Laboratory Management of patients treated with Daratumumab: the Irish Experience.

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Background

Daratumumab (DARA) is an anti-CD38 human monoclonal antibody licensed for use in relapsed/refractory MM. CD38 is expressed on myeloma cells and other haemopoietic cells, including red cells. DARA binds CD38 on red cells (RBCs) causing *in vitro* pan-agglutination in the indirect antiglobulin test (IAT), which can mask red cell allo-antibodies. Various methods for mitigating Daratumumab interference have been published (Chapuy & et al., 2015).

DARA became available in the Republic of Ireland (RoI) as part of clinical trials early in 2016 followed by compassionate use in March 2016. It was agreed that all pre-compatibility testing for patients receiving DARA in the RoI would be performed by the IBTS laboratories (Red Cell Immunohaematology (RCI) and Diagnostic Laboratories), requiring the validation of a satisfactory technique.

Materials & Methods

The RCI Laboratory evaluated five different methods to detect known alloantibodies in the presence of DARA prior to its release. These included :

1. Allo-adsorption studies
2. Cord cells (which have low expression of CD38) as reagent red cells

3-5. DTT treatment of reagent red cells using three different techniques.

Testing was performed on plasma containing known antibodies spiked with DARA at three different concentrations 1µg/ml, 10µg/ml & 35µg/ml.

Each method was assessed for specificity, sensitivity, reproducibility and repeatability.

Allo adsorption studies were performed using various ratios of red cells to plasma, papain treated and untreated cells and the use of LISS versus non-LISS methods.

A selection of phenotyped cord cells were recovered and used as an antibody screening panel.

The DTT methods evaluated were: direct tube IAT technique, Low Ionic Strength Saline (LISS) tube IAT technique and gel column (GC) IAT techniques.

References

- Kelly , M. (2010) 'Diagnosis and management of multiple myeloma.' *Irish Medical Times.* Accessed 12/09/17.
- Al-Farsi, Khalil. "Multiple Myeloma: An Update." *Oman Medical Journal* 28.1 (2013): 3–11. *PMC*. Web. 27 Feb. 2017.
- Chapuy, C., Nicholson, R., Aguad, M., Chapuy, B., Laubach, J., Richardson, P., Doshi, P. and Kaufman, R. (2015) 'Resolving the daratumumab interference with blood compatibility testing', *Transfusion.*, 55(6pt2), 1545–54.
- Guidelines for pre-transfusion compatibility procedures in blood transfusion laboratories. British Committee for standards in Haematology.

Results: Evaluation

Allo-adsorption studies are summarised in Table 1 and all methods in Table 2.

Ratio *(Red cells: Plasma: LISS)*	DARA conc. 1ug/ml	DARA conc. 35ug/ml
1.1.1	Failed to remove interference	Failed to remove interference
2.1.1	Failed to remove interference	Failed to remove interference
3.1.1	Failed to remove interference	Failed to remove interference
4.1.1	Removed interference	Failed to remove interference

Table 1: The optimum adsorption technique was found to be the LISS addition method using a ratio of 4:1:1 with untreated cells.
This removed DARA spiked plasma conc. 1µg/ml following four adsorptions but not at the concentration seen in patients plasma which can be as high as 35µg/ml.

Method	Advantages	Disadvantages
Allo-Adsorption	Method is validated and used routinely.	A high volume of adsorptions cells are required at removing DARA. Method is not reliable with higher concentrations of DARA.
Cord Cells	No / very weak expression of CD38 on cord red cells. Easy and quick to use.	Phenotyped cord cells not readily available. Would have to meet BSH guidelines for 3 cell screen.
DTT Treatment	Both card and tube method can be used.	DTT sensitive blood group systems are removed. Time consuming and labour intensive.

Table 2: Identifies the advantages and disadvantages of the three methods evaluated

Of the DTT methods evaluated, the direct tube method was not sufficiently sensitive when detecting underlying antibodies and was rejected. The DTT LISS tube and GC IAT method were satisfactory with the GC method the most sensitive method. These two DTT method, were chosen as the method of choice and validated for use in the laboratory.

Performance monitoring was performed on all 137 samples referred to the IBTS from May 2015 to March 2017 (see below).

Results: Patient Samples

Following the release of DARA in March 2016 until March 2017, samples from 28 patients were tested using both LISS tube and GC IAT methods. From 28 patients, we received 137 cross-match samples and issued a total of 301 units.

Despite DTT treatment, the GC method remained positive by IAT in 16/28 patients tested (Figure 1).

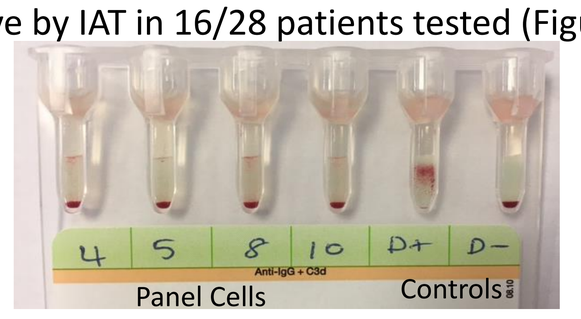

Figure 1. The interference remaining post DTT treatment

Further testing was performed on 9/16 patients. Eight were tested for the presence of antibodies at 18°C: a cold reactive antibody was detected in all eight tested. Rouleaux formation was observed in 5/9 patients tested and four out of the five also had reactivity detectable at 18°C.

No transfusion reactions have been reported to date nor has alloantibody formation been observed in any of the patients.

Conclusions

1. As previously reported, the DTT method was the most useful for mitigating DARA interference.

2. In some instances interference remained with the DTT GC IAT technique. This appears to be due to rouleaux and/or cold reactive antibodies present in the patient's plasma. (Rouleaux are a feature of active MM).

3. The DTT LISS tube method is adaptable when a cold reactive antibodies are present, by performing the technique at 37°C

It is hypothesised that interference is not observed with the DTT LISS tube IAT method due to the multiple washing phases in the technique as the washing phases dissipate rouleaux formation.

While DTT has its limitations, it is currently the most efficient way of dealing with this cohort of patients.

Discussion

We now request baseline (pre-DARA) samples and perform a group, antibody screen, extended phenotype, cold panel and rouleaux check on these – the latter two investigations based on our experience described above. Once the patient has been administered DARA, on initial patient referral both DTT IAT methods are performed. If residual reactivity in gel column is observed then the LISS tube technique is employed thereafter in these patients.

If the history of DARA administration is unknown, valuable time may be wasted in fruitless investigations resulting in delays in issuing blood. If we suspect DARA use, we use cord cells of known phenotype as a confirmation/exclusion step. Fortunately, only two patients (to date) receiving DARA have been referred without a history.

DTT treatment cleaves certain blood group system antigens from RBCs : these are listed in each patient report. BSH (UK) Guidelines recently recommended that rare k- units be given to all patients who type as k-. This approach mitigates against the possibility of missing an anti-k, however, this antibody can be excluded with the use of suitable cord cells, if available, reserving the use of these uncommon units for those with an allo-antibody.

Cord banks may wish to consider investigating the provision of cord red cells of known phenotypes, especially those negative for the antigens which DTT cleaves.

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