

Belantamab Mafodotin detection by protein electrophoresis: Assessing interference for defining clinical response

Poster No. 407

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Background

- Multiple Myeloma (MM) is a life threatening, incurable cancer of the bone marrow resulting in plasma cell expansion and over-production of plasma proteins ('M' proteins). Belantamab Mafodotin (GSK2857916) is an antibody drug conjugate (ADC) consisting of an ADCC-enhanced humanised anti-B-cell maturation antigen monoclonal antibody that is conjugated to the microtubule inhibitor monomethylauristatin F (MMAF) for the treatment of multiple myeloma. The antibody component is an afucosylated IgG1 directed against B-cell maturation antigen, a protein expressed on normal B lymphocytes and multiple myeloma cells. Current criteria for assessing clinical responses, include changes in serum or urine M-protein levels by serum protein electrophoresis and immunofixation electrophoresis. Therapeutic use of ADC has the potential to interfere with 'M' protein assessment by electrophoresis, thus confounding clinical trial results.

Plasma Cell expansion Leading to monoclonal antibody production: 'M' proteins



<http://www.medifree.com/blog/wp-content/uploads/2016/05/multiple-myeloma-1.jpg>
http://www.cancernetwork.com/sites/default/files/cn_import/2147922.png

Objectives

- We aimed to modify an existing electrophoresis (EPS) platform (SAS-1 plus) to identify Belantamab Mafodotin in both saline and patients' sera using a specific Anti-mcMMAF antibody:
 - To determine if Belantamab Mafodotin can be detected by serum protein electrophoresis and/or immunofixation electrophoresis methods that are used to measure 'M' protein in MM patients.
 - If Belantamab Mafodotin can be detected this way, then determine if the detectable levels could interfere with clinical outcomes such as complete response.

Methods

- Varying concentrations of Belantamab Mafodotin were spiked into saline alone and also serum of 7 healthy subjects and 2 multiple myeloma patients.
- These samples were then processed by gel EPS in the same way as diagnosing multiple myeloma. Process refinements employed to optimise the sensitivity of the EPS included modified washing procedures, stain intensity and ratio of ADC to detection Ab.



- The SAS-1 plus (Helena Biosciences) is a microprocessor-controlled instrument designed to automatically apply clinical samples and controls on Helena Biosciences agarose gel products and perform gel-electrophoresis, including serum protein electrophoresis (SPE) and immunofixation electrophoresis (IFE).

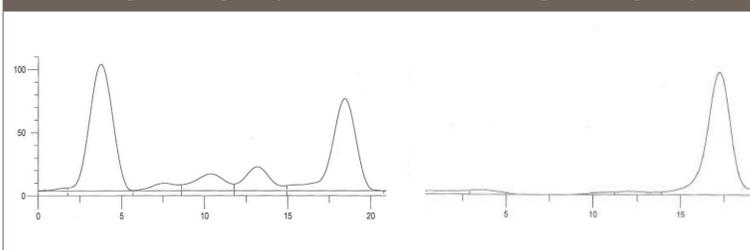
IFE is a two-stage process that uses high resolution agarose electrophoresis in the first stage followed by immunoprecipitation in the second stage. Specific monoclonal antibodies can be used to detect IgG, IgA, IgM, IgKappa and IgLambda in the second stage.

- Specific detection of Belantamab Mafodotin in patients' sera can only be achieved by anti-mcMMAF as anti-IgG and Igkappa antibodies will detect those proteins present in the patients' sera both MM and healthy patients.
- Anti-mcMMAF from Seattle Genetics is a specific antibody that will detect Belantamab Mafodotin thought to recognise the linker/toxin complex of the ADC. This Ab was used during the immunoprecipitation stage of IFE.

Results

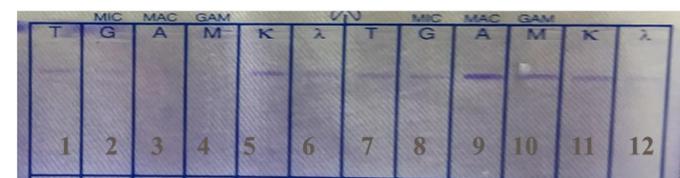
- The SAS-1 plus was able to detect Belantamab Mafodotin by SPE and IFE at concentrations down to 0.5 and 0.2 g/L in saline respectively, the latter being detected using Helena Biosciences IgG antibody, N.B. this is a non-specific IgG detection Ab. This is consistent with published data for the Sebia Hydrasys system, used for clinical trials samples. (Tsui, Thomas et al. 2018).

Abnormal 'M' protein in myeloma patient detected in gamma region by EPS | Belantamab Mafodotin in saline detected in gamma region by EPS



- Anti-mcMMAF from Seattle Genetics is a specific antibody that can detect Belantamab Mafodotin by IFE at concentrations down to 0.5 g/L in saline.
- Belantamab Mafodotin can be detected by IFE down to concentrations of 0.5 g/L with anti-mcMMAF In healthy patients sera (total protein concentration 65–75 g/L).
- In multiple myeloma patients whose total protein concentrations are in excess of 90 g/L, Belantamab Mafodotin can be detected down to concentrations of 1.5 g/L but not 0.5 g/L. Concentrations in between were not assessed.

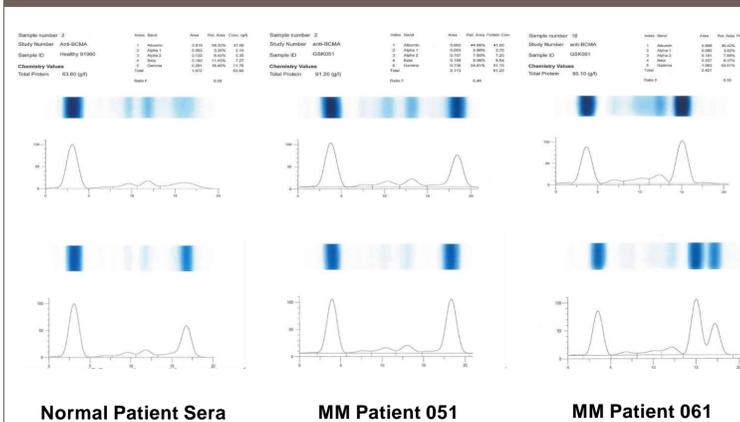
Detection of Belantamab Mafodotin in normal patients' sera by IFE Channels 1, 5 and 9 show 1.5 g/L and Channels 4, 8 and 12 show 0.5 g/L



Optimisation

- Although Belantamab Mafodotin was detected in MM patient's sera down to concentrations of 1.5 g/L, the gamma region of patient 061 spiked with Belantamab Mafodotin showed two distinct peaks. Whereas Belantamab Mafodotin is indistinguishable from patient 051's 'M' protein peak within the gamma region, and as such SPE alone could not identify Belantamab Mafodotin in this patient's serum. Bespoke Immunofixation electrophoresis was required to differentiate Belantamab Mafodotin from abnormal monoclonal proteins within the gamma electrophoretic region.
- Optimisation for individual MM patients' samples is recommended and will be dependent on the concentration and class of protein present.
- Process refinements employed to optimise the sensitivity of the EPS included modified washing procedures, stain intensity and ratio of ADC to detection Ab.
- Further refinement of the SAS-1 plus system may achieve greater sensitivity down to 0.2 g/L (0.2 g/L was detected using IgG specific Ab), however increased sensitivity and optimisation is highly unlikely to detected therapeutic concentrations of Belantamab Mafodotin.

Detection of Belantamab Mafodotin in MM patients samples by EPS



Conclusions

- Therapeutic monoclonal antibodies have the potential to be identified by SPE and IFE, and as such can confound the International Myeloma Working Group consensus criteria for response and minimal residual disease assessment in patients with multiple myeloma (Kumar S, et al. (2016):
 - Criteria for partial response includes at least 50% reduction in serum M-component
 - Very good partial response of 90% or greater reduction in serum M-component
 - Complete responses in patients with CR show that no abnormal monoclonal proteins (also called M-proteins) are present in the blood serum or urine.
- By modifying and refining an existing platform and using an mcMMAF-specific antibody we were able to detect Belantamab Mafodotin in patients' sera with no 'M' protein at concentrations down to 0.5 g/L by IFE.
- Patients in cycle 1 who received the recommended dose of 2.5 mg/kg Belantamab Mafodotin demonstrated a C_{max} of 0.042 g/L (BLENREP Prescribing information Reference ID: 4652412). This therapeutic concentration will not be detected by the SAS-1 plus IFE system and should not interfere with the clinical response criteria.
- Clinical assessment of patients' samples is frequently performed using the Sebia Hydraplus/Hydrasys and the detection limit is between 12 and 25 mg/dL (0.12–0.25 g/L) depending on specific conditions at that time, including, but not limited to, staining process, protein migration position or polyclonal background level.
- The Sebia Hydraplus/Hydrasys clinical platform, with quoted but unconfirmed greater sensitivity, will not have the sensitivity to detect Belantamab Mafodotin at the expected therapeutic doses.

References

- Kumar S, et al. (2016). "International Myeloma Working Group consensus criteria for response and minimal residual disease assessment in multiple myeloma." *Lancet Oncol* 17(8): e328–e346.
- Tsui AKY, et al. (2018). "Analytical sensitivity and diagnostic performance of serum protein electrophoresis on the HYDRAGEL 30 PROTEIN(E) beta1-beta2 Sebia Hydrasys system." *Clin Biochem* 51: 80–84.

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- Drug linker technology licensed from Seattle Genetics; monoclonal antibody produced using POTELLIGENT Technology licensed from BioWa
- The human biological samples were sourced ethically and their research use was in accord with the terms of the informed consents under an IRB/EC approved protocol.

