Irish Blood Transfusion Service
Seirbhís Fuilaistriúcháin na hÉireann

Mitigating the Interference of Anti-CD47 in Pre-Transfusion Serological Testing

Julie Long
Medical Scientist
Irish Blood Transfusion Service
James' St.
Dublin 8

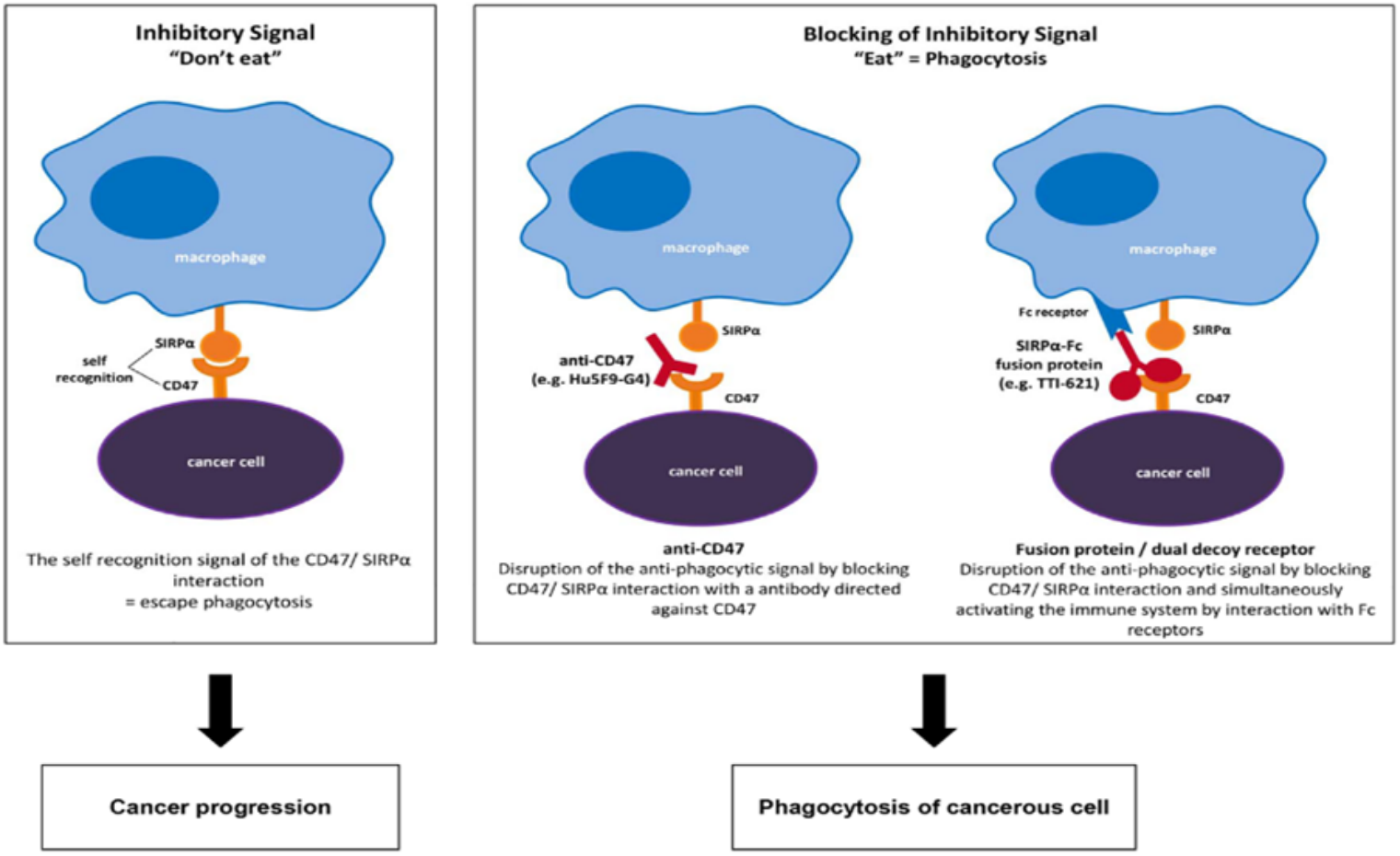
Magrolimab

- First in class human monoclonal IgG subclass 4 (IgG4) anti-CD47 antibody created by Gilead Sciences.
- Magrolimab was designed to treat haematological and solid tumours.
- ENHANCE Clinical trial has recently been terminated for treating Myelodyplastic syndrome (MDS) and paused for treating acute myeloid leukaemia (AML) due to futility.
- Extended access has been granted in America.

CD47

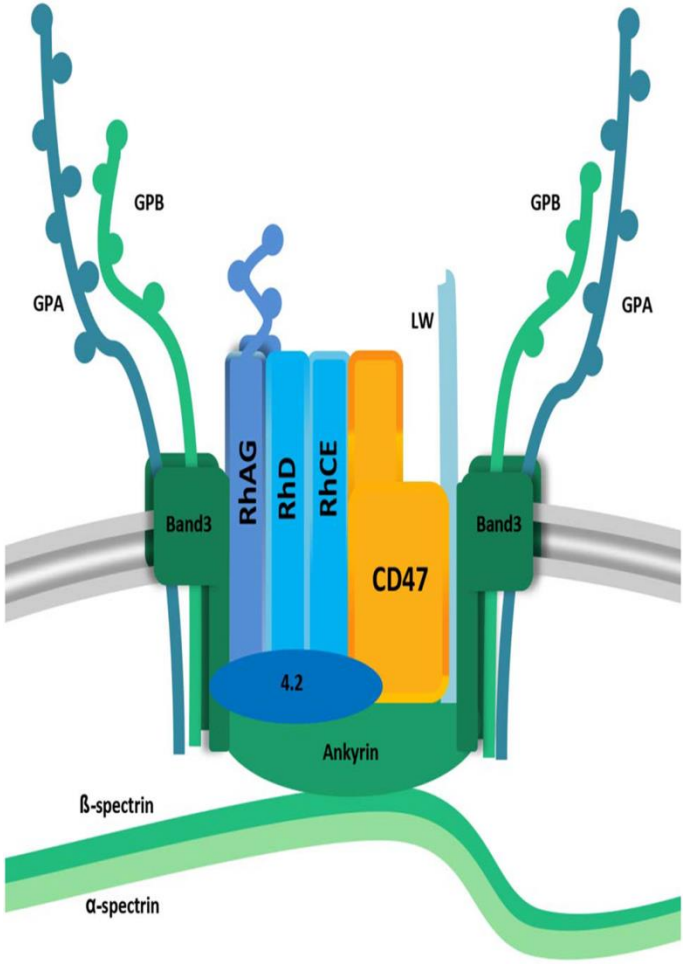
- CD47 is a glycoprotein expressed on all human cells and acts as an immune checkpoint.
- CD47 prevents phagocytic removal of normal cells by binding to it's receptor, single regulatory protein α (SIRPα), on macrophages. Upon binding the macrophages receive a protective 'do not eat me' signal preventing apoptosis by phagocytosis.
- Increased CD47 expression has been detected on tumour cells which supports them in avoiding phagocytosis.

CD47 Antagonists



Interference in Pre-Transfusion Serological Testing

- CD47 is expressed on the RBC surface as part of the Rh complex.
- Similarly to DARA binding to CD38 , Magrolimab will bind to CD47 on reagent red cells *in vitro.*
- It has been reported, patients receiving Magrolimab will show reactivity with all reagent red cells in Indirect Antiglobulin Test (IAT) and may show interference in forward and reverse ABO grouping.



Study Aims

- Determine the extent of Magrolimab interference in pre-transfusion testing in the Red Cell Immunohematology laboratory.

- Find a viable approach to negate Magrolimab interference in pre-transfusion testing, ensuring safe and timely blood transfusions for this specific patient cohort.

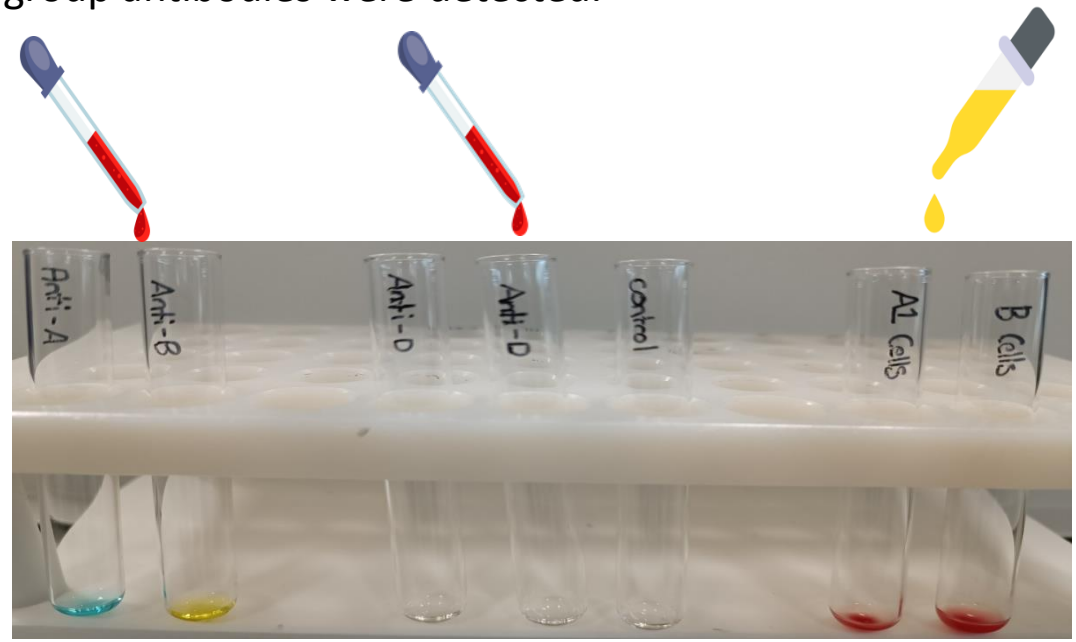
Magrolimab Samples

- Live patient samples were unavailable for testing.
- Plasma and whole blood EDTA samples were spiked with Magrolimab (provided by Gilead Sciences).
- It has been reported that patient samples have an anti-CD47 doubling dilution titre of between 4,096 and 16,384 (Velliquette, 2019).
- Magrolimab was provided in 10ug/ml suspension.
- It was established that 6.5ul of Magrolimab in 1ml of plasma resulted in a titre of 16,384.
- A lower concentration of 0.5ul/3ml resulted in a titre of 4,096 and was used when mitigation techniques were ineffective.

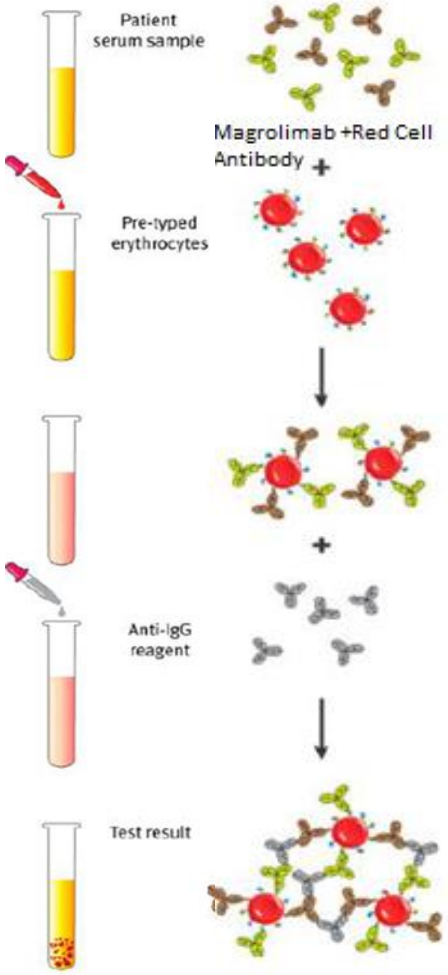
Plasma (concentration)	2	4	8	16	32	64	128	256	512	1024	2048	4096	8192	16384	32,768
P-1 (6.5µl/ml)	+4	+4	+4	+4	+4	+4	+4	+4	+3	+3	+3	+3	+3	+2	0
P-2 (6.5µl/ml)	+3	+3	+3	+3	+3	+3	+3	+3	+3	+3	+3	+3	+3	+2	+w
P-5 (0.5/ 3ml)	+3	+3	+3	+3	+3	+3	+3	+3	+3	+2	+2	+1	+w	NT	NT

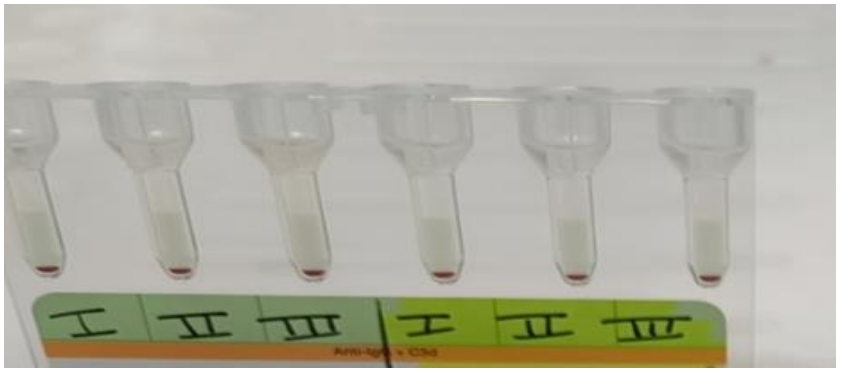
ABO Group

- Manual tube ABO group was performed on spiked EDTA samples.
- Pan-agglutination (+4/+3 reactions) was observed in the forward and reverse group of all 12 spiked samples (A, B, AB, O).
- Following warm washing of patient RBCs, anti-CD47 interference remained in the forward group.
- Following serial allo-adsorption of patient plasma interference was resolved in the reverse group and ABO reverse group antibodies were detected.



Interference in Antibody Identification





Neat plasma

Magrolimab spiked plasma

Mitigation Techniques

- Immucor AHG
- Allo-Adsorption
- SrCD47

LISS Tube IAT Method

- Routine Ortho AHG reagents were replaced with Immucor AHG reagents that do not detect IgG4 antibodies.

- Magrolimab is an IgG4 monoclonal antibody and clinically significant red cell antibodies do not tend to be IgG4 in nature.

- LISS Tube IAT technique was tested using 27 spiked plasma (Consisting of antibody screen negative, Anti-D, Anti-C, anti-c, anti-E, anti-e, anti-K, anti-Fya, anti-Fyb, anti-Jka, anti-Jkb, anti-M and anti-S).

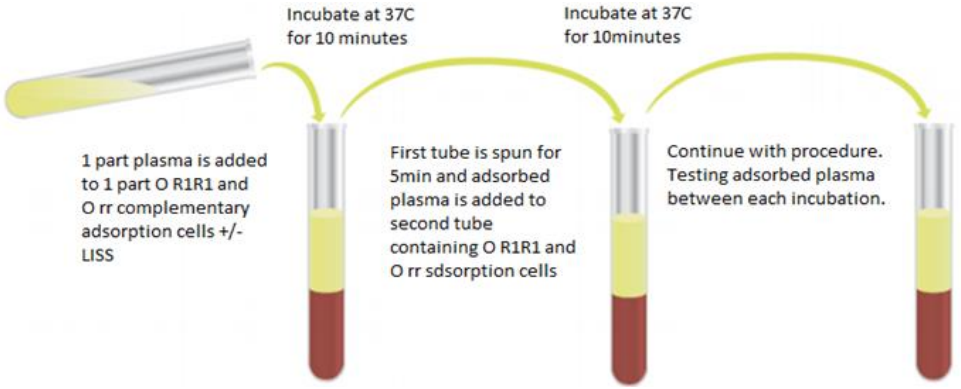
- Two drops of patient plasma were incubated with 2 drops of 3-5% Quotient reagent red cells in LISS at 37°C for 20 minutes. Following incubation the test was washed 4 times in PBS and 2 drops of anti-human globulin reagent were added. Tubes were read macroscopically with visible agglutination being assigned a +2, +3 or +4 result.

LISS Tube IAT Results

Plasma concentration	Immucor Reagent	Number of samples tested	Interference resolved	Partially resolved	Complete interference remained
6.5µl/ml	Anti-IgG, C3d	17	8	7	2
6.5µl/ml	Anti-IgG	8	3	4	1
0.5µl /3ml	Anti-IgG, C3d	2	0	2	0
0.5µl/3ml	Anti-IgG	3	1	1	1

Allo-Adsorption Technique

- Complementary phenotypically matched donor cells are used to adsorb out auto-antibodies, allowing for the detection of any underlying allo-antibodies.

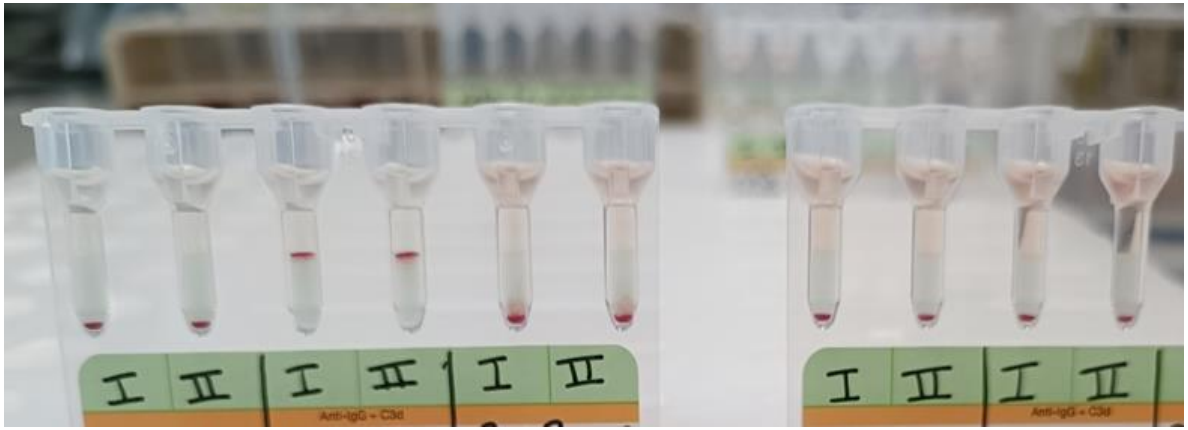


- Papain treated and untreated adsorption cells were used and serial rounds were carried out until anti-CD47 interference was removed.

Allo-Adsorption Results

- Following four rounds of adsorptions with either papinized or untreated RBCs, anti-CD47 interference in BioRad gel card IAT was eliminated in all 22 plasmas.

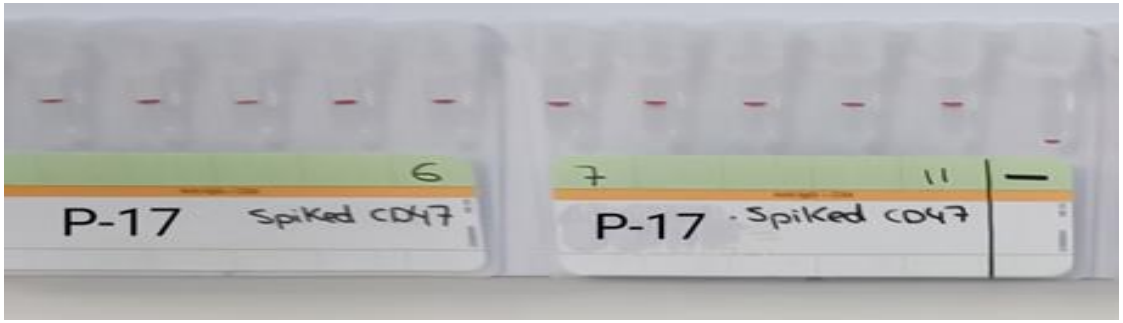
- Weak reactions remained following three rounds of allo-adsorption.

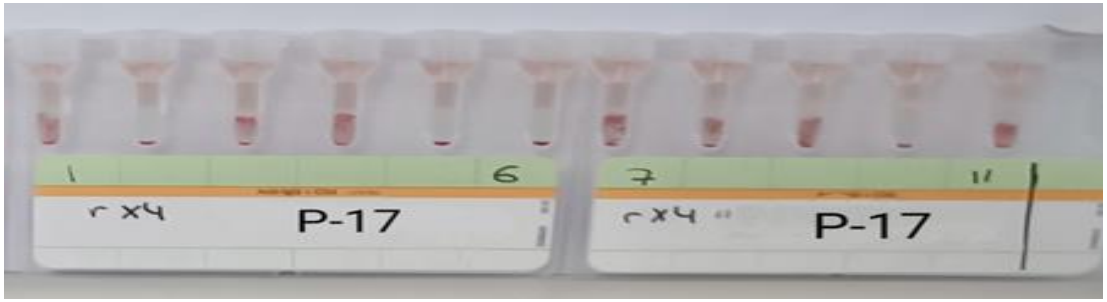


Neat/ Spiked/ R1R1 Ads x 3 R1R1 Ads x4/ rr Adsx4

Allo-Adsorption Results

- Underlying clinically significant antibodies (Anti-D, Anti-C, anti-c, anti-E, anti-K, anti-Fya, anti-Fyb, anti-Jka, anti-Jkb, anti-M and anti-S) were detectable by gel card IAT following removal of anti-CD47 by allo-adsorption technique





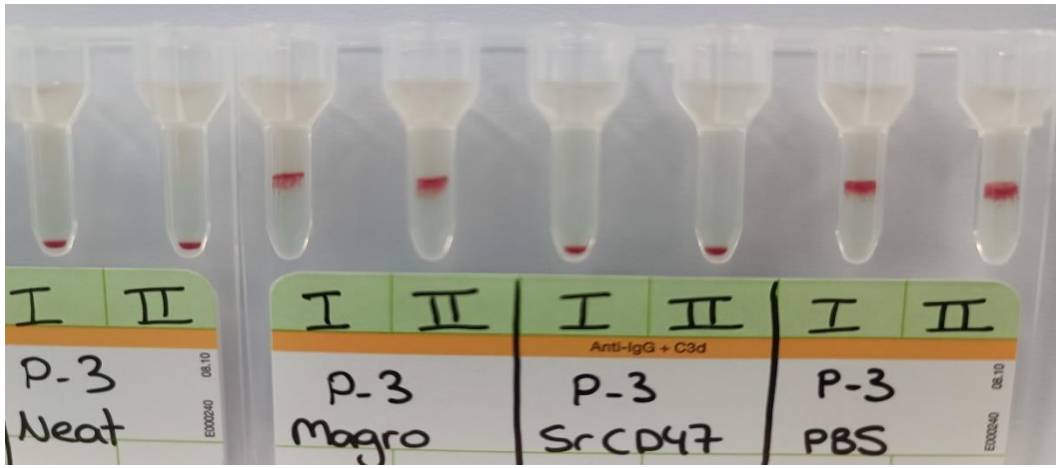
Jka+	Jka-	Jka+	Jka+	Jka-	Jka-	Jka+	Jka+	Jka+	Jka+	Jka-	Jka+

SrCD47 Technique

- Thirty microliter vials of srCD47 in PBS pH 7.2-7.4 were provided by the National Health Service Blood and Transplant.
- As per manufactures instructions 60µl of Magrolimab spiked plasma was added to the 30µl vial of srCD47.
- A dilution control was also set up of 60µl of plasma and 30µl of PBS.
- The solutions were mixed well and incubated at room temperature for 15 minutes.
- The solutions were analysed by IAT using BioRad gel cards.

SrCD47 Results

- Soluble recombinant CD47 was effective at inhibiting anti-CD47 in the two samples tested.



- Further analysis and validation would be required to ensure this was an effective method at removing anti-CD47 interference, allowing for the identification of underlying clinically significant antibodies.

- The major benefit of srCD47 is that it has the potential to be effective at mitigating several drug types such as mAbs and fusion proteins

- Major problem is it is not yet commercially available.

Conclusion

- If a patient's blood group cannot be determined the patient must receive group O red cell units for transfusion.
- As per the British Society for Haematology (BSH) guidelines the reverse group may be dropped from subsequent patient samples following an internal validation and risk assessment plan as long as the patient samples are tested solely on an automatic system.
- Results from this study found that using Immucor AHG reagents that do not detect IgG4 antibodies for LISS tube IAT technique is not a reliable antibody identification method as anti-CD47 may still intervene.
- This study determined that differential LISS allo-adsorptions are a reliable, robust and safe method for anti-CD47 mitigation and antibody identification, and compatibility testing.
- Soluble recombinant CD47 was effective at inhibiting anti-CD47 but further analysis and validation would be required.

Patient Management

- Clear communication and strong collaboration between clinicians, local transfusion laboratories and the RCI laboratory will be central to managing patients taking anti-CD47 therapy.

- Pre-Transfusion baseline testing:
  - ABO group and RhD typing
  - Antibody screen and identification
  - DAT
  - Phenotyping or genotyping for Rh (DCcEe), K, Jk^a, Jk^b, Fy^a, Fy^b, S and s

- Close monitoring of patient hemoglobin level is advised to anticipate transfusion requirements and provide the transfusion laboratory with maximum notice of expected transfusions.

Acknowledgements

Gilead Sciences

Aisling Costelloe

Edel Scally

Dr Kieran Morris

Staff at the RCI Laboratory, National Blood Centre

NHS Blood and Transplant

References

- Brierley C.K., Staves J., Roberts C., Johnson H., Vyas P., Goodnough L.T. and Murphy M.F. (2019) The effects of monoclonal anti-CD47 on RBCs, compatibility testing, and transfusion requirements in refractory acute myeloid leukemia. *Transfusion,* 59: 2248-2254

- Du C., Sui W., Huang H., Zhang Y., Ding X., Gao C. and Wang Y. (2022) Effect of clinical application of anti-CD38 and anti-CD47 monoclonal antibodies on blood group detection and transfusion therapy and treatment. *Leukemia Research*,122, 106953, 0145-2126

- Kim T.Y., Yoon M.S., Hustinx H., Sim J., Wan H.I. and Kim H. (2020) Assessing and mitigating the interference of ALX148, a novel CD47 blocking agent, in pretransfusion compatibility testing. *Transfusion,* 60(10):2399-2407

- Velliquette R.W., Aeschlimann J., Kirkegaard J., Shakarian G., Lomas-Francis C. and Westhoff C.M. (2019) Monoclonal anti-CD47 interference in red cell and platelet testing. *Transfusion*, 59(2):730-737

Thank you!

WE IMPROVE PATIENTS
LIVES, TOGETHER

Irish Blood Transfusion Service
Seirbhís Fuilaistriúcháin na hÉireann

IBTS Research & Development

Irish Blood Transfusion Service
Seirbhís Fuilaistriúcháin na hÉireann

IBTS Research & Development