Red blood cell antibody assays in DARA-treated patients

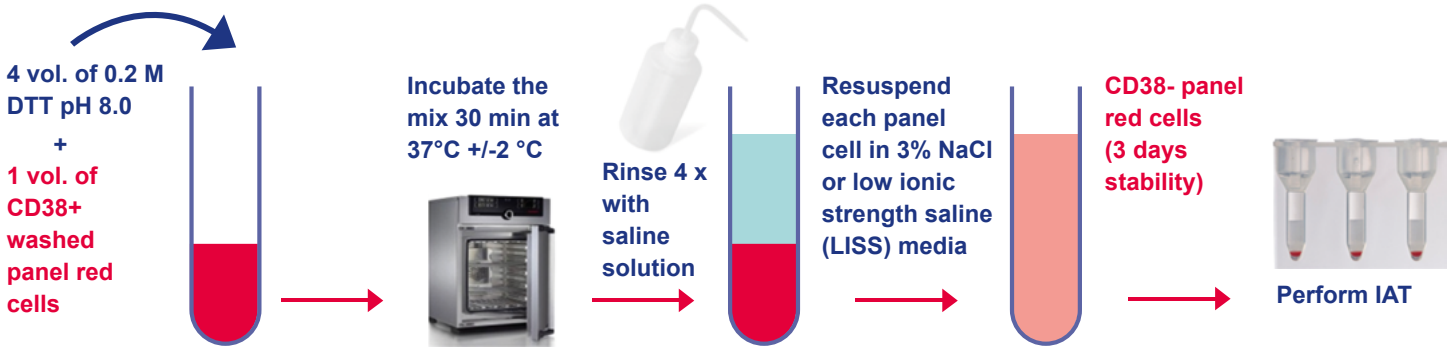
E. RABUT, A. CASTRO-FERNANDEZ, N. MEKNACHE
Hematology Department
Eurofins Biomnis laboratory, Ivry-Sur-Seine, France

Introduction

Daratumumab (DARA) is a treatment of multiple myeloma refractory to conventional chemotherapy prescribed since 2015. DARA is an experimental human monoclonal anti-CD38 IgG1K: by binding with high affinity to tumor cells expressing the CD38 signaling molecule on their surface, DARA destroys these plasma cells by various mechanisms. Through its anti-CD38 activity, DARA also recognizes red blood cells (RBC) as they weakly express the CD38 target molecule. This leads to turn positive RBC antibody screening and identification by indirect antiglobulin test (IAT) up to 6 months after the last injection of DARA. This interference may mask the presence of RBC alloantibodies when classical methods of RBC antibodies screening are performed.

Method

We describe the RBC screening and identification profiles of 29 DARA-treated patients, performed by IAT (solid-phase immunocapture technique and gel column filtration), enzymatic method (papain treatment) and dithiotreitol (DTT) treatment of panel cells. Papain and DTT treatments both destroy CD38 antigen, removing the artifactual panagglutinin. Commercial papain treated panels have been used. DTT treatment is prepared in such a way:



For DTT treatment 3 or 4 panel cells are selected according to their extended phenotype: homozygous cells for FY1, FY2, JK1, JK2, MNS3 and MNS4 are necessary. As DTT destroys KEL2 antigen, KEL2 phenotype of panel cells is analyzed before and after DTT treatment to prove treatment effectiveness. Anti-FY1 internal quality control is also performed to ensure the quality of the method.

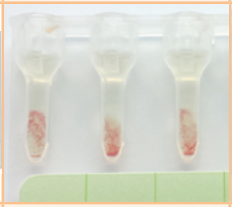
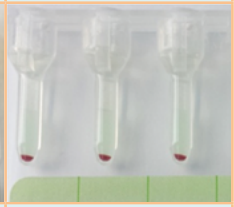
A stability study of DTT treated panel cells in LISS has been carried out and show a stability of 3 days at refrigerated storage.

Results

Immunohematological profile of 29 DARA treated patients :

Autocontrol	Direct Antiglobulin Test	Blood grouping/ phenotyping
Mostly negative (26/29)	Mostly negative (26/29)	Undisturbed

One patient had unexplained reactions on papain treated panel and another patient unexplained and weak reactions on DTT treated cells.
In these cases other panel cells may be treated by DTT to formally exclude alloantibodies.

IAT by solid phase (Neo, Immucor)	IAT by gel column filtration	Papain-treated panel	DTT-treated panel cells
Image / Score / Intensity Cell 1: 30.8 / 1+ Cell 2: 58.8 / 2+ Cell 3: 15.5 / 0 Cell 4: 24.5 / 2+			
Unconsistent panagglutinin or heterogenous reactions (negative to 3+/4+ reactions)	Consistent homogenous and weak panagglutinin (29/29)	Undisturbed: negative or presence of allo-Ab	Undisturbed: negative or presence of allo-Ab

Conclusion

A homogeneous and mostly low intensity panagglutinin at column filtration IAT is characteristic of DARA interference. As transfusions are often necessary in patients with myeloma, removing the interference is required to check possibly masked alloantibodies. **For this purpose an enzymatic treatment (trypsin or papain) AND a DTT treatment are necessary.**

Benefits of combining DTT + enzymatic treatment : effective, costless, quick implementation

Limitations: KEL antigens are damaged by DTT treatment / FY and MNS antigens are destroyed by papain
▶ KEL1 antigen matched RBC transfusions are recommended in chronically transfused patients. If possible extended phenotype matched transfusions are recommended to prevent RBCs alloimmunization.

Oostendorp M, Lammerts van Bueren JJ, Doshi P, Khan I, Ahmadi T, Parren P, et al. When blood transfusion medicine becomes complicated due to interference by monoclonal antibody therapy. Transfusion 2015;55(6 Pt 2):1555-62

Chapuy CI, Aguad MD, Nicholson RT, Aubuchon JP, Cohn CS, Delaney M, et al. International validation of a dithiothreitol (DTT)-based method to resolve the daratumumab interference with blood compatibility testing.

Oral and Poster Abstracts presented December 7, 2015 at 57th Annual American Society of Hematology meeting.Blood 2015;26(Suppl):Abstract 3571.