# Applicable Laboratory(s)):

[x]  North Carolina Baptist Hospital (NCBH)

[ ]  Lexington Medical Center (LMC)

[ ]  Davie Medical Center (DMC)

[ ]  Wilkes Medical Center (WMC)

[ ]  High Point Medical Center (HPMC)

[ ]  Westchester

[ ]  Clemmons

# Procedure Statement

Monoclonal antibody-based cancer therapies are under development. Some of these therapies may interfere with blood bank tests. Guidelines are developed to determine the best approach to testing in the presence of these new drugs.

# Scope

Procedure Owner/Implementer: Blood Bank Management

Procedure Prepared by: Julie Simmons

Who Performs Procedure: Blood Bank Staff

# Definitions

1. Procedure: A process or method for accomplishing a specific task or objective.
2. WFBH Lab System: Wake Forest Baptist Lab System is a health system that includes Wake Forest Baptist Medical Center and all affiliated organizations including Wake Forest University Health Sciences (WFUHS), North Carolina Baptist Hospital (NCBH), Lexington Medical Center (LMC), Davie Medical Center (DMC), Wilkes Medical Center (WMC), High Point Medical Center (HPMC), Lab at Westchester and Lab at Clemmons.

# Sections

1. Policy Guidelines
2. Use of DTT Treated Red Cells for patients receiving DARA
3. Patients Receiving anti-CD-47 (Hu5F9-G4) Magrolimab

# Policy Guidelines

1. **General Guidelines**
2. Monoclonal antibodies directed against specific markers expressed on tumor cells are an active area in new drug development to treat hematologic malignancies and solid tumors.
3. Two monoclonal antibodies currently undergoing use are anti-CD38 (DARA) and anti-CD47 (Hu5F9-G4) Magrolimab.
4. Patients receiving treatment with monoclonal antibody drugs (DARA – anti-CD38 and Hu5F9-G4 – anti-CD47) should have samples submitted for ABO, Rh, antibody screen and DNA genotyping prior to start of therapy.
5. Many other antibody-based cancer therapies are under development. Some of these may also interfere with blood bank tests.
6. **DARA (Anti-CD38)**
7. Anti-CD38 (DARA: Daratumumab) is a monoclonal antibody drug that is a new treatment for multiple myeloma (MM).
8. CD38 is highly expressed on myeloma cells.
9. CD38 is expressed at low levels on the surface of red blood cells.
10. Anti-CD38 kills by complement mediated cytotoxicity through phagocytosis.
11. Approximately 1/3 of patients with refractory Multiple Myeloma have responded to anti-CD38.
12. Patients being treated with Anti-CD38 have anti-CD38 in their plasma that may or often reacts with all red cells.
13. Patients usually undergo treatment with DARA for approximately 8 weeks.
14. Level of anti-CD38 may be a high titer and cannot be easily adsorbed.
15. There are several published approaches to resolving the reactivity of the anti-CD38:
	1. Denature CD38 (protein) on red cells with Dithiothreitol (DTT).
	2. Neutralize the anti-CD38 in the plasma.
	3. Genotype/phenotype patients and give phenotypically matched units
	4. Cord red blood cells that have been phenotyped for testing may be used for antibody screening.
16. DTT is a reducing agent that disrupts the tertiary structure of proteins by reducing the disulfide bonds to free sulfhydryl groups.
	1. Protein antigens cannot bind to their specific antibodies.
	2. DTT destroys antigens in the Kell system, most antigens in the Knops system and most antigens below:
17. Cartwright, LW, Scianna, Dombrock (Doa/Dob), Lutheran, JMH, Indian, Gregory (Gya), Holly (Hy), and Joseph (Joa) are sensitive to DTT treatment.
18. Prior to starting any patient on anti-CD38, a baseline type and screen and a phenotype or genotype will be performed.
	1. The comment DARA/date should be added to the patient’s computer record to flag future work-ups within the 6 month period that DARA may be causing interference.
19. DTT treatment of reagent red cells will be the first approach.
20. After receiving DARA, the patient’s plasma is expected to react with all red cells and the DAT may also be positive.
21. The patient’s antibody screen should be set up in PEG.
22. If the neat PEG testing is reactive with all cells, the 3% screening cells should be treated with DTT. The DTT treated cells are tested with patient’s plasma.
23. Reactivity with the untreated and negative with the treated suggest DARA interference.
24. The eluate should be prepared if the DTT treated DAT is positive and tested in parallel with the DTT treated and untreated screening cells.
25. Patients should receive Kell negative units if they are Kell negative. Management/Medical Director will determine if phenotypically matched transfusions should occur.
26. **Anti-CD47 (Hu5F9-G4) Magrolimab**
27. CD47 is a cell surface glycoprotein expressed on all cell types including red blood cells and platelets.
28. CD47 is expressed as a member of the Rh complex in the membrane so expression of CD47 varies according to Rh phenotype.
29. CD47 is involved in regulation of cell survival and cell death because it acts as a signal-regulatory protein expressed on macrophages. Blocking CD47 is thought to enhance phagocytosis and promote antitumor responses.
30. Anti-CD47 (Hu5F9-G4) now known as Magrolimab, is an IgG4 monoclonal antibody in clinical trials to treat hematologic and solid organ malignancies.
	1. Multiple Myeloma patients may receive Magrolimab alone or in combination with DARA
	2. AML patients enrolled in ENHANCE-3 clinical trial may receive Magrolimab.
31. Nearly all patients receiving anti-CD47 experience an initial drop in hemoglobin with thrombocytopenia occurring infrequently.
32. Plasma from patients receiving Anti-CD47 reacts in all phases with all red blood cells including immediate spin, i.e. panreactivity in all phases of pretransfusion testing including antibody screen, crossmatch and ABO reverse type.
33. Plasma is reactive with DTT, trypsin, papain.
34. Antibody may be very high titer.
35. Patients may have weak spontaneous agglutination of their red cells causing a weak false-positive reaction in ABO forward typing and Rh typing.
36. Immucor gamma clone anti-IgG lacks IgG4 and reactions appear weaker.
37. Direct antiglobulin testing and auto control were negative or weak with reactive eluates. The negative DATs are thought to be due to blocking or steric hindrance, i.e. the antibody is present but a falsely negative DAT occurs.
38. PEG adsorptions are often invalid due to precipitation of antibody.
39. Reactivity has been removed by multiple allo adsorptions with papain-treated cells or pooled platelets.
40. ARC currently uses pooled platelets to perform adsorptions when testing is positive.
41. Multiple Myeloma patients receiving BOTH Dara and Magrolimab:
42. Require testing in PEG with Immucor Gamma Clone anti-IgG.
43. May require adsorptions as stated above if Peg testing is positive
44. Shall receive Kell negative units if they are Kell negative

**D. ENHANCE-3 Clinical Trial**

1. ENHANCE-3 is a Phase 3, randomized, double-blind, placebo-controlled study evaluating the safety and efficacy of Magrolimab versus placebo in combination with Venetoclax and Azacitidine in newly diagnosed, previously untreated patients with Acute Myeloid Leukemia (AML) who are ineligible for intensive chemotherapy.
2. An email to the blood bank group will be sent out alerting BB staff that a patient has been enrolled into this trial.
3. Add special message: BCD47 (Blinded Study Patient: Anti-CD47) to Patient Caution Window when email is received.
4. All patients in this trial will require irradiated blood. Add “IRR” to PCW if necessary.
5. Upon receipt of the email a blood product release form should be sent to the clinical team stating “Blood released on this patient in the duration of the clinical trial may be incompatible due to the presence of interfering substances.”
6. We will get a signed BPR on every patient regardless of their randomization assignment
7. Give all completed signed release forms to management
8. Baseline TSX will be sent on all patients enrolled in the study. (They may be randomized to Magrolimab or placebo).
9. Subsequent antibody screen testing can be performed in gel. If patient results indicate a pan-reactive antibody with gel screen and panel, a PEG screen using Immucor Gamma Clone shall be performed.
10. If PEG screen is negative, assume patient received Magrolimab and enter note into PCW indicating patient is receiving anti-CD47 therapy.
11. Antibody screen tests can be resulted based on PEG testing
12. Antibody ID should NOT be resulted as anti-CD47 as this will unblind the patient to the clinical team
13. ABO/Rh testing may be pan-reactive. Patients with pan-reactive ABO/Rh tests receiving Magrolimab will have the ABO/Rh resulted as NOGR and receive group O units.
14. NOGR patients require ISXMs. ISXMs may be incompatible. Result incompatible crossmatches, signed BPR should be on file.
15. Patients receiving anti-CD47 enrolled in the ENHANCE-3 shall receive Irradiated Rh/Kell matched units.
16. When anti-CD47 is identified comments placed into PCW should include “Patient receiving anti-CD47” AND “match Rh/Kell”
17. Plasma given shall be type specific.
18. Platelets given shall be plasma compatible whenever possible.

**II. Use of DTT treated Red Cells for Patients receiving DARA**

Chemical Risk Assessment: low

Biological Risk Assessment: low

Protective Equipment: Lab coat, gloves

Reagents: DTT treated RBCs, Normal Saline, PEG, Anti-IgG, Check Cells

Supplies: pipettes, centrifuge, test tubes

Equipment: Light magnifying lamp

Serofuge or CW3 centrifuge

Specimen Requirements: Patient Plasma

| **STEPS** | **INSTRUCTIONS** | **CHANGE /** **APPROVAL** |
| --- | --- | --- |
| **1.0** | **Test on each day of use the 1ml aliquots:*** 1. Test the controls (Anti-K+DTT treated cells positive for K antigen and anti-

E+DTT treated cell positive for E antigen).* 1. Refer to Steps 8-9 in Section I of BB-SOP-0115.

1.3 Place 2 drops of the DTT treated cells in a properly labeled test tube and  wash one (1) time prior to adding patient specimen or control sera.* 1. Note: To save time and if adequate patient sample, patient specimen

testing may be set up while controls are being tested. |  |
| **2.0** | **Proceed to testing of patient specimen.**2.1 Perform ABO/RH and antibody screen. Obtain Genotype if not already done.  *Refer to BB-SOP-0003, BB-SOP-0004, BB-SOP-0006, BB-SOP-0105** + If PEG screen is negative, STOP.
	+ If PEG screen is positive and showing specificity perform a panel to identify antibody and DAT profile.

 *Refer to BB-SOP-0067* *Refer to BB-SOP-0064** + If PEG screen is positive with all cells repeat PEG screen with DTT treated screening cells and perform a Gel DAT.
	+ If DTT treated screening cells are negative and Gel DAT is negative perform AHG crossmatch with untreated red blood cells.
	+ If DTT treated screening cells are positive Refer to step 3.0
	+ If DAT is positive Refer to step 4.0
* **Antibody Identification may be resulted as DARA if reactivity**

**disappears when tested with DTT treated cells.** * **Select K negative units if patient is Kell negative and requires red cell transfusions since DTT destroys Kell antigens.**

NOTE: Refer to step 1.3 above. DTT treated cells should be washed once prior to addition of specimen. |  |
| **3.0** | **DTT treat panel cells and test plasma in parallel with treated and untreated panel.*** 1. If PEG panel is positive and showing specificity identify the antibody and provide antigen negative units if indicated. (Give Kell negative RBCs if patient is Kell negative).
1. AHG crossmatch with untreated red blood cells.
	1. If PEG panel is positive with all cells this could indicate a possible autoantibody.
	2. Phenotype patient cells and adsorb plasma and test with DTT treated cells.
	3. AHG crossmatch with untreated and DTT treated donor unit red blood cells.
* Report untreated donor unit results and issue with Emergency Release.
 |  |
| **4.0** | **DTT treat patient cells and repeat gel DAT.***Refer to BB-SOP-0064*4.1 If DTT treated patient DAT is negative, Stop.4.2 If DTT treated patient DAT is positive perform an elution and test eluate in parallel with DTT treated and untreated screening cells. a. If both DTT treated and untreated screening cells are negative, STOP.b. If all DTT treated cells are negative and all untreated cells are positive, STOP. c. If DTT treated and/ or untreated cells are showing specificity identify the antibody and provide antigen negative units if indicated. d. If all DTT treated cells and all untreated cells are positive, phenotype the patient cells and adsorb the eluate and test with DTT treated cells. * AHG crossmatch with untreated and DTT treated donor unit red blood cells.
* Report untreated donor unit results and issue with Emergency Release.
 |  |
| **5.0** | **Result and charge for workup.**5.1 Neat results should be reported.a. Consult with management/medical director on what to result/report in computer if unclear.5.3 Antibody Identification may be resulted as DARA if reactivity disappears when tested with DTT treated cells.5.4 Charge for each cell treated with an SCC action “BCHEM” and charge for the actual test performed (e.g. DARA screen “RSCRN”, panel “BPANL”, DAT, crossmatch “RXM”). |  |

**III. Patients Receiving anti-CD-47 (Hu5F9-G4) Magrolimab**

Chemical Risk Assessment: low

Biological Risk Assessment: low

Protective Equipment: Lab coat, gloves

Reagents: Immucor Gamma Clone anti-IgG, PEG, reagent red cells, DTT reagent red cells

Supplies: test tubes, pipettes

Equipment: Light magnifying lamp

Serofuge or CW3 centrifuge

Specimen Requirements: Red cells/plasma to be tested

| **STEPS** | **INSTRUCTIONS** | **CHANGE /** **APPROVAL** |
| --- | --- | --- |
| **1.0** | Perform testing ordered.* 1. Antibody screen should be testing in tube using PEG and Immucor Gamma Clone IgG.
	2. Report results if antibody screen is negative and no discrepancies in ABO/Rh.
	3. If antibody screen is positive with all cells and/or ABO/Rh discrepancy,

*Refer to Attachment 2: Flow Chart for Anti-CD47 (Hu5F9-G4) Patients DAT and Screen.**Refer to Attachment 3: Flow Chart for ENHANCE-3 clinical trial patients* |  |

# Results Reporting

See Section I, 5.0.

*Refer to Attachments 2 and 3: Flow Charts for Anti-CD47*

# Interfering Substances/Test Method Limitations

Hu5F9-G4 (magrolimab) and Daratumumab bind to red blood cells (RBCs) and may lead to erythrophagocytosis and autoimmune haemolytic anemia. Blood typing and antibody screens may be problematic; treatment with monoclonal antibodies may interfere with ABO typing and/or the detection of underlying RBC alloantibodies.

DTT destroys Kell blood group antigens making detection of antibodies to this blood group system unreliable.

Immucor Gamma Clone anti-IgG does not detect pure IgG4 antibodies.

# Downtime

DARA patients: Revert to gel and LISS testing.

CD47 patients: Revert to gel testing, consult with management about need for send out testing.

# References

Roback, John D. et al. ***TECHNICAL MANUAL***. Bethesda, MD: American Association of Blood Banks, updated periodically.

*‘Monoclonal anti-CD47 interference in red cell and platelet testing.’* AABB Transfusion 2018;9999, 1-8

*‘HU5F9-G4 monoclonal Anti-CD47 therapy; A First Experience with Interference in Antibody Identification.* Illinois Association of blood Banks

# Related procedures/policies in Navex/Policy Tech:

# Attachments/Linked documents (title 21)

Attachment 1: Flow Chart for DARA Patients DAT and Screen
Attachment 2: Flow Chart for Anti-CD47 (Hu5F9-G4) Patients DAT and Screen

Attachment 3: Flow Chart for Anti-CD47 ENCHANCE-3 Patients

BB-SOP-0003

BB-SOP-0004

BB-SOP-0006

BB-SOP-0105

BB-SOP-0064

BB-SOP-0067

# Revision Dates: Review Change Summary as represented in Title 21.