumab IFE Reflex Assay (DIRA) to Distinguish Malignant M-protein From Therapeutic Antibody

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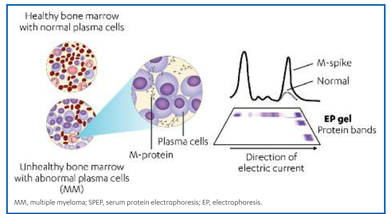
INTRODUCTION

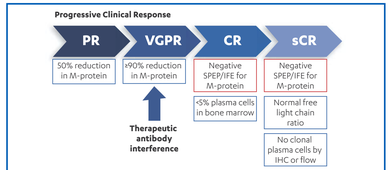
- In multiple myeloma (MM), malignant plasma cells secrete high levels of monoclonal immunoglobulin protein (M-protein) that are detectable by serum protein electrophoresis (SPEP) or immunofixation electrophoresis[1] (IFE; **Figure 1**)



MM, multiple myeloma; SPEP, serum protein electrophoresis; EP, electrophoresis.

Figure 1. MM cells secrete high levels of M-protein detectable by SPEP.

- International Myeloma Working Group (IMWG) criteria require that patients' serum samples are negative for M-protein by SPEP/IFE in order to claim complete response (CR) or stringent CR[2] (sCR; **Figure 2**)



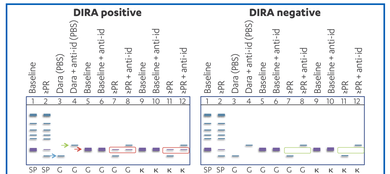
PR, partial response; VGPR, very good partial response; CR, complete response; sCR, stringent complete response; SPEP, serum protein electrophoresis; IFE, immunofixation electrophoresis; IHC, immunohistochemistry.

Figure 2. Therapeutic antibodies may interfere with the ability to confirm clinical outcomes deeper than very good partial responses.

- Monoclonal antibodies (mAbs) have shown therapeutic efficacy in a number of malignancies, but they may interfere with the interpretation of IFE data[3,4]
- Daratumumab is a CD38 IgG1κ mAb in clinical development for the treatment of MM[5]
- Daratumumab has demonstrated clinical responses that deepen over time, necessitating evaluation of CR/sCR by SPEP/IFE[6,7]
  - Approximately 50% of patients with MM produce an IgGκ M-protein. As an immunoglobulin, daratumumab may be detected by IFE and may co-migrate with endogenous M-protein in a subset of patients[8]
- Steady-state concentrations of daratumumab (dosed at 16 mg/kg weekly, bi-monthly, and then monthly) are readily detectable on most SPEP and IFE assays[8]

OBJECTIVE

- Validate and implement a daratumumab IFE reflex assay (DIRA) that distinguishes M-protein from daratumumab, as assessed by IFE, in order to determine if additional testing to assess CR/sCR is warranted (ie, bone marrow examination)
  - Schematics of idealized gels for DIRA-negative (no remaining M-protein) and DIRA-positive (M-protein present) samples are shown in **Figure 3**



DIRA, daratumumab IFE reflex assay; PR, partial response; Dara, daratumumab; PBS, phosphate-buffered saline; anti-id, anti-idiotype; SP, total serum protein fix; G, anti-IgG antisera; κ, kappa antisera.

Figure 3. Schematic presentation of DIRA-positive and DIRA-negative daratumumab-treated patient samples.

METHODS

Patient Samples

- Human serum samples from patients with MM (n = 51) were acquired from a commercial source or from patients treated with daratumumab in clinical trials (n = 33)

IFE

- Serum IFE assays were performed using Maxikit Hydragel 9IF Kits (Sebia Electrophoresis, Norcross, GA) according to the manufacturer's specifications
- Antisera against immunoglobulins gamma (IgG), alpha, mu heavy chains, and free and bound kappa (κ) and lambda light chains were used to characterize the monoclonal protein present in each sample, and visualized by staining

DIRA

- Serum samples for baseline and daratumumab-treated patients were incubated with or without an anti-idiotype mAb (mouse-anti-HuMax-CD38; clone 5-3-9-4) at room temperature for 15 minutes and analyzed by IFE with IgG and κ antisera

Specificity

- To demonstrate that the anti-idiotype antibody binds and shifts daratumumab without affecting detection and migration of endogenous M-protein, commercially available serum samples from patients with MM (n = 51) were spiked with daratumumab, anti-idiotype, or daratumumab + anti-idiotype (500 and 1,000 µg/mL; 1:1 ratio) IgG and κ, and were then analyzed by IFE to assess changes in migration of M-protein

Lower Limit of Detection

- Lower limit of detection (LOD) was determined by evaluating daratumumab ± anti-idiotype over a clinically relevant dynamic range to determine the lowest concentration detected by ≥1 parameter (daratumumab IgG, daratumumab + anti-idiotype complex IgG, daratumumab κ, or daratumumab + anti-idiotype κ by IFE; daratumumab or daratumumab + anti-idiotype by SPEP)

Reproducibility

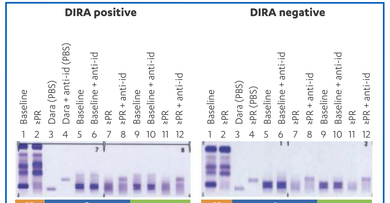
- Three independent runs of 10 samples from daratumumab-treated patients, who had achieved partial response (PR) or better and M-protein ≤0.5 g/dL by SPEP, were performed using DIRA, and the results (DIRA positive or DIRA negative) were assessed for reproducibility

Concordance

- Two independent reviewers interpreted all results

RESULTS

- The DIRA template utilized daratumumab ± anti-idiotype as controls for migration of the therapeutic antibody and the daratumumab–anti-idiotype shifted complexes. Baseline and post-treatment serum ± anti-idiotype were compared to determine whether M-protein remained after shifting daratumumab. DIRA-positive results showed M-protein, whereas DIRA-negative results showed only a shift in daratumumab but no remaining M-protein (lanes 8, 12; **Figure 4**)

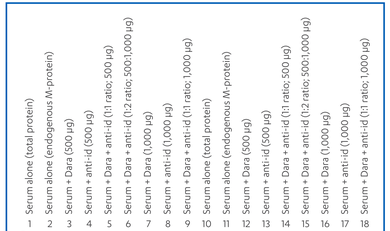


DIRA, daratumumab IFE reflex assay; PR, partial response; Dara, daratumumab; PBS, phosphate-buffered saline; anti-id, anti-idiotype; SP, total serum protein fix; G, IgG antisera; κ, kappa antisera.
Patient samples shown.

Figure 4. Example of DIRA-positive and DIRA-negative daratumumab-treated patient samples.

Specificity

- Daratumumab was shifted by the anti-idiotype at all concentrations in 51 of 51 samples (100%)
- In 47 of 51 samples (92%), no alteration in banding patterns occurred when either concentration of anti-idiotype (500 and 1,000 µg/mL) was introduced, indicating that no nonspecific binding was observed
- In 4 of 51 samples (8%), a faint band appeared with the addition of anti-idiotype at both concentrations with IgG antisera
  - A representative gel, with no change in banding pattern, is shown in **Figure 5A**; the faint band is apparent in **Figure 5B**, lanes 4 and 8



mAb, monoclonal antibody; G, IgG antisera; κ, kappa antisera; Dara, daratumumab; anti-id, anti-idiotype; SP, total serum protein fix.
Commercial samples 35 (A) and 27 (B) are shown.

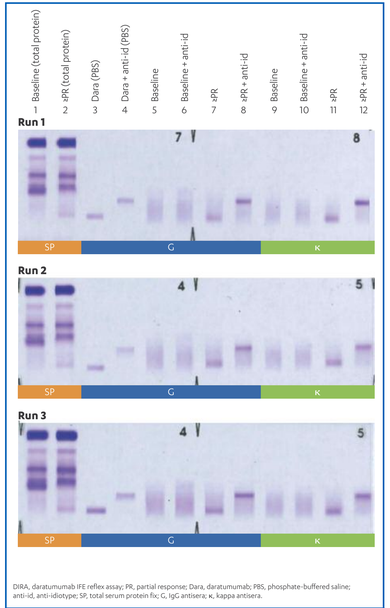
Figure 5. Specificity of anti-idiotype mAb.

Lower Limit of Detection

- In MM serum samples, daratumumab could be detected by IFE at 100 µg/mL in 9 of 10 samples by using ≥1 parameter, and at 200 µg/mL in 10 of 10 samples
  - When the same samples were analyzed by SPEP, either daratumumab and/or daratumumab + anti-idiotype complex could be identified at 100 µg/mL in 3 of 10 samples, and by 200 µg/mL in 10 of 10 samples
- Co-migration with M-protein and varying polyclonal background affect the LOD by some parameters but, in all cases, daratumumab is detectable below the predicted serum levels in treated patients

DIRA Reproducibility and Concordance

- In 10 of 10 (100%) daratumumab-treated patient samples, results were consistent across all 3 independent runs
  - Results from all repetitions from a representative patient sample are shown in **Figure 6**
- There was 100% concordance between the evaluations of both independent reviewers
  - Reviewer evaluations were standardized using a brief form with set assessment criteria; those criteria, and the reviewers' responses assessing the sample shown in **Figure 6**, are tallied in **Table 1**



DIRA, daratumumab IFE reflex assay; PR, partial response; Dara, daratumumab; PBS, phosphate-buffered saline; anti-id, anti-idiotype; SP, total serum protein fix; G, IgG antisera; κ, kappa antisera.

Figure 6. Reproducibility of DIRA results between independent experiments.

Identification of Clinical Responses

- DIRA differentiated daratumumab-treated patient samples that contained residual M-protein (DIRA positive) from those containing no M-protein (DIRA negative)
- 33 samples from daratumumab-treated patients from a number of different studies were assessed for clinical response using DIRA
- 13 patients (39%) were DIRA negative, 10 of whom were confirmed as having achieved CR based on bone marrow and free light chains

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Table 1. Concordance of Reviewer Assessments Between Experiments

Reviewer 1	Lane	Run 1	Run 2	Run 3
Migration of Dara + anti-id in control?	4 vs 3	Y	Y	Y
Migration of endogenous M-protein at baseline?	6 and 10	N	N	N
Migration of Dara in +PR due to the disappearance of Dara (DD) or the appearance of Dara + anti-id complex (AC)?	8 vs 7 and 12 vs 11	Y DD + AC	Y DD + AC	Y DD + AC
Presence of M-protein after migration of Dara?	8 and 12	N	N	N
M-protein (M) or Dara (D)?		D	D	D
Conclusion		Negative	Negative	Negative
Reviewer 2	**Lane**	**Run 1**	**Run 2**	**Run 3**
Migration of Dara + anti-id in control?	4 vs 3	Y	Y	Y
Migration of endogenous M-protein at baseline?	6 and 10	N	N	N
Migration of Dara in +PR due to the disappearance of Dara (DD) or the appearance of Dara + anti-id complex (AC)?	8 vs 7 and 12 vs 11	Y DD + AC	Y DD + AC	Y DD + AC
Presence of M-protein after migration of Dara?	8 and 12	N	N	N
M-protein (M) or Dara (D)?		D	D	D
Conclusion		Negative	Negative	Negative

Dara, daratumumab; anti-id, anti-idiotype; Y, yes; N, no; PR, partial response.

CONCLUSIONS

- **DIRA is a specific, reproducible method to confirm the interference of daratumumab on serum IFE at clinically relevant concentrations**
- **DIRA-negative status warrants additional testing to confirm CR/sCR**
- **IMWG response criteria may require modification as mAbs receive approval for the treatment of MM**

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