Deciphering the Clinical Clues

Updates to Protocols and Procedures for Anti-CD47 Agents in Clinical Laboratories

Supported by an educational grant from Gilead Sciences, Inc.

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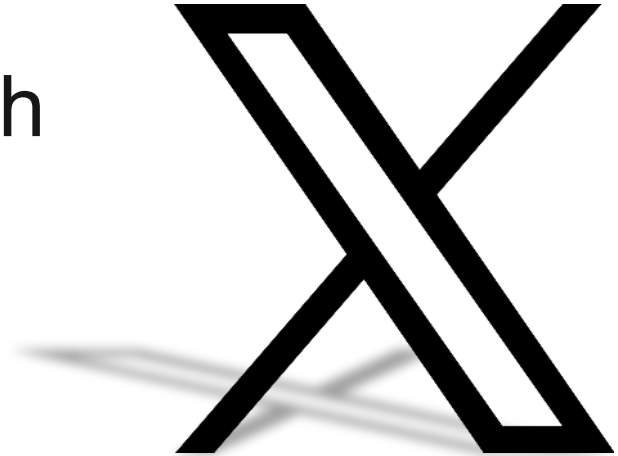


David A. Sallman, MD

Leukemia and MDS Section Head
Associate Member, Department
of Malignant Hematology
H. Lee Moffitt Cancer Center
and Research Institute
Associate Professor, Department
of Oncologic Sciences
University of South Florida
Tampa, Florida

Christine Lomas-Francis
MSc, FIBMS

Scientist Emeritus, Lindsley F. Kimball
Research Institute (LFKRI)
of New York Blood Center
New York, New York

Lynsi Rahorst
MHPE, MLS(ASCP)SBB^{CM}

Manager, Education and Training,
Immunohematology Reference
and Genomics Laboratories
New York Blood Center Enterprises
Kansas City, Missouri

Learning Objectives

1. Evaluate the impact of anti-CD47 agents on blood compatibility testing.
2. Implement laboratory protocols and procedures to facilitate timely transfusion support for patients taking anti-CD47 agents.
3. Use collaborative care strategies to ensure patients receive timely transfusions as part of supportive care.

Targeting CD47

David A. Sallman, MD

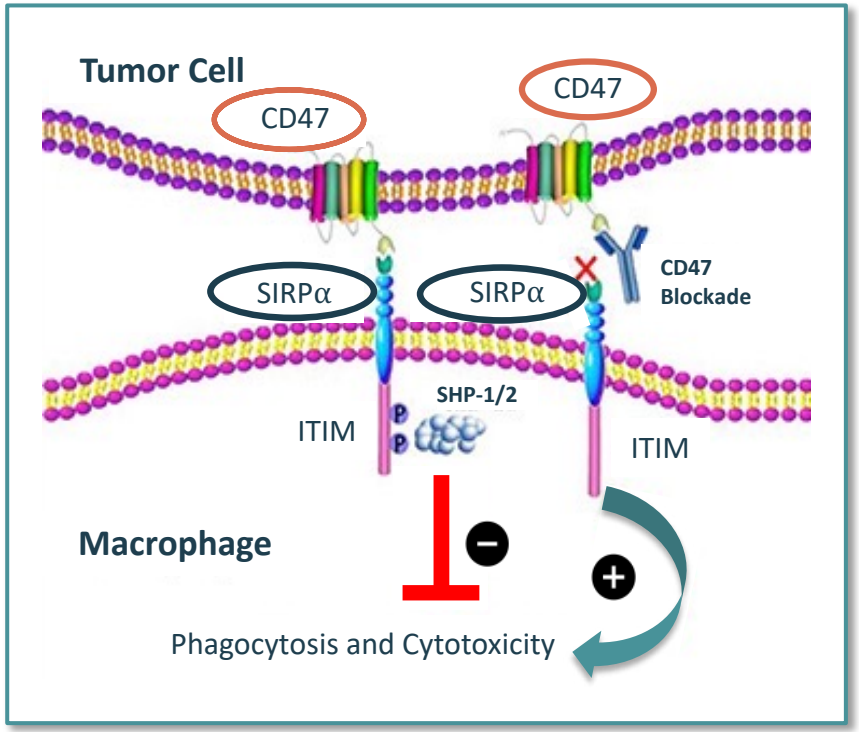
Structure and Function of CD47 and SIRPα

CD47

- Widely expressed transmembrane protein
- Serves as the ligand for SIRPα

SIRPα

- Expressed on phagocytic cells, including macrophages and dendritic cells



CD47, cluster of differentiation 47; ITIM, immunoreceptor tyrosine-based inhibitory motif; SIRPα, signal regulatory protein alpha; SHP-1/2, protein tyrosine phosphatase substrate-1/2.

Zhang W, et al. *Front Immunol*. 2020;11:18. Barclay AN, Brown MH. *Nat Rev Immunol*. 2006;6:457–464. Brown EJ, Frazier WA. *Trends Cell Biol*. 2001;11:130–135. Blazer BR, et al. *J Exp Med*. 2001;194(4):541–549.

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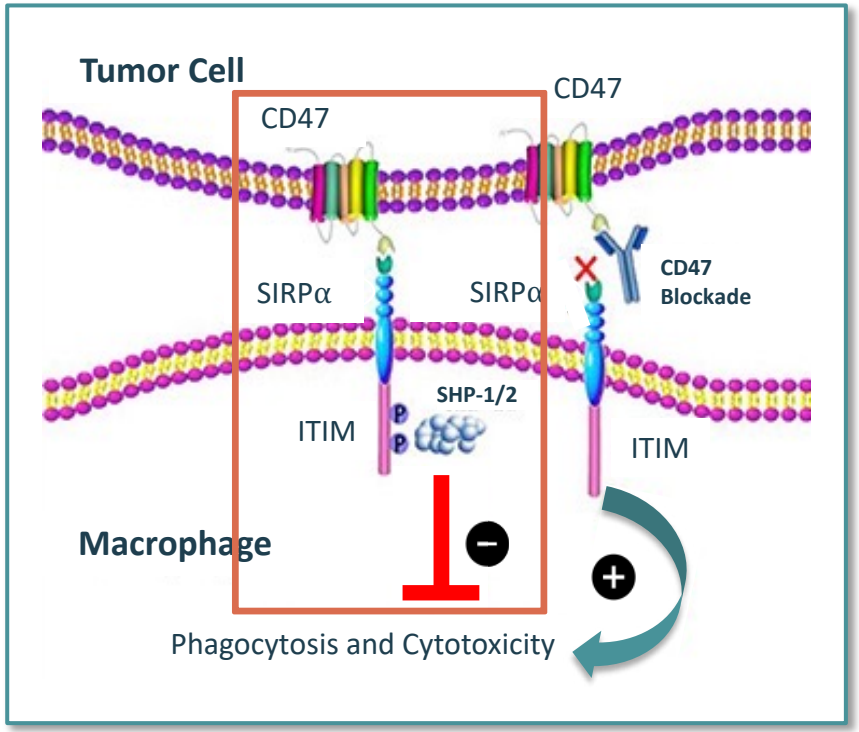
Structure and Function of CD47 and SIRPα

CD47/SIRPα Binding

- Initiates a signal transduction cascade
- Results in SHP-1/2 activation and consequent inhibition of phagocytosis



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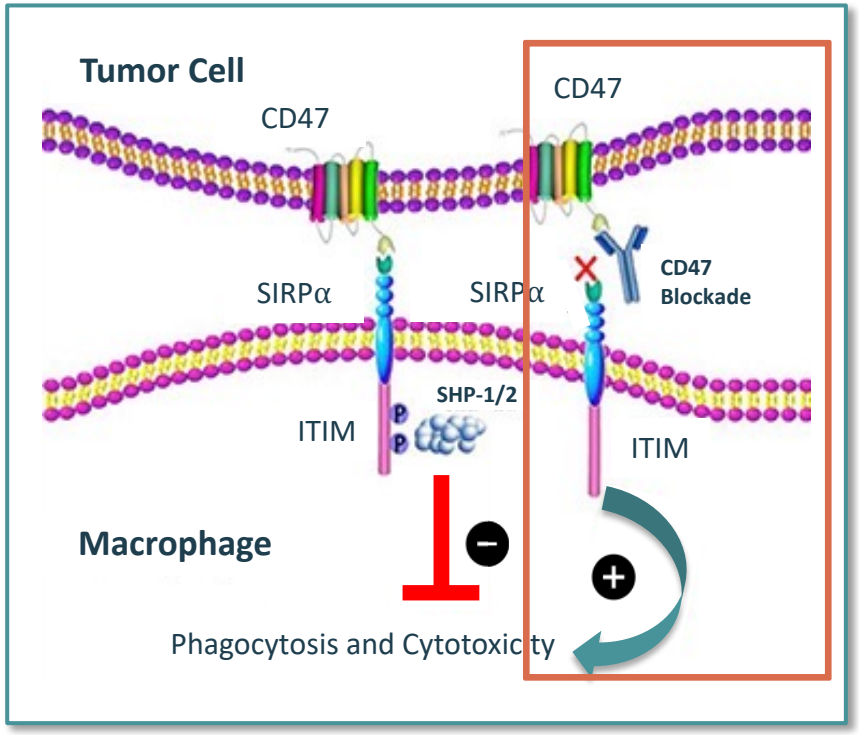
Structure and Function of CD47 and SIRPα

CD47

- Helps maintain immunotolerance by non-malignant cells under physiological conditions
- Blockade can abrogate this suppression signal



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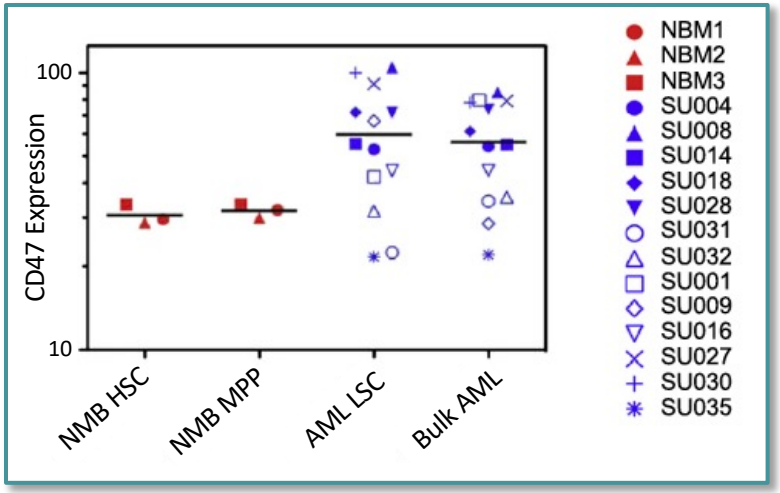
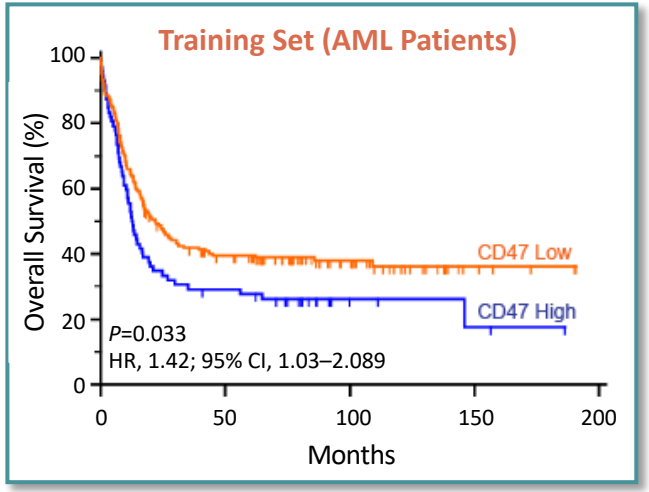
Innate Immune System Evasion via CD47





- CD47 is a “don’t eat me” signal on cancers that enables macrophage immune evasion
- CD47 is the dominant macrophage checkpoint overexpressed on most cancers
- In acute myeloid leukemia (AML), CD47 expression is overexpressed on leukemia stem cells (LSC)/bulk AML vs normal hematopoietic stem cell (HSC)/multipotent progenitor (MPP)
- CD47 leads to a strong fitness advantage in AML LSCs
- Increased CD47 expression predicts worse prognosis in AML patients

Majeti R, et al. *Cell.* 2009;138:286–299. Jaiswal S, et al. *Cell.* 2009;138;271–285.

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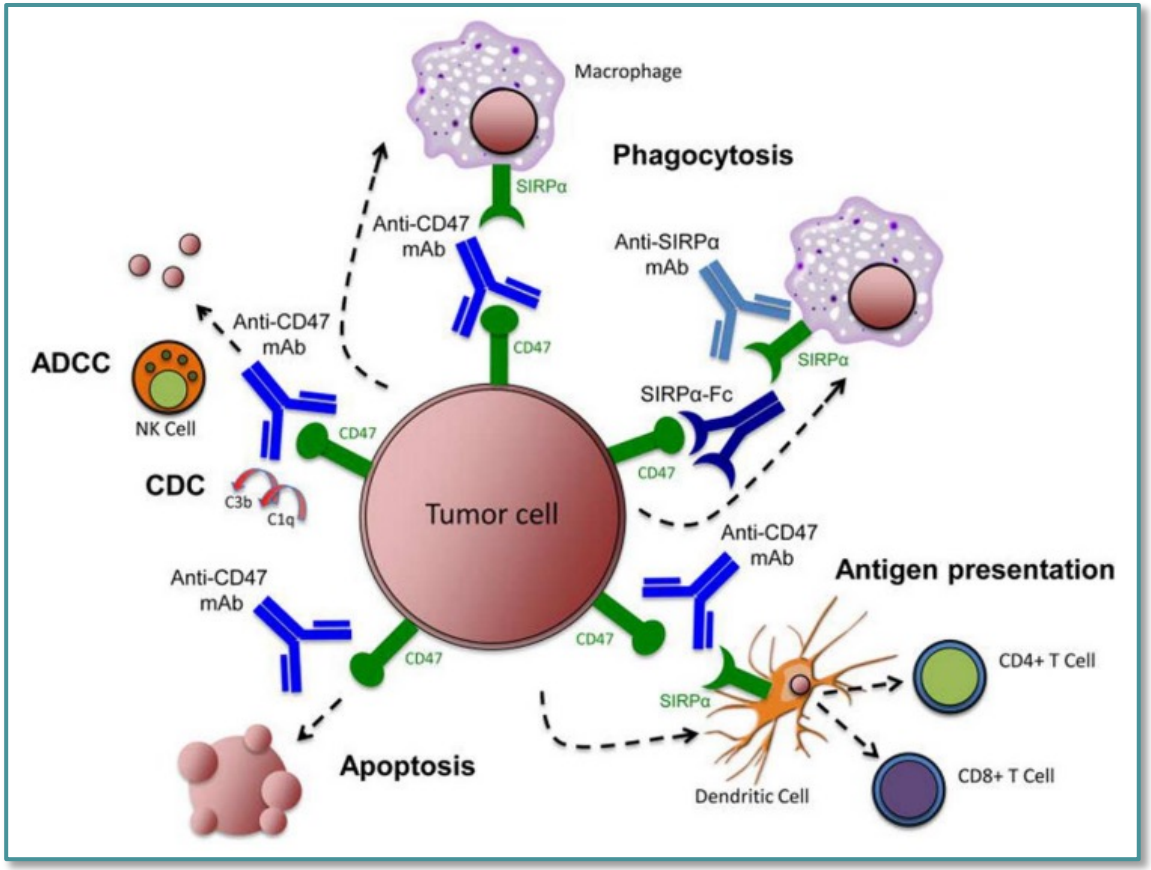
Therapeutic Impact of CD47/SIRPα Blockade in Cancer



Zhang W, et al. *Front Immunol.* 2020;11:18.

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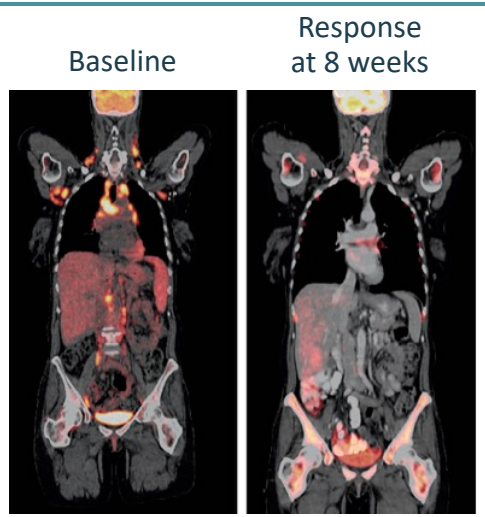
Efficacy of Magrolimab + Rituximab in NHL

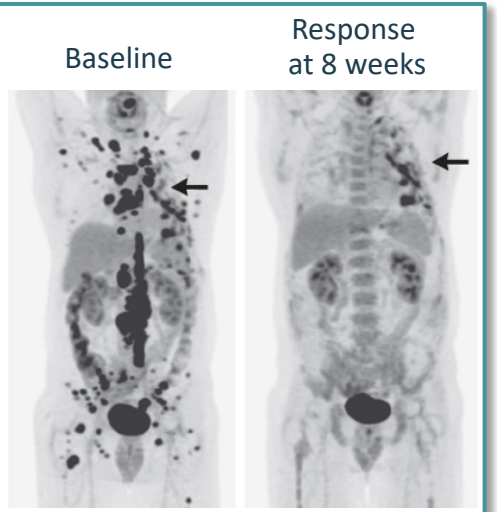
Clinical Responses to Combination Therapy with 5F9 and Rituximab

Response	All Patients (N=22)	Patients with DLBCL (N=15)	Patients with Follicular Lymphoma (N=7)
Objective response	11 (50)	6 (40)	5 (71)
Complete response	8 (36)	5 (33)	3 (43)
Partial response	3 (14)	1 (7)	2 (29)
Stable disease	3 (14)	3 (20)	0
Progressive disease	8 (36)	6 (40)	2 (29)
Disease control	14 (64)	9 (60)	5 (71)

Complete Response in Female Patient with DLBCL



Complete Response in Male Patient with DLBCL



DLBCL, diffuse large B-cell lymphoma.

Advani R, et al. *N Engl J Med.* 2018;379:1711–1721.

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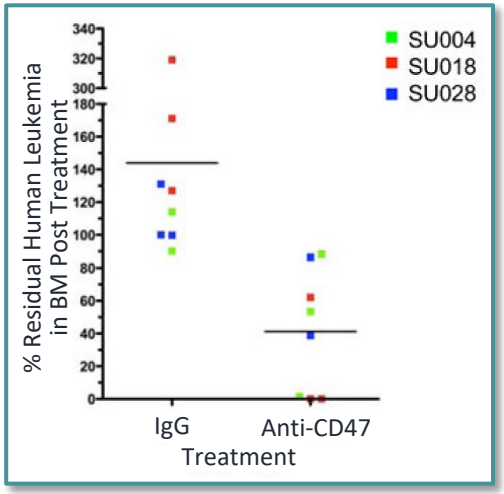
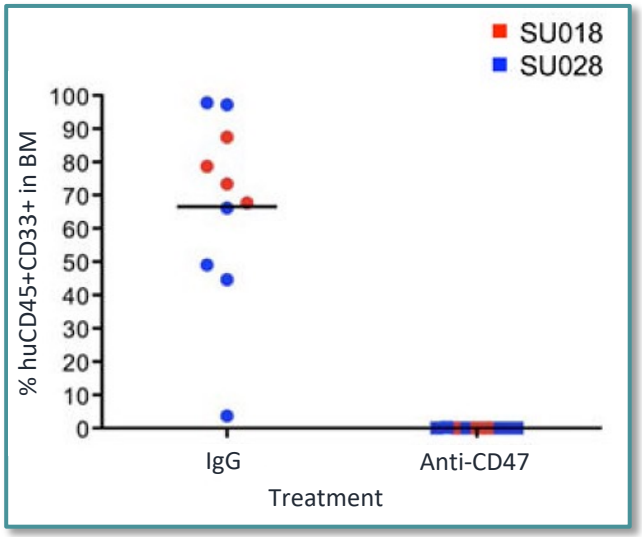
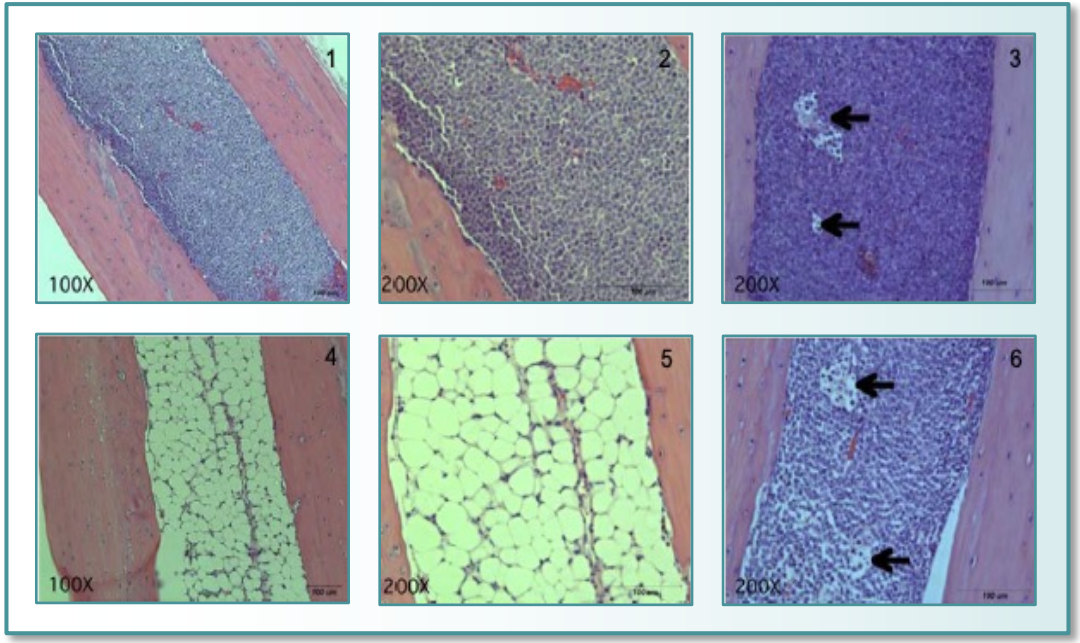
Preclinical Efficacy of CD47 and AML







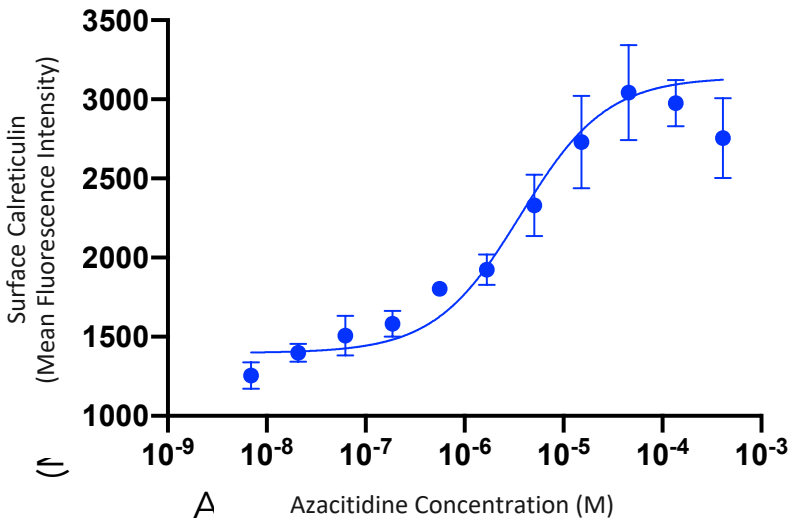
Majeti R, et al. *Cell.* 2009;138(2):286–299.

CME OUTFITTERS

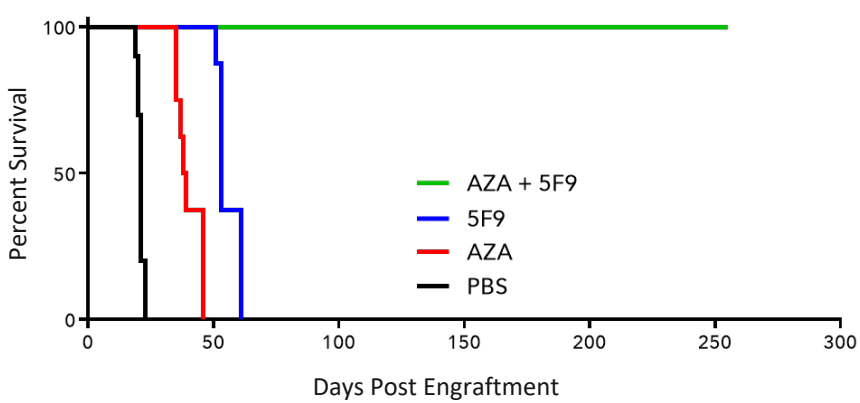
Magrolimab Synergizes with Azacitidine to Induce Remissions in AML Xenograft Models

- Azacitidine (AZA) induces pro-phagocytic “eat me” signals like calreticulin on cancer cells
- Increased “eat me” signals induced by azacitidine synergizes with CD47 blockade of the “don’t eat me” signal leading to enhanced phagocytosis

Calreticulin Expression in AML



AML Xenograft Model



Feng D, et al. *Blood*.2018;132(Suppl 1):2729. Vyas P, et al. *Eur Hem Assoc.* 2018;PF232.

CME OUTFITTERS

5F9005 Study Design
Magrolimab in Combination with AZA in MDS and AML

Untreated AML ineligible for induction chemotherapy or untreated myelodysplastic syndromes (MDS) intermediate to very high risk by IPSS-R

Magrolimab + AZA Combo Safety Evaluation (N=6)

Magro: 1, 30 mg/kg* weekly
AZA: 75 mg/m^2
D1–7

Expansion

Magro: 1, 30 mg/kg* weekly or Q2W
AZA: 75 mg/m^2
D1–7

- A magrolimab priming dose (1 mg/kg) and dose ramp-up was utilized to mitigate on-target anemia
- Data from the expansion cohort is presented

*Dose ramp-up from 1 mg/kg to 30 mg/kg by week 2, then 30 mg/kg maintenance dosing or 30 mg/kg Q2W starting Cycle 3+.

DoR, duration of response; IPSS-R, Revised International Prognostic Scoring System; OS, overall survival; PFS, progression-free survival; Q2W, every 2 weeks.

Sallman DA, et al. *J Clin Oncol.* 2023;41(15):2815–2826.

Primary Objectives

1. Safety of magrolimab alone or with AZA
2. Efficacy of magrolimab + AZA in untreated AML/MDS

Secondary Objectives

1. Pharmacokinetics, pharmacodynamics, and immunogenicity of 5F9
2. Additional measures of efficacy (DoR, PFS, OS)

Exploratory Objective

1. To assess CD47 receptor occupancy, markers of immune cell activity, and molecular profiling in AML/MDS

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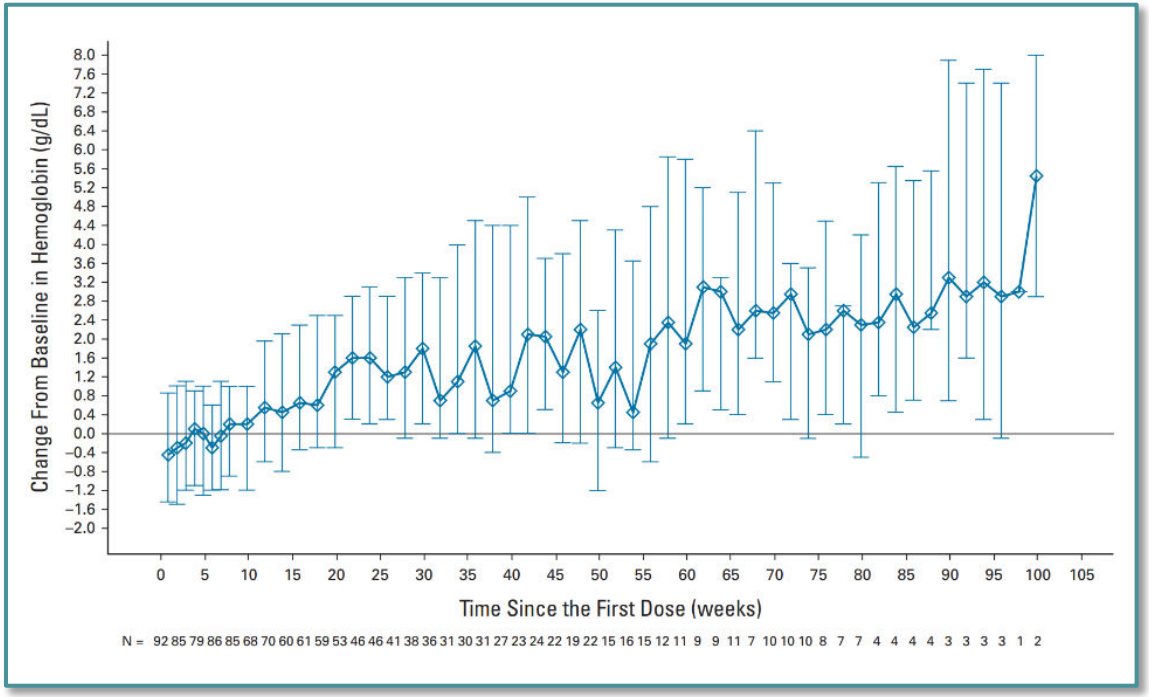
On-Target Anemia

A Pharmacodynamic Effect Mitigated with Magrolimab Priming and Maintenance Dosing Regimen



- Initial priming dose mitigated on-target anemia by CD47 blockade, resulting in a transient hemoglobin drop
- Median hemoglobin change from baseline was -0.7 g/dL (range -3.1 to +2.4) at first post-treatment visit
- 37 (38.9%) patients were transfusion dependent at baseline; 13 (35.1%) of these converted to red blood cell (RBC) transfusion independence

Sallman DA, et al. *J Clin Oncol.* 2023;41(15):2815–2826.

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Efficacy of Azacitidine + Magrolimab in HR-MDS

	All (N=95)	**TP53-wt MDS** (N=61)	**TP53-mut MDS** (N=25)
OR rate, %	74.7	78.7	68.0
CR, % (95% CI)	32.6 (23.4–43.0)	31.1 (19.9–44.3)	40.0 (21.1–61.3)
mCR, %	31.6	37.7	20
PR, %	0	0	0
SD with HI, %	10.5	9.8	8.0
Duration of CR, months, median (95% CI)	11.1 (7.6–13.4)	12.9 (8.0–NR)	7.6 (3.1–13.4)
Time to CR, months, median (range)	3.7 (1.7–72)	4.6(1.7–7.2)	3.1 (1.9–4.0)
Duration of OR, months, median (95% CI)	9.8 (8.8–12.9)	9.8 (8.5–18.5)	9.2 (5.0–12.2)
Time to OR, months, median (range)	1.9 (0.7–10.9)	1.9 (0.7–5.5)	1.9 (1.8–10.3)
mCR with HI/Any HI, %	16.8/58.9	19.7/60.7	12.0/56.0
Converted to RBC transfusion independence, %	35.1	26.1	46.2
PFS, months, median (95% CI)	11.6 (9.0–14.0)	11.8 (8.8–16.6)	11.0 (6.3–12.8)
OS, months, median (95% CI)	NR (16.3–NR)	NR (21.3–NR)	16.3 (10.8–NR)

CR, complete remission; HI, hematologic improvement; HR-MDS, high-risk myelodysplastic syndrome; mCR, marrow CR; NR, not reached; OR, objective response; PR, partial remission; SD, stable disease.

Sallman DA, et al. *J Clin Oncol.* 2023;41(15):2815–2826.

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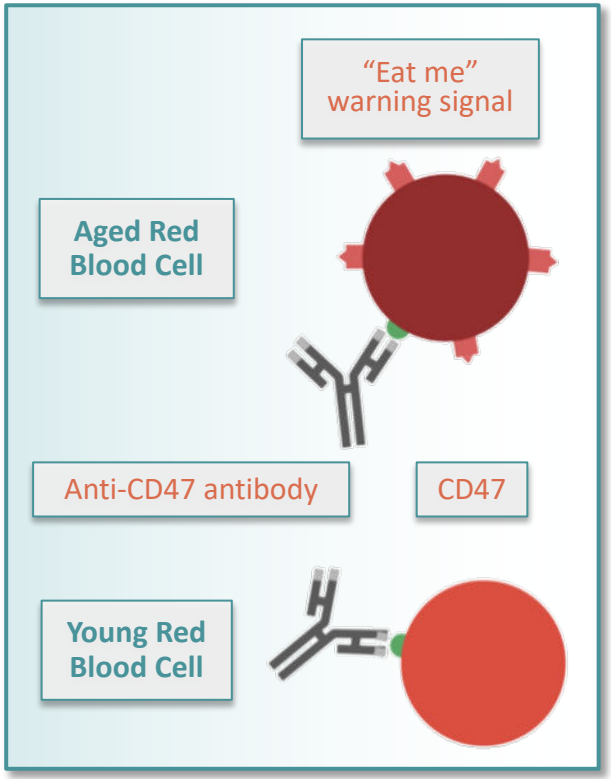
On Target Anemia and Mitigation Strategies

- Aged RBCs express pro-“eat me” signals, whereas young RBCs do not, leading to clearance of senescent RBCs
- Anemia mitigation via
  - Priming strategy (e.g., magrolimab)
  - RBC pruning process of CD47
  - Decrease/eliminate RBC affinity (e.g., TTI-621/622, ALX-147, and others)
  - Novel platforms (prodrug or tumor targeted nanoparticles)



Advani R, et al. *N Engl J Med*. 2018;379(18):1711–1721. Chao MP, et al. *Curr Opin Immunol*. 2012;24(2):225–232. Ishikawa-Sekigami T, et al. *Biochem Biophys Res Commun.* 2006;343(4):1197–1200.

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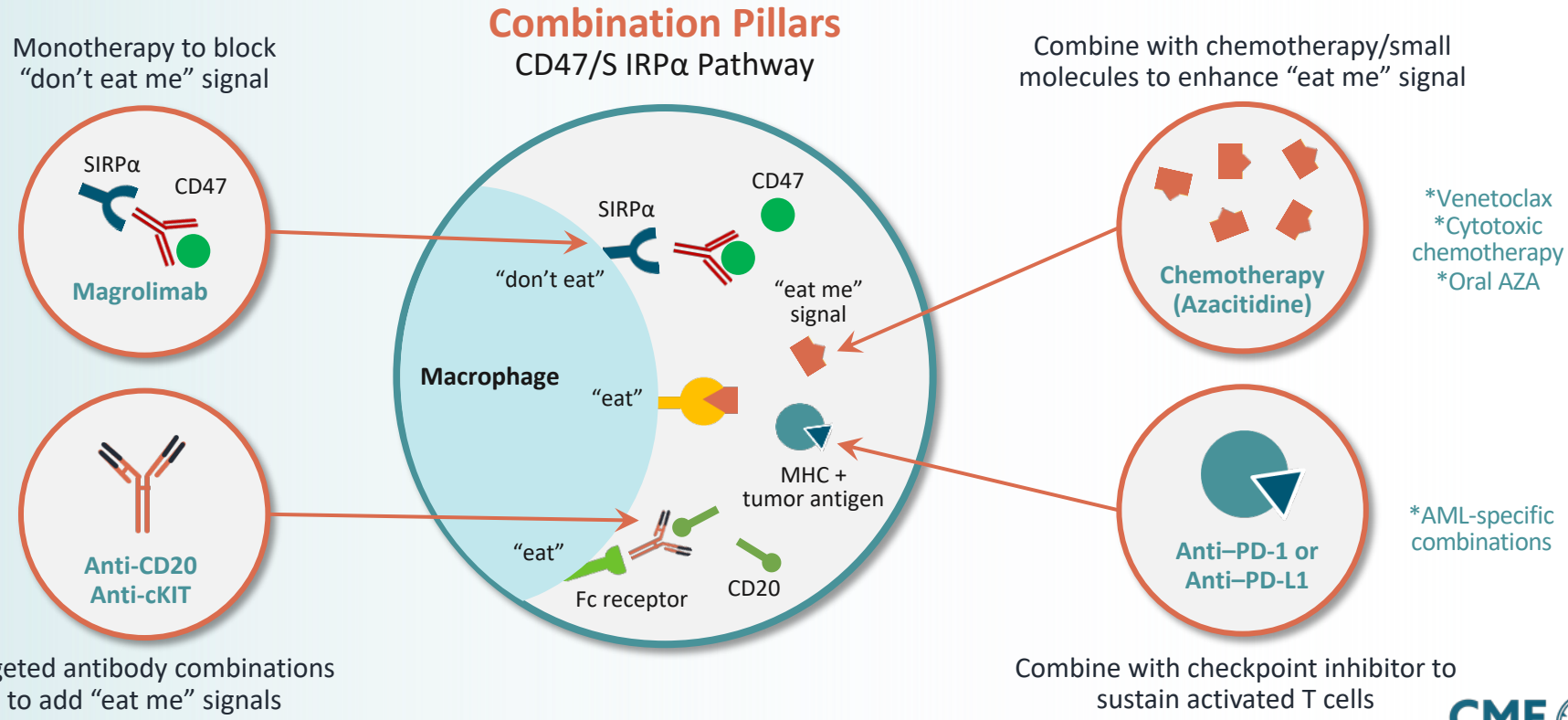
Combination Therapy with CD47 Targeted Therapy



Chao MP, et al. *Cell.* 2010;142(5):699–713. Sikic BI, et al. *J Clin Oncol.* 2019;37(12):946–953.

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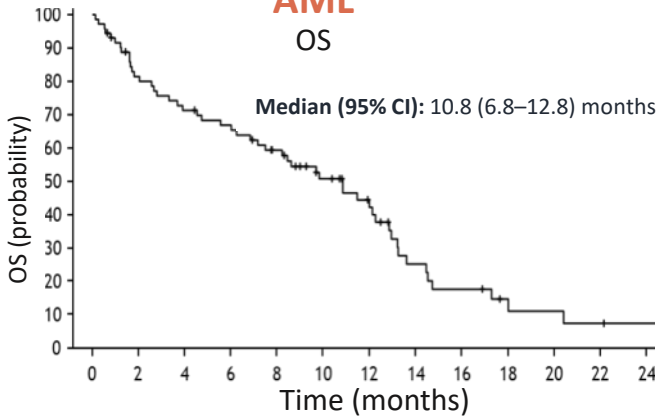
Overall Survival (OS) Is Encouraging in *TP53*-mut MDS/AML

AML

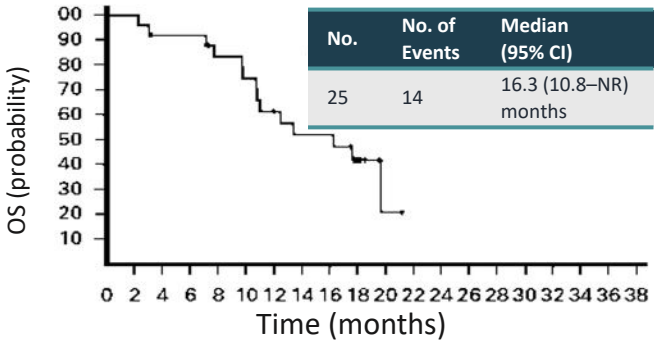
OS



MDS

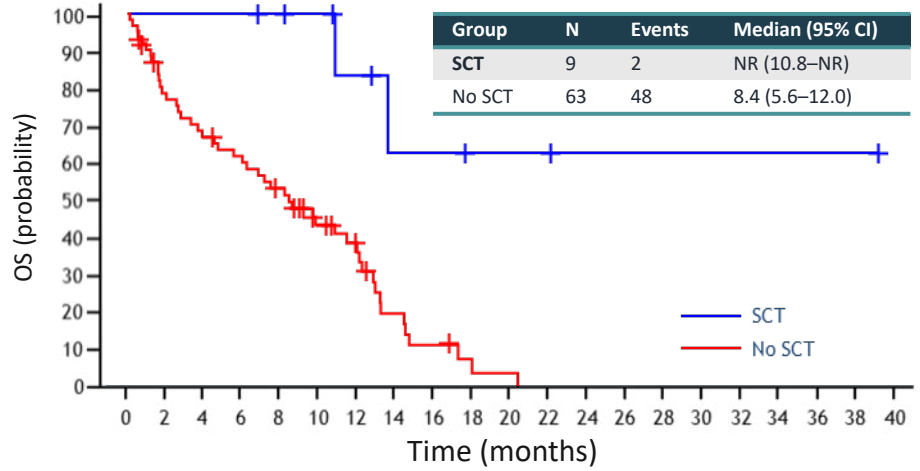
TP53-mutant

No.	No. of Events	Median (95% CI)
25	14	16.3 (10.8–NR) months



OS with and without Stem Cell Transplant (SCT)

Group	N	Events	Median (95% CI)
SCT	9	2	NR (10.8–NR)
No SCT	63	48	8.4 (5.6–12.0)



1-year OS 83% (27%–98%) vs 36% (23%–49%)

In *TP53wt* AML patients (n=15), overall response rate (ORR) similar with median OS of 18.9 months.

Sallman DA, et al. *J Clin Oncol*. 2023;41(15):2815–2826.

CME OUTFITTERS

Triplet Azacitidine + Venetoclax + Magrolimab

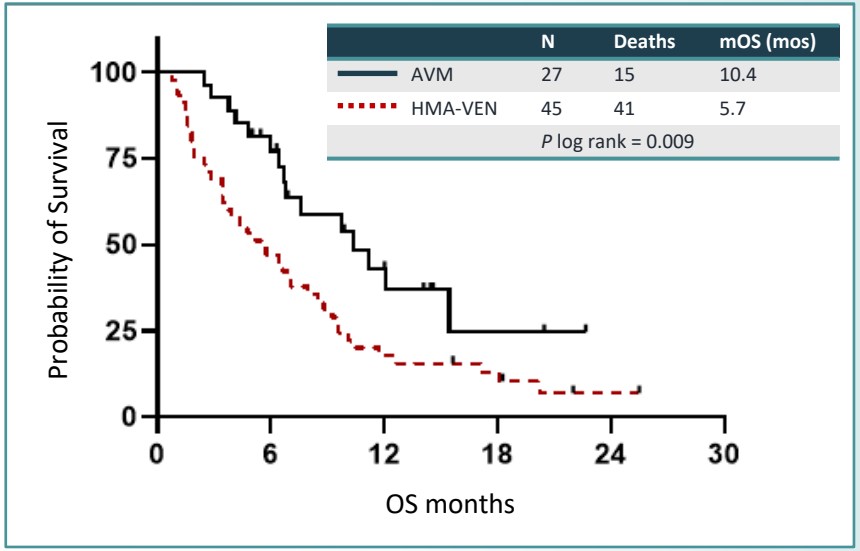
Parameters		Full Frontline (N=43)
Overall response	CR	21 (49)
	CRi	10 (23)
	CR + CRi	31 (72)
	MLFS	4 (9)
MRD-ve best responses[#]	FCM-CR/CRi	16/28 (67)[#]
Cytogenetic responses	CCyR	11/21 (52)
Time to response (days)	First response	23 (19–105)
	Best response	51 (20–130)
Counts recovery (days)	ANC ≥500/cu mm	36 (16–88)
	Platelet ≥100 × 10^9/L	32 (0–74)
Cycles on therapy		3 (1–17)
Mortality		
4 week		0 (0)
8 week		0 (0)

[#] Among CR/CRi patients with longitudinally MRD evaluable samples

Adjusted HR for AVM arm

for death = 0.41; 95% CI, 0.18–0.88

Comparison of overall survival (unmatched groups)



ANC, absolute neutrophil count; AVM, azacitidine + venetoclax + magrolimab; CCyR, complete cytogenetic response; CR, complete remission; CRi, complete remission with incomplete count recovery; FCM, fludarabine, cyclophosphamide, and mitoxantrone; HMA, hypomethylating agents; MLFS, morphologic leukemia-free state; MRD-ve, minimal residual disease venetoclax-exposed; VEN, venetoclax.

Daver N, et al. 2022 American Society of Hematology (ASH) Meeting and Exposition. Abstract 61.

CME OUTFITTERS

Ongoing Phase 3 Trials with Magrolimab in FL AML

Phase III AZA+ Magro vs Investigator Choice in TP53 AML (ENHANCE-2)

Study Design

Trial Population

Previously untreated AML with presence of at least one TP53 mutation that is not benign or likely benign

Physician's choice

- Appropriate for non-intensive treatment
  - 1:1 Randomization
    - **Control** Venetoclax + azacitidine
    - **Experimental** Magrolimab + azacitidine
- Appropriate for intensive treatment
  - 1:1 Randomization
    - **Control** 7+3 chemo

Sample size*: N=~346

*Study will enroll a minimum of 228 TP53mut AML patients appropriate for non-intensive treatment.

Stratification

1. Appropriateness for non-intensive therapy
2. Age <75 vs ≥75
3. Geographic region (U.S. vs outside the U.S.)

Endpoints

- Primary endpoint: OS in TP53mut AML population appropriate for non-intensive treatment
- First secondary endpoint (alpha controlled): OS in all TP53mut AML population
- Other key secondary endpoints (alpha controlled): Event free survival (EFS), transfusion independence, CR/CR_{MRD^-} patient reported outcomes (PRO) in all TP53mut AML population

Phase III AZA + VEN + Magro vs AZA + VEN in Older/Unfit AML (ENHANCE-3)

Study Design

Trial Population

Previously untreated adult AML patients unfit to receive intensive treatment due to age (≥75 years) or comorbidities

1:1 Randomization

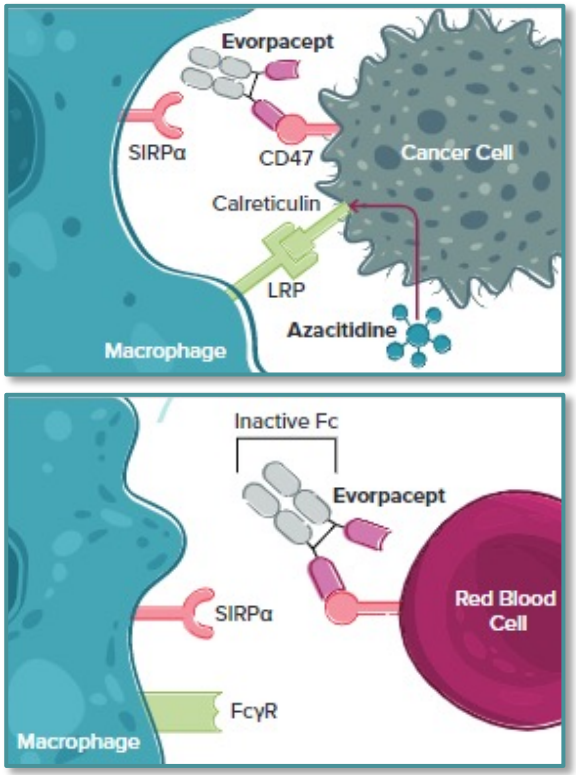
- **Experimental** Magrolimab + venetoclax + azacitidine
- **Control** Placebo + venetoclax + azacitidine

Endpoints

- Primary endpoint: CR, overall survival
- Secondary endpoints
  1. MRD-ve CR
  2. CR + CR with partial hematologic recovery (CRh)
  3. Duration of CR
  4. Duration of CR + CRh
  5. Transfusion independence
  6. Quality of life (QoL)/PRO

ClinicalTrials.gov. Identifiers: NCT04778397, NCT05079230.

Evorpacept (ALX148)
ASPEN-02 Study



	Previously Untreated HR-MDS (N=6)	Previously Untreated HR-MDS with TP53 Mutation (N=5)	Relapsed/Refractory MDS (N=9)[#]
ORR	3 (50%)	3 (60%)	5 (56%)*
CR	2 (33%)	2 (40%)	0
PR	0	0	0
Marrow CR	1 (17%) with HI	1 (20%) with HI	5 (56%)*
HI	0	0	0
SD	2 (33%)	1 (20%)	2 (22%)
PD	1 (17%)	1 (20%)	1 (11%)

Data cutoff: October 25, 2021. Response evaluable population (n=15): *Includes 3 unconfirmed responses; [#]One subject had G5 event unrelated to treatment prior to first disease assessment.

ASPEN-05 triplet study with VEN + AZA is recruiting.

PD, progressive disease.

Garcia-Manero G, et al. 2021 American Society of Hematology (ASH) Annual Meeting and Exposition. Abstract 2601.

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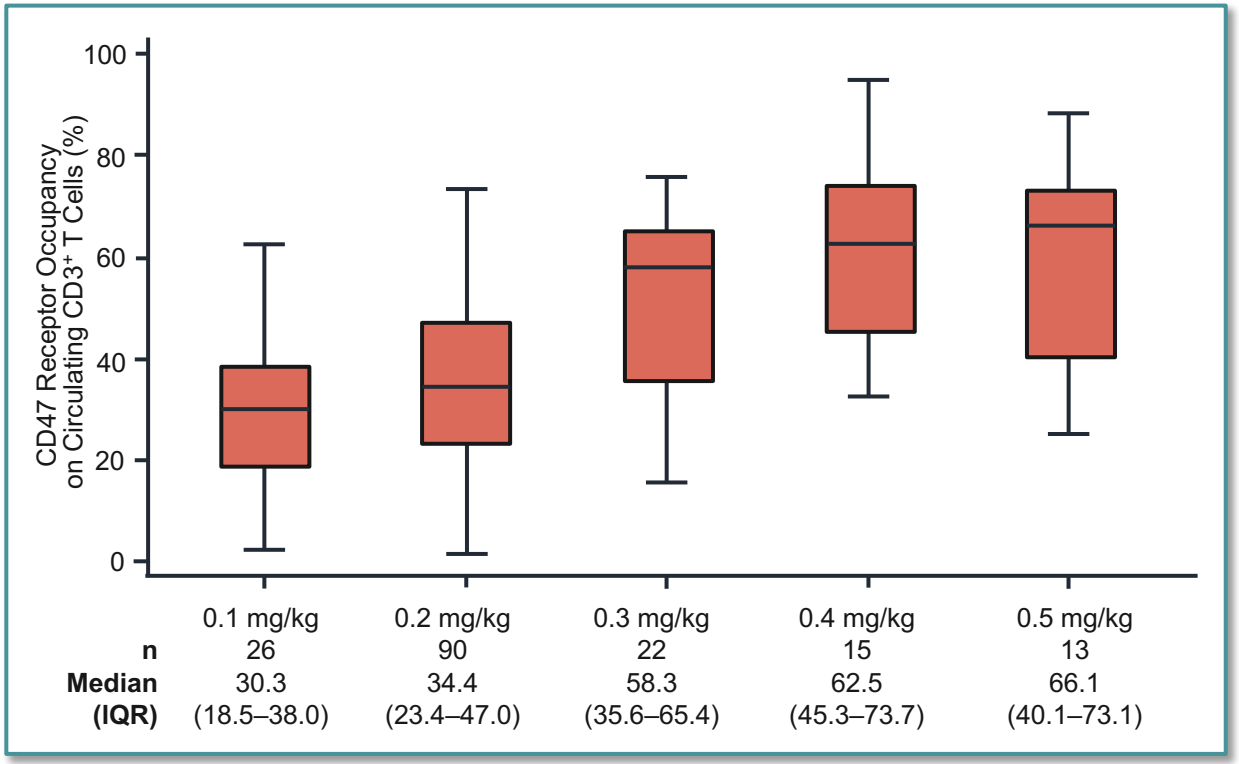
CD47 Blocker TTI-621 and TTI-622 in Patients with Relapsed or Refractory Hematologic Malignancies



TTI-622 *TP53* AML study with azacitidine or *TP53* wildtype triplet with azacitidine + venetoclax started accrual late 2021.

Ansell SM, et al. *Clin Cancer Res.* 2021;27(8):2190–2199. Daver N, et al. *Hemasphere*. 2022;6(Suppl):1700–1701.

CME OUTFITTERS

Novel CD47 Modalities and Combination Possibilities in Myeloid Neoplasms

- Synergy with Fc receptor of mAbs targeting myeloid antigens (e.g., CD33/CD123/TIM3/CLL1/CD70)
- Ongoing/possible triplet strategies that could include
  - AZA + magrolimab + VEN in AML (NCT04435691)
  - Combination with traditional PD-1/PD-L1 adaptive immune checkpoints (NCT03922477)
  - Combination of AZA + magrolimab + APR-246 for *TP53* mutant patients
  - Combination with synergistic combinations in MDS/AML (such as HMA + MBG-453; phase 1 in 2022)

mAbs, monoclonal antibodies.

Zhang W, et al. *Front Immunol*. 2020;11:18.
Swoboda DM, Sallman DA. *Best Pract Res Clin Haematol*. 2020;33(4):101221.
Yang H. *Biomark Res*. 2023;11(1):15.

CME OUTFITTERS

Novel CD47 Modalities and Combination Possibilities in Myeloid Neoplasms (…cont'd)

- HMBD004 is a bispecific anti-CD47xCD33 antibody that has shown decreased tumor burden and increased PFS in CD47+CD33+ AML mouse models
- CD47 directed CAR T cells
- Currently at least 13 CD47/SIRPα agents in clinical trial with ~50 agents in preclinical development

Zhang W, et al. *Front Immunol*. 2020;11:18.
Swoboda DM, Sallman DA. *Best Pract Res Clin Haematol*. 2020;33(4):101221.
Yang H. *Biomark Res*. 2023;11(1):15.

CME OUTFITTERS

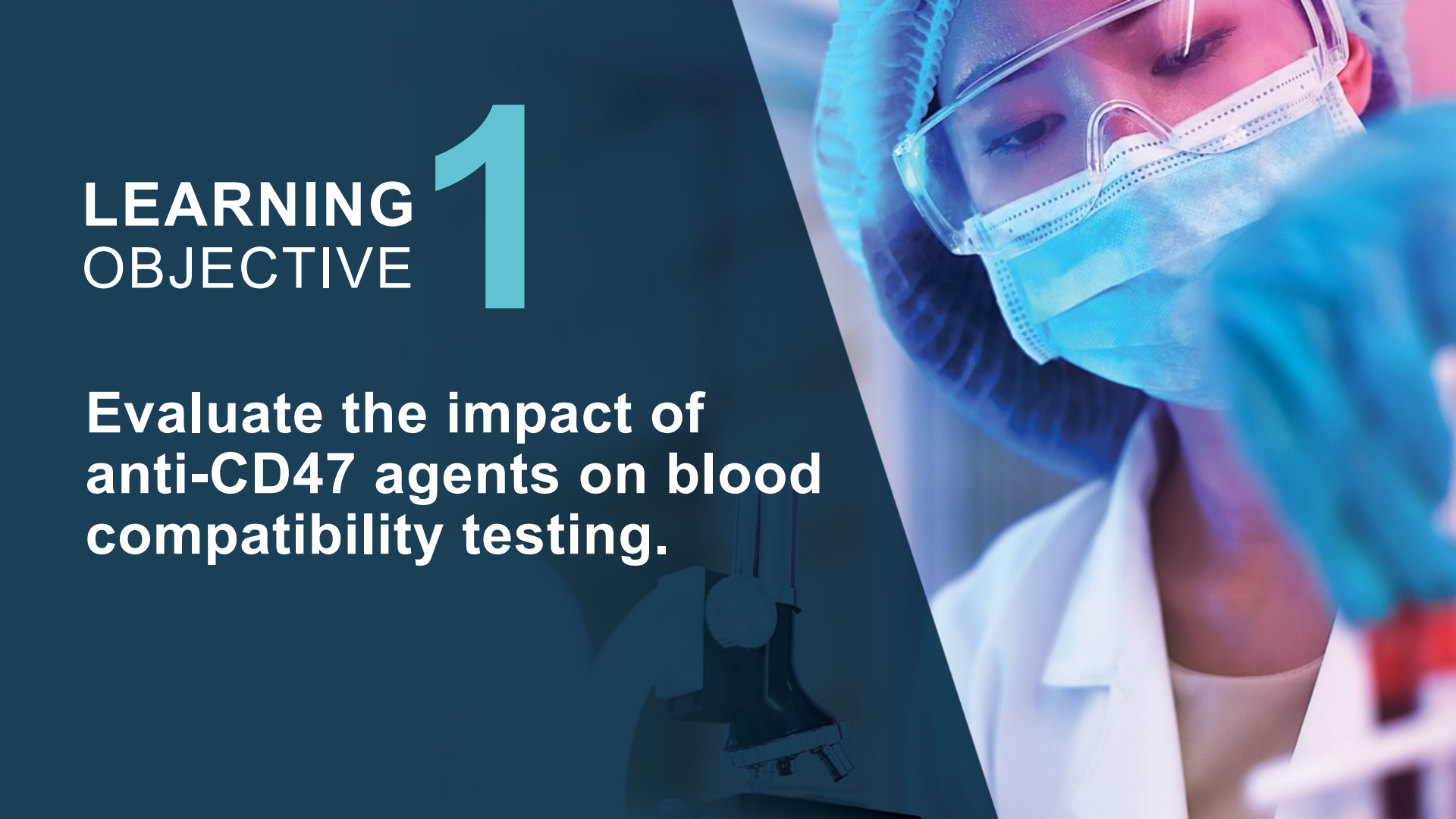
Interference with Standard Serologic Techniques for Blood Compatibility Testing

Christine Lomas-Francis, MSc, FIBMS

CME OUTFITTERS

LEARNING OBJECTIVE 1

Evaluate the impact of anti-CD47 agents on blood compatibility testing.

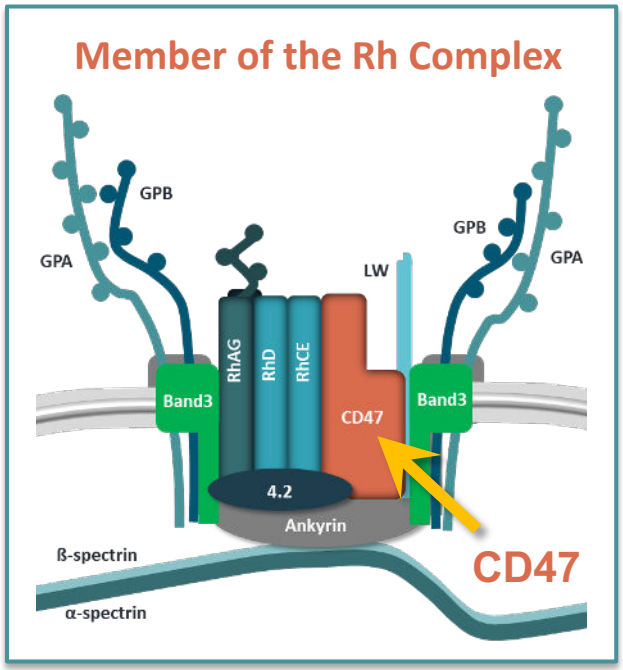


Audience Response

The potential for CD47 therapeutics to interfere in pretransfusion testing depends on which of the following?

A. ABO type
B. CD47 dose, but not the individual therapeutic
C. Hematologic condition being treated
D. Individual CD47 therapeutic and its dose
E. I’m not sure

CD47 is Highly Expressed on RBCs

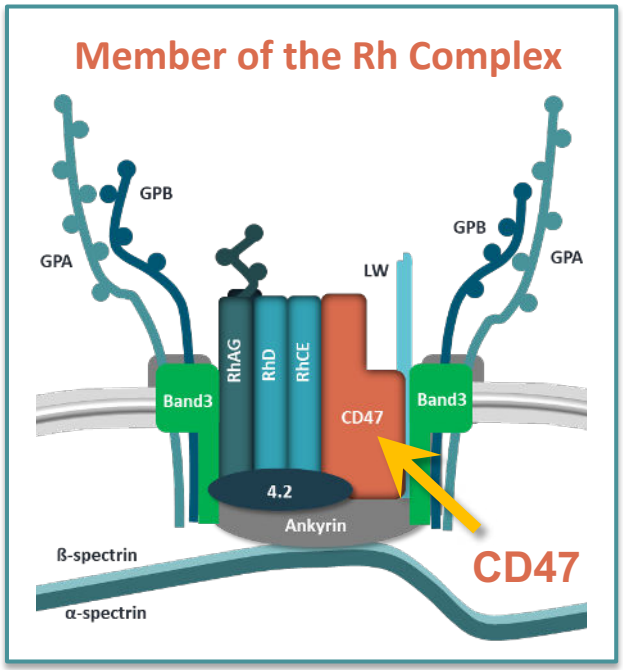


- Expression on RBCs varies by Rh phenotype
  - Rh negative → highest expression
  - Rh positive, especially R_2R_2 → lower expression
  - Related to RhCE in the membrane: rr (dce/dce) > R_1r/R_2r > R_2R_2> –D–
  - Weakest expression on Rh_{null} (lack all Rh antigens)
- **CD47 therapeutic in patient plasma**
  - Potential to interfere in pretransfusion testing
  - Impact depends on CD47 therapeutic and dose

Velliquette RW, et al. *Transfusion.* 2019;59(2):730–737. Yang H. *Biomark Res*. 2023;11(1):15.

CME OUTFITTERS

CD47 Therapeutics That May Be Encountered



- **Magrolimab (Hu5F9-G4)**
- Lemzoparlimab (TJ011133)
- Evorpacept (ALX148)
- TTI-621, TTI-622
- Many more in development

Velliquette RW, et al. *Transfusion*. 2019;59(2):730–737.

CME OUTFITTERS

Magrolimab IgG4 Anti-CD47
Interference in Pretransfusion Testing

Antibody Screen/Panel/Crossmatch

- Panreactivity in all phases and by all methods: gel, tube, solid phase
  - 3 to 4+ initial spin; IgM-like; 4+ in IAT
  - Stronger reactivity with D– (rr) RBCs compared to D+
  - Reactivity enhanced with treated RBCs (enzymes, dithiothreitol [DTT])
  - As soon as 1-hour post-infusion

IAT, indirect antiglobulin test.

Velliquette RW, et al. *Transfusion*. 2019;59(2):730–737.

CME OUTFITTERS

Magrolimab IgG4 Anti-CD47
Interference in Pretransfusion Testing (…cont'd)

Interference in ABO Typing

- Reverse/back type strongly reactive with A and B cells
- Spontaneous agglutination in front/forward typing may be observed
- May not be possible to obtain a valid ABO type
- Dependent on timing, dose, and circulating plasma drug concentration

ABO	IS
Anti-A	4+
Anti-B	0
A1 cells	4+
A2 cells	3+
B cells	4+
Interpretation	NTD

Velliquette RW, et al. *Transfusion*. 2019;59(2):730–737.

CME OUTFITTERS

Magrolimab IgG4 Anti-CD47
Interference in Pretransfusion Testing (…cont'd)

Direct Antiglobulin Test (DAT)

- Negative or weak positive DAT and auto control

Eluate Testing

- 4+ panreactivity
- Anti-CD47 coating patient RBCs causing steric interference or blocking in DAT and auto control tests

Velliquette RW, et al. *Transfusion*. 2019;59(2):730–737.

CME OUTFITTERS

CD47
Expressed on Virtually All Cells and Tissues

Platelet Antibody Testing

- False positive reactions with solid phase red cell adherence assays to detect platelet antibodies
- Avoid false positive reactions with commercially available enzyme-linked immunosorbent assay (ELISA) test utilizing glycoprotein molecules rather than intact platelets

Neutrophil Antibody Testing

- False positive results, depending on methodology

Velliquette RW, et al. *Transfusion*. 2019;59(2):730–737. Schuller et al. *Transfusion.* 2019;59(S3):136A–137A.

CME OUTFITTERS

Other CD47 Therapeutic Agents

Name	Molecule	Interference	DAT/auto control
ALX148 (Evorpacept)	CD47-blocking molecule Modified CD47 SIRPα D1 domain fused to inactive human IgG1	No interference in ABO testing No IS/RT/37C interference **Interference in all antiglobulin testing (strong)**	Both positive
TJ011133 (Lemzoparlimab)	IgG4 antibody	No interference in ABO testing No IS/RT interference **Interference in antiglobulin testing if "total anti-IgG" used**	Weak positive DAT Auto control strongly reactive with "total anti-IgG"
TTI-621	CD47-blocking molecule Modified CD47-binding domain of human SIRPα fused to human IgG1	No interference observed	Both negative
TTI-622	CD47-blocking molecule Modified CD47-binding domain of human SIRPα fused to human IgG4	No interference in ABO testing No interference IS/RT/37C No interference in **tube** antiglobulin testing **Interference in gel testing**	DAT may be weakly positive Auto control positive in gel testing

Velliquette RW, et al. *Transfusion.* 2019;59:136A. Kim TY, et al. *Transfusion.* 2020;60:1–9, Tahiri T, et al. *Transfusion.* 2022;62(S2):132A–133A. Velliquette RW, et al. *Transfusion*. 2019;59(2):730–737. Velliquette RW, et al. *Transfusion*. 2022;62(S2):146A.

CME OUTFITTERS

Mitigation Strategies

Lynsi Rahorst, MHPE, MLS(ASCP)SBB^{CM}

LEARNING OBJECTIVE 2

Implement laboratory protocols and procedures to facilitate timely transfusion support for patients taking anti-CD47 agents.

Audience Response

Which of the following is an effective mitigation strategy for magrolimab interference?

A. Change enhancement media
B. Change testing methodologies
C. Treat reagent RBCs
D. Use anti-IgG that does not react with IgG4
E. I'm not sure

Mitigating Magrolimab Interference
What Doesn't Work?

- Changing testing methodologies (tube, gel, solid phase)
- Changing enhancement media (PEG, LISS, albumin) or not using enhancement media (saline testing)
- Cord RBCs
- Treatment of reagent RBCs (DTT, ficin/papain, trypsin, α-chymotrypsin)

	Tube			Gel	SPRCA	Treated RBCs			
	LISS IAT	PEG IAT	Saline IAT	IgG		DTT IAT	Ficin/Papain IAT	Trypsin IAT	α-chymotrypsin IAT
1	4+	4+	4+	4+	2+	4+	4+	4+	4+
2	4+	4+	4+	4+	2+	4+	4+	4+	4+
3	4+	4+	4+	4+	2+	4+	4+	4+	4+
4	4+	4+	4+	4+	2+	4+	4+	4+	4+
5	4+	4+	4+	4+	2+	4+	4+	4+	4+
6	4+	4+	4+	4+	2+	4+	4+	4+	4+
7	4+	4+	4+	4+	2+	4+	4+	4+	4+
8	4+	4+	4+	4+	2+	4+	4+	4+	4+
9	4+	4+	4+	4+	2+	4+	4+	4+	4+
10	4+	4+	4+	4+	2+	4+	4+	4+	4+
11	4+	4+	4+	4+	2+	4+	4+	4+	4+
Auto	0√	0√	0√	0					
Cord	4+	4+	4+	4+					

LISS, low-ionic saline solutions; PEG, polyethylene glycol.

Velliquette RW, et al. *Transfusion*. 2019;59(2):730–737.

CME OUTFITTERS

Mitigating Magrolimab Interference
What Works?

- AHG reagent (anti-IgG) lacking reactivity with IgG4
  - Negative to very weakly positive results reported
- Adsorption
  - Multiple adsorptions (4) with enzyme-treated RBCs
  - Multiple adsorptions (4) with platelets
    - Pooled single-donor apheresis platelets (expired)
    - Commercially available human platelet concentrate (HPC)
- One study reported lack of panreactivity detected in automated solid phase
  - 3/18 samples demonstrated nonspecific reactivity

DO NOT PERFORM PEG ADSORPTIONS
Precipitation of antibody occurs, invalidating procedure

	IAT (total anti-IgG)	IAT (anti-IgG lacking reactivity with IgG4)
1	4+	0v
2	4+	0v
3	4+	0v
4	4+	0v
5	4+	0v
6	4+	0v
7	4+	0v
8	4+	0v
9	4+	0v
10	4+	0v
11	4+	0v
Auto	0v	

Allows valid ABO reverse typing

Velliquette RW, et al. *Transfusion*. 2019;59(2):730–737. Carl T, et al. *Transfusion*. 2022;62:916–918.

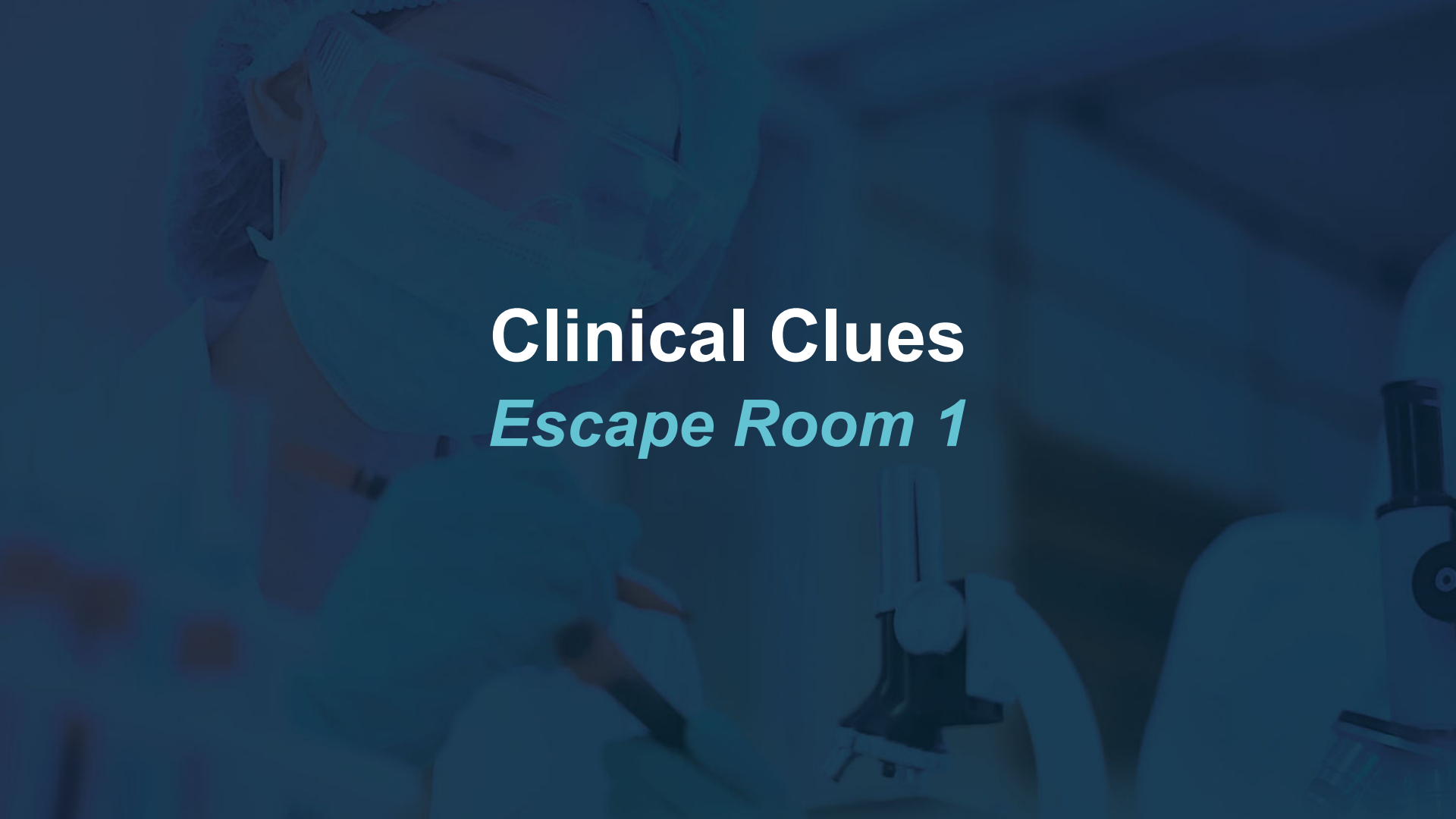
CME OUTFITTERS

Mitigating Interference of Other CD47 Therapies

Name	Molecule	Interference	Mitigation Strategy
ALX148 (Evorpacept)	CD47-blocking molecule Modified CD47 SIRPα D1 domain fused to inactive human IgG1	No interference in ABO testing No IS/RT/37C interference **Interference in all antiglobulin testing (strong)**	• 5–6 adsorptions with papain-treated RBCs—results variable • Neutralization with soluble CD47 (not widely available) **(AHG reagent lacking reactivity with IgG4 not effective)**
TJ011133 (Lemzoparlimab)	IgG4 antibody	No interference in ABO testing No IS/RT interference **Interference in antiglobulin testing if "total anti-IgG" used**	AHG reagent lacking reactivity with IgG4
TTI-621	CD47-blocking molecule Modified CD47-binding domain of human SIRPα fused to human IgG1	No interference observed	N/A
TTI-622	CD47-blocking molecule Modified CD47-binding domain of human SIRPα fused to human IgG4	No interference in ABO testing No interference IS/RT/37C No interference in **tube** antiglobulin testing **Interference in gel testing**	Utilize tube testing (LISS or PEG enhancement)

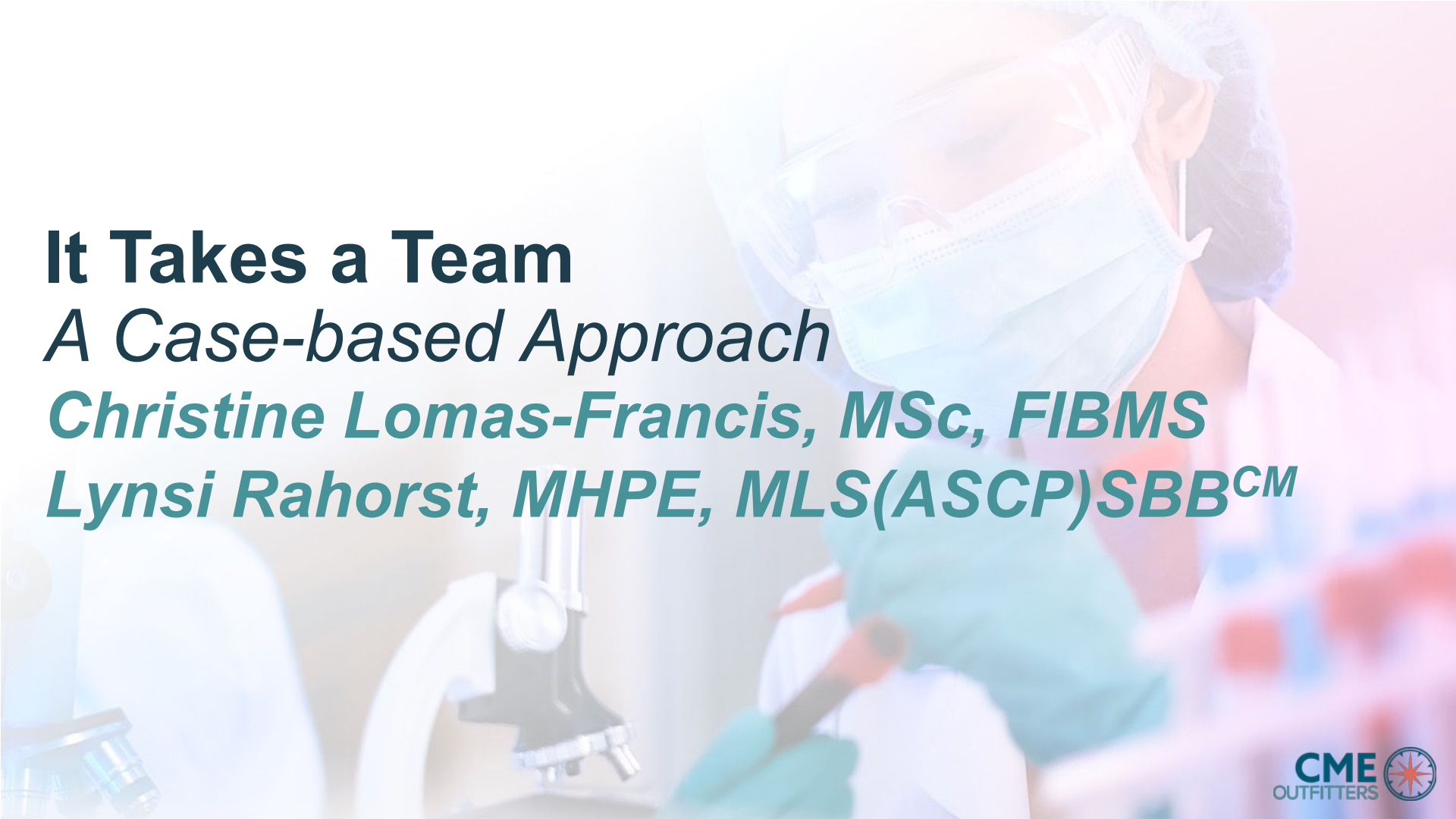
Velliquette RW, et al. *Transfusion.* 2019; 59:136A. Kim TY, et al. *Transfusion.* 2020;60:1–9, Tahiri T, et al. *Transfusion.* 2022;62(S2):132A–133A. Velliquette RW, et al. *Transfusion*. 2019;59(2):730–737. Velliquette RW, et al. *Transfusion*. 2022;62(S2):146A.

CME OUTFITTERS



Clinical Clues

Escape Room 1



It Takes a Team

A Case-based Approach

Christine Lomas-Francis, MSc, FIBMS

Lynsi Rahorst, MHPE, MLS(ASCP)SBB^{CM}

CME OUTFITTERS

LEARNING OBJECTIVE 3

Use collaborative care strategies to ensure patients receive timely transfusions as part of supportive care.

Audience Response

For patients on a CD47 therapeutic, multidisciplinary communication is critical to achieve which of the following?

A. Elimination of chimerism in fraternal twins
B. Timely and safe transfusions for patients
C. Need to resolve ABO discrepancies
D. Reduced transfusion of blood components
E. I’m not sure

That's Life in the Reference Laboratory….

The patient is a 71-year-old male who is multiply transfused.

- Diagnosis: Anemia; Hgb: 7g/dL!
- Blood type: Historical A+; currently, discrepancy between forward and reverse typing
- RBCs: DAT-negative

His plasma reacts strongly with all panel cells in the IgG gel test.

- Hospital requests antibody identification
- STAT DNA-based testing for extended phenotype also requested
- Further transfusion(s) likely needed

Patient received monoclonal therapy?

Midnight on-call sample

CME OUTFITTERS

That's Life in the Reference Laboratory….

The patient is a 71-year-old male who is multiply transfused.

- Diagnosis: Anemia; Hgb: 7g/dL!
- Blood type: Historical A+; currently, discrepancy between forward and reverse typing
- RBCs: DAT-negative

His plasma reacts strongly with all panel cells in the IgG gel test.

- Hospital requests antibody identification
- STAT DNA-based testing for extended phenotype also requested
- Further transfusion(s) likely needed

Patient received monoclonal therapy?

Midnight on-call sample

Oh, another patient on CD38 monoclonal therapy!

CME OUTFITTERS

Patient: Initial Testing Results

DAT	IS	5 min
POLY	0	0
IgG	0	NT
C3	0	0
Control	0	0

Anti-D	
IS	4+

		Rh-hr					MNS				P1	Lewis		Luth			Kell			Duffy		Kidd		Domb			SAL	LISS		PEG	IgG Gel	PAPAIN	
		D	C	E	c	e	M	N	S	s	P1	L e a	L e b	L u a	LK u b	k	K p a	J s a	J s b	F y a	F y b	J k a	J k b	D o a	D o b	X g a	IS	37C	IAT IgG (no anti-IgG4)	IAT IgG (no anti-IgG4)	IAT (anti-total IgG)	37C	IAT IgG (no anti-IgG4)
1	R^1R^1	+	+	0	0	+	0	+	0	+	0	0	+	0	+ 0	0	+	0	0	+	+	0	0	+	0	+	+	4+	4+	+w	+w	4+	4+	4+
2	R^1R^1	+	+	0	0	+	0	+	+	+	+	0	0	0	+ +	0	0	0	0	+	0	+	+	0	+	+	+	3+	4+	+w	+w	4+	4+	4+
3	R^2R^2	+	0	+	+	0	0	+	0	+	+	0	+	0	+ 0	0	+	0	0	+	+	0	+	0	0	+	+	3+	4+	m+	m+	4+	4+	4+
4	R^2R^2	+	0	+	+	0	+	0	0	+	+	+	0	0	+ 0	0	+	0	0	+	0	+	0	+	0	+	+	3+	4+	m+	m+	4+	4+	4+
5	R^o	+	0	0	+	+	0	+	0	0	+	0	+	+	+ 0	0	+	0	0	+	0	0	+	+	0	+	+	3+	4+	+w	+w	4+	4+	4+
6	rr	0	0	0	+	+	+	0	+	0	+	0	0	0	+ 0	0	+	0	0	+	0	+	+	0	+	0	+	4+	4+	1+	1+	4+	4+	4+
7	rr	0	0	0	+	+	+	0	+	0	+	0	+	0	+ 0	0	+	0	0	+	+	0	0	+	0	+	+	4+	4+	1+	1+	4+	4+	4+
8	rr	0	0	0	+	+	+	+	+	+	w	+	0	0	+ +	0	0	0	0	+	+	+	0	+	0	+	+	4+	4+	1+	1+	4+	4+	4+
Auto			3+ MF	1+ MF	4+	4+																					0	0	0	0	0	0	0
Cord																											3+	4+	m+	m+	4+	4+	4+

Patient: Initial Testing Results

ABO	IS
Anti-A	4+
Anti-B	0
A1 cells	4+
A2 cells	3+
B cells	4+
Interpretation	NTD

DAT	IS	5 min
POLY	0	0
IgG	0	NT
C3	0	0
Control	0	0

Anti-D	
IS	4+

		Rh-hr					MNS				P1	Lewis		Luth			Kell			Duffy		Kidd		Domb			SAL	LISS		PEG	IgG Gel	PAPAIN	
		D	C	E	c	e	M	N	S	s	P1	Le a	Le b	u a	u b	k	K a	J a	J b	F y a	F y b	J a	J b	D o a	D o b	X g a	IS	37C	IAT IgG (no anti-IgG4)	IAT IgG (no anti-IgG4)	IAT (anti-total IgG)	37C	IAT IgG (no anti-IgG4)
1	R^1R^1	+	+	0	0	+	0	+	0	+	0	0	+	0	+	0	+	0	0	+	+	0	0	+	0	+	+	4+	4+	+w	+w	4+	4+	4+
2	R^1R^1	+	+	0	0	+	0	+	+	+	+	0	0	0	+	+	0	0	0	+	0	+	+	0	+	+	+	3+	4+	+w	+w	4+	4+	4+
3	R^2R^2	+	0	+	+	0	0	+	0	+	+	0	+	0	+	0	+	0	0	+	+	0	+	0	0	+	+	3+	4+	m+	m+	4+	4+	4+
4	R^2R^2	+	0	+	+	0	+	0	0	+	+	+	0	0	+	0	+	0	0	+	0	+	0	+	0	+	+	3+	4+	m+	m+	4+	4+	4+
5	R^o	+	0	0	+	+	0	+	0	0	+	0	+	+	+	0	+	0	0	+	0	0	+	+	0	+	+	3+	4+	+w	+w	4+	4+	4+
6	rr	0	0	0	+	+	+	0	+	0	+	0	0	0	+	0	+	0	0	+	0	+	+	0	+	0	+	4+	4+	1+	1+	4+	4+	4+
7	rr	0	0	0	+	+	+	0	+	0	+	0	+	0	+	0	+	0	0	+	+	0	0	+	0	+	+	4+	4+	1+	1+	4+	4+	4+
8	rr	0	0	0	+	+	+	+	+	+	w	+	0	0	+	+	0	0	0	+	+	+	0	+	0	+	+	4+	4+	1+	1+	4+	4+	4+
Auto			3+ MF	1+ MF	4+	4+																					0	0	0	0	0	0	0
Cord																											3+	4+	m+	m+	4+	4+	4+

Patient: Initial Testing Results

ABO	IS
Anti-A	4+
Anti-B	0
A1 cells	4+
A2 cells	3+
B cells	4+
Interpretation	NTD

DAT	IS	5 min
POLY	0	0
IgG	0	NT
C3	0	0
Control	0	0

Anti-D	
IS	4+

		Rh-hr					MNS				P1	Lewis		Luth			Kell			Duffy		Kidd		Domb			SAL	LISS		PEG	IgG Gel	PAPAIN	
		D	C	E	c	e	M	N	S	s	P1	Le^a	Le^b	Lu^a	Lu^b	k	K	Jp^a	Js^a	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	Do^a	Do^b	Xg^a	IS	37C	IAT IgG (no anti-IgG4)	IAT IgG (no anti-IgG4)	IAT (anti-total IgG)	37C	IAT IgG (no anti-IgG4)
1	R^{1}R^1	+	+	0	0	+	0	+	0	+	0	0	+	0	+	0	+	0	0	+	+	0	0	+	0	+	+	4+	4+	+w	+w	4+	4+	4+
2	R^{1}R^1	+	+	0	0	+	0	+	+	+	+	0	0	0	+	+	0	0	0	+	0	+	+	0	+	+	+	3+	4+	+w	+w	4+	4+	4+
3	R^{2}R^2	+	0	+	+	0	0	+	0	+	+	0	+	0	+	0	+	0	0	+	+	0	+	0	0	+	+	3+	4+	m+	m+	4+	4+	4+
4	R^{2}R^2	+	0	+	+	0	+	0	0	+	+	+	0	0	+	0	+	0	0	+	0	+	0	+	0	+	+	3+	4+	m+	m+	4+	4+	4+
5	R^o	+	0	0	+	+	0	+	0	0	+	0	+	+	+	0	+	0	0	+	0	0	+	+	0	+	+	3+	4+	+w	+w	4+	4+	4+
6	rr	0	0	0	+	+	+	0	+	0	+	0	0	0	+	0	+	0	0	+	0	+	+	0	+	0	+	4+	4+	1+	1+	4+	4+	4+
7	rr	0	0	0	+	+	+	0	+	0	+	0	+	0	+	0	+	0	0	+	+	0	0	+	0	+	+	4+	4+	1+	1+	4+	4+	4+
8	rr	0	0	0	+	+	+	+	+	+	w	+	0	0	+	+	0	0	0	+	+	+	0	+	0	+	+	4+	4+	1+	1+	4+	4+	4+
Auto			3+ MF	1+ MF	4+	4+																						0	0	0	0	0	0	0
Cord																												3+	4+	m+	m+	4+	4+	4+

Patient: Initial Testing Results

- Does not look like anti-CD38 interference
- Does not appear to be autoantibody
- Strong immediate spin reactivity
- ABO discrepancy

ABO	IS
Anti-A	4+
Anti-B	0
A1 cells	4+
A2 cells	3+
B cells	4+
Interpretation	NTD

DAT	IS	5 min
POLY	0	0
IgG	0	NT
C3	0	0
Control	0	0

Anti-D	
IS	4+

		Rh-hr					MNS				P1	Lewis		Luth			Kell			Duffy		Kidd		Domb			SAL	LISS		PEG	IgG Gel	PAPAIN	
		D	C	E	c	e	M	N	S	s	P1	Le^a	Le^b	u	LK	k	K	J	J	F	F	J	J	D	D	X	IS	37C	IAT IgG (no anti-IgG4)	IAT IgG (no anti-IgG4)	IAT (anti-total IgG)	37C	IAT IgG (no anti-IgG4)
1	R^{1}R^1	+	+	0	0	+	0	+	0	+	0	0	+	0	+	0	0	0	0	+	+	0	0	+	0	+	+	4+	4+	+w	+w	4+	4+	4+
2	R^{1}R^1	+	+	0	0	+	0	+	+	+	+	0	0	0	+	+	0	0	0	+	0	+	+	0	+	+	+	3+	4+	+w	+w	4+	4+	4+
3	R^{2}R^2	+	0	+	+	0	0	+	0	+	+	0	+	0	+	0	0	0	0	+	+	0	+	0	0	+	+	3+	4+	m+	m+	4+	4+	4+
4	R^{2}R^2	+	0	+	+	0	+	0	0	+	+	+	0	0	+	0	0	0	0	+	0	+	0	+	0	+	+	3+	4+	m+	m+	4+	4+	4+
5	R^o	+	0	0	+	+	0	+	0	0	+	0	+	+	+	0	+	0	0	+	0	0	+	+	0	+	+	3+	4+	+w	+w	4+	4+	4+
6	rr	0	0	0	+	+	+	0	+	0	+	0	0	0	+	0	+	0	0	+	0	+	+	0	+	0	+	4+	4+	1+	1+	4+	4+	4+
7	rr	0	0	0	+	+	+	0	+	0	+	0	+	0	+	0	+	0	0	+	+	0	0	+	0	+	+	4+	4+	1+	1+	4+	4+	4+
8	rr	0	0	0	+	+	+	+	+	+	w	+	0	0	+	+	0	0	0	+	+	+	0	+	0	+	+	4+	4+	1+	1+	4+	4+	4+
Auto			3+ MF	1+ MF	4+	4+																					0	0	0	0	0	0	0
Cord																											3+	4+	m+	m+	4+	4+	4+

Patient: Initial Testing Results

ABO	IS
Anti-A	4+
Anti-B	0
A1 cells	4+
A2 cells	3+
B cells	4+
Interpretation	NTD

DAT	IS	5 min
POLY	0	0
IgG	0	NT
C3	0	0
Control	0	0

- Does not look like anti-CD38 interference
- Does not appear to be autoantibody
- Strong immediate spin reactivity
- ABO discrepancy

Interesting: Difference between tube and gel testing
Interesting: Rh-negative RBCs stronger than Rh-positive

Anti-D	
IS	4+

		Rh-hr					MNS				P1	Lewis		Luth			Kell			Duffy		Kidd		Domb			SAL	LISS		PEG	IgG Gel	PAPAIN	
		D	C	E	c	e	M	N	S	s	P1	Le a	Le b	u a	u b	k	K a	J a	J b	F y a	F y b	J a	J b	D o a	D o b	X g a	IS	37C	IAT IgG (no anti-IgG4)	IAT IgG (no anti-IgG4)	IAT (anti-total IgG)	37C	IAT IgG (no anti-IgG4)
1	R¹R¹	+	+	0	0	+	0	+	0	+	0	0	+	0	+	0	+	0	0	+	+	0	0	+	0	+	+	4+	4+	+w	+w	4+	4+	4+
2	R¹R¹	+	+	0	0	+	0	+	+	+	+	0	0	0	+	+	0	0	0	+	0	+	+	0	+	+	+	3+	4+	+w	+w	4+	4+	4+
3	R²R²	+	0	+	+	0	0	+	0	+	+	0	+	0	+	0	+	0	0	+	+	0	+	0	0	+	+	3+	4+	m+	m+	4+	4+	4+
4	R²R²	+	0	+	+	0	+	0	0	+	+	+	0	0	+	0	+	0	0	+	0	+	0	+	0	+	+	3+	4+	m+	m+	4+	4+	4+
5	Rº	+	0	0	+	+	0	+	0	0	+	0	+	+	+	0	+	0	0	+	0	0	+	+	0	+	+	3+	4+	+w	+w	4+	4+	4+
6	rr	0	0	0	+	+	+	0	+	0	+	0	0	0	+	0	+	0	0	+	0	+	+	0	+	0	+	4+	4+	1+	1+	4+	4+	4+
7	rr	0	0	0	+	+	+	0	+	0	+	0	+	0	+	0	+	0	0	+	+	0	0	+	0	+	+	4+	4+	1+	1+	4+	4+	4+
8	rr	0	0	0	+	+	+	+	+	+	w	+	0	0	+	+	0	0	0	+	+	+	0	+	0	+	+	4+	4+	1+	1+	4+	4+	4+
Auto			3+ MF	1+ MF	4+	4+																					0	0	0	0	0	0	0
Cord																											3+	4+	m+	m+	4+	4+	4+

Patient: Initial Testing Results

- Does not look like anti-CD38 interference
- Does not appear to be autoantibody
- Strong immediate spin reactivity
- ABO discrepancy

Interesting: Difference between tube and gel testing
Interesting: Rh-negative RBCs stronger than Rh-positive

ABO	IS
Anti-A	4+
Anti-B	0
A1 cells	4+
A2 cells	3+
B cells	4+
Interpretation	NTD

DAT	IS	5 min
POLY	0	0
IgG	0	NT
C3	0	0
Control	0	0

Anti-D	
IS	4+

		Rh-hr					MNS				P1	Lewis		Luth		Kell			Duffy		Kidd		Domb			SAL	LISS		PEG	IgG Gel	PAPAIN	
		D	C	E	c	e	M	N	S	s	P1	L	I	I	LK			J				J	D	D	X	IS	37C	IAT IgG (no anti-IgG4)	IAT IgG (no anti-IgG4)	IAT IgG (anti-total IgG)	37C	IAT IgG (no anti-IgG4)
																						k	o	o b	g a							
1	R¹R¹	+	+	0	0																			+	+	4+	4+	+w	+w	4+	4+	4+
2	R¹R¹	+	+	0																				+	+	3+	4+	+w	+w	4+	4+	4+
3	R²R²	+	0	+	+																			+	+	3+	4+	m+	m+	4+	4+	4+
4	R²R²	+	0	+	+																	0	+	0	+	+	3+	4+	m+	m+	4+	4+	4+
5	Ro	+	0	0	+	+																0	+	+	0	+	+	3+	4+	+w	+w	4+	4+	4+
6	rr	0	0	0	+	+	+	0	+		0					0	0	+	0	+	+	0	+	0	+	4+	4+	1+	1+	4+	4+	4+
7	rr	0	0	0	+	+	+	0	+	0	+	0	+	0	+	0	+	0	0	+	+	0	0	+	0	+	4+	4+	1+	1+	4+	4+	4+
8	rr	0	0	0	+	+	+	+	+	+	w	+	0	0	+	+	0	0	0	+	+	+	0	+	0	+	+	4+	4+	1+	1+	4+	4+	4+
Auto			3+ MF	1+ MF	4+	4+																				0	0	0	0	0	0	0
Cord																										3+	4+	m+	m+	4+	4+	4+

Hospital blood bank: *No additional information*

What can we do to investigate further?

		Rh-hr					MNS				P1	Lewis		Luth.		Kell			Duffy		Kidd		Domb			PLASMA			ELUATE	
		D	C	E	c	e	M	N	S	s	P1	Le a	Le b	Lu a	Lu b	K	k	Kpa	Js a	Js b	Fy a	Fy b	Jk a	Jk b	Do a	Do b	Xg a	Trypsin	α-chymo	DTT	PEG IAT	
																												IAT IgG (no anti-IgG4)			IgG (no IgG4)	Total IgG
1	R_1R_1	+	+	0	0	+	0	+	0	+	0	0	+	0	+	0	+	0	0	0	+	+	0	0	+	0	+	+	4+	4+	4+	0	4+
2	R_1R_1	+	+	0	0	+	0	+	+	+	+	0	0	0	+	+	0	0	0	+	0	+	+	0	+	+	+	4+	4+	4+	0	4+
3	R_2R_2	+	0	+	+	0	0	+	0	+	+	0	+	0	+	0	+	0	0	0	+	+	0	+	0	0	+	+	4+	4+	4+	0	4+
4	R_2R_2	+	0	+	+	0	+	0	0	+	+	+	0	0	+	0	+	0	0	0	+	0	+	0	+	0	+	+	4+	4+	4+	0	4+
5	R_o	+	0	0	+	+	0	+	0	0	+	0	+	+	+	0	+	0	0	0	+	0	0	+	+	0	+	+	4+	4+	4+	0	4+
6	rr	0	0	0	+	+	+	0	+	0	+	0	0	0	+	0	+	0	0	0	+	0	+	+	0	+	0	+	4+	4+	4+	0	4+
7	rr	0	0	0	+	+	+	0	+	0	+	0	+	0	+	0	+	0	0	0	+	+	0	0	+	0	+	+	4+	4+	4+	0	4+
8	rr	0	0	0	+	+	+	+	+	+	w	+	0	0	+	+	0	0	0	0	+	+	+	0	+	0	+	+	4+	4+	4+	0	4+
Auto			3+ MF		1+ MF	4+	4+																					0	0	0	0	0

What can we do to investigate further?

		Rh-hr					MNS				P1	Lewis		Luth.		Kell			Duffy		Kidd		Domb			PLASMA			ELUATE	
		D	C	E	c	e	M	N	S	s	P1	Le a	Le b	Lu a	Lu b	K	k	Kpa	Js a	Js b	Fy a	Fy b	Jk a	Jk b	Do a	Do b	Xg a	Trypsin	α-chymo	DTT	PEG IAT	
																												IAT IgG (no anti-IgG4)			IgG (no IgG4)	Total IgG
1	R1R1	+	+	0	0	+	0	+	0	+	0	0	+	0	+	0	+	0	0	+	+	0	0	+	0	+	+	4+	4+	4+	0	4+
2	R1R1	+	+	0	0	+	0	+	+	+	+	0	0	0	+	+	0	0	0	+	0	+	+	0	+	+	+	4+	4+	4+	0	4+
3	R2R2	+	0	+	+	0	0	+	0	+	+	0	+	0	+	0	+	0	0	+	+	0	+	0	0	+	+	4+	4+	4+	0	4+
4	R2R2	+	0	+	+	0	+	0	0	+	+	+	0	0	+	0	+	0	0	+	0	+	0	+	0	+	+	4+	4+	4+	0	4+
5	Ro	+	0	0	+	+	0	+	0	0	+	0	+	+	+	0	+	0	0	+	0	0	+	+	0	+	+	4+	4+	4+	0	4+
6	rr	0	0	0	+	+	+	0	+	0	+	0	0	0	+	0	+	0	0	+	0	+	+	0	+	0	+	4+	4+	4+	0	4+
7	rr	0	0	0	+	+	+	0	+	0	+	0	+	0	+	0	+	0	0	+	+	0	0	+	0	+	+	4+	4+	4+	0	4+
8	rr	0	0	0	+	+	+	+	+	+	w	+	0	0	+	+	0	0	0	+	+	+	0	+	0	+	+	4+	4+	4+	0	4+
Auto			3+ MF	1+ MF	4+	4+																						0	0	0	0	0

What can we do to investigate further?

		Rh-hr					MNS				P1	Lewis		Luth.		Kell			Duffy		Kidd		Domb			PLASMA			ELUATE	
		D	C	E	c	e	M	N	S	s	P1	Le a	Le b	Lu a	Lu b	K	k	Kpa	Js a	Js b	Fy a	Fy b	Jk a	Jk b	Do a	Do b	Xg a	Trypsin	α-chymo	DTT	PEG IAT	
																												IAT IgG (no anti-IgG4)			IgG (no IgG4)	Total IgG
1	R_1R_1	+	+	0	0	+	0	+	0	+	0	0	+	0	+	0	+	0	0	0	+	+	0	0	+	0	+	+	4+	4+	4+	0	4+
2	R_1R_1	+	+	0	0	+	0	+	+	+	+	0	0	0	+	+	0	0	0	+	0	+	+	0	+	+	+	4+	4+	4+	0	4+
3	R_2R_2	+	0	+	+	0	0	+	0	+	+	0	+	0	+	0	+	0	0	0	+	+	0	+	0	0	+	+	4+	4+	4+	0	4+
4	R_2R_2	+	0	+	+	0	+	0	0	+	+	+	0	0	+	0	+	0	0	0	+	0	+	0	+	0	+	+	4+	4+	4+	0	4+
5	R_o	+	0	0	+	+	0	+	0	0	+	0	+	+	+	0	+	0	0	0	+	0	0	+	+	0	+	+	4+	4+	4+	0	4+
6	rr	0	0	0	+	+	+	0	+	0	+	0	0	0	+	0	+	0	0	0	+	0	+	+	0	+	0	+	4+	4+	4+	0	4+
7	rr	0	0	0	+	+	+	0	+	0	+	0	+	0	+	0	+	0	0	0	+	+	0	0	+	0	+	+	4+	4+	4+	0	4+
8	rr	0	0	0	+	+	+	+	+	+	w	+	0	0	+	+	0	0	0	0	+	+	+	0	+	0	+	+	4+	4+	4+	0	4+
Auto			3+ MF		1+ MF	4+	4+																					0	0	0	0	0

RBC Treatments
No Help

What can we do to investigate further?

		Rh-hr					MNS				P1	Lewis		Luth.			Kell			Duffy		Kidd		Domb			PLASMA			ELUATE	
		D	C	E	c	e	M	N	S	s	P1	Le a	Le b	Lu a	Lu b	K	k	Kpa	Js a	Js b	Fy a	Fy b	Jk a	Jk b	Do a	Do b	Xg a	Trypsin	α-chymo	DTT	PEG IAT	
																												IAT IgG (no anti-IgG4)			IgG (no IgG4)	Total IgG
1	R_1R_1	+	+	0	0	+	0	+	0	+	0	0	+	0	+	0	+	0	0	+	+	0	0	+	0	+	+	4+	4+	4+	0	4+
2	R_1R_1	+	+	0	0	+	0	+	+	+	+	0	0	0	+	+	0	0	0	+	0	+	+	0	+	+	+	4+	4+	4+	0	4+
3	R_2R_2	+	0	+	+	0	0	+	0	+	+	0	+	0	+	0	+	0	0	+	+	0	+	0	0	+	+	4+	4+	4+	0	4+
4	R_2R_2	+	0	+	+	0	+	0	0	+	+	+	0	0	+	0	+	0	0	+	0	+	0	+	0	+	+	4+	4+	4+	0	4+
5	R_o	+	0	0	+	+	0	+	0	0	+	0	+	+	+	0	+	0	0	+	0	0	+	+	0	+	+	4+	4+	4+	0	4+
6	rr	0	0	0	+	+	+	0	+	0	+	0	0	0	+	0	+	0	0	+	0	+	+	0	+	0	+	4+	4+	4+	0	4+
7	rr	0	0	0	+	+	+	0	+	0	+	0	+	0	+	0	+	0	0	+	+	0	0	+	0	+	+	4+	4+	4+	0	4+
8	rr	0	0	0	+	+	+	+	+	+	w	+	0	0	+	+	0	0	0	+	+	+	0	+	0	+	+	4+	4+	4+	0	4+
Auto			3+ MF		1+ MF	4+	4+																					0	0	0	0	0

Eluate made as patient was transfused

RBC Treatments
No Help

What can we do to investigate further?

		Rh-hr					MNS				P1	Lewis		Luth.		Kell			Duffy		Kidd		Domb			PLASMA			ELUATE	
		D	C	E	c	e	M	N	S	s	P1	Le a	Le b	Lu a	Lu b	K	k	Kpa	Js a	Js b	Fy a	Fy b	Jk a	Jk b	Do a	Do b	Xg a	Trypsin	α-chymo	DTT	PEG IAT	
																												IAT IgG (no anti-IgG4)			IgG (no IgG4)	Total IgG
1	R_1R_1	+	+	0	0	+	0	+	0	+	0	0	+	0	+	0	+	0	0	+	+	0	0	+	0	+	+	4+	4+	4+	0	4+
2	R_1R_1	+	+	0	0	+	0	+	+	+	+	0	0	0	+	+	0	0	0	+	0	+	+	0	+	+	+	4+	4+	4+	0	4+
3	R_2R_2	+	0	+	+	0	0	+	0	+	+	0	+	0	+	0	+	0	0	+	+	0	+	0	0	+	+	4+	4+	4+	0	4+
4	R_2R_2	+	0	+	+	0	+	0	0	+	+	+	0	0	+	0	+	0	0	+	0	+	0	+	0	+	+	4+	4+	4+	0	4+
5	R_o	+	0	0	+	+	0	+	0	0	+	0	+	+	+	0	+	0	0	+	0	0	+	+	0	+	+	4+	4+	4+	0	4+
6	rr	0	0	0	+	+	+	0	+	0	+	0	0	0	+	0	+	0	0	+	0	+	+	0	+	0	+	4+	4+	4+	0	4+
7	rr	0	0	0	+	+	+	0	+	0	+	0	+	0	+	0	+	0	0	+	+	0	0	+	0	+	+	4+	4+	4+	0	4+
8	rr	0	0	0	+	+	+	+	+	+	w	+	0	0	+	+	0	0	0	+	+	+	0	+	0	+	+	4+	4+	4+	0	4+
Auto			3+ MF	1+ MF	4+	4+																						0	0	0	0	0

RBC Treatments
No Help

Eluate made as patient was transfused

ABO	IS
Anti-A	4+
Anti-B	0
Anti-D	4+
4X alloadsorbed plasma	
A1 cells	0
A2 cells	0
B cells	4+
Interpretation	A Pos

What can we do to investigate further?

		Rh-hr					MNS				P1	Lewis		Luth.		Kell			Duffy		Kidd		Domb			PLASMA			ELUATE	
		D	C	E	c	e	M	N	S	s	P1	Le a	Le b	Lu a	Lu b	K	k	Kpa	Js a	Js b	Fy a	Fy b	Jk a	Jk b	Do a	Do b	Xg a	Trypsin	α-chymo	DTT	PEG IAT IgG (no IgG4)	PEG IAT Total IgG
																												IAT IgG (no anti-IgG4)				
1	R1R1	+	+	0	0	+	0	+	0	+	0	0	+	0	+	0	+	0	0	+	+	0	0	+	0	+	+	4+	4+	4+	0	4+
2	R1R1	+	+	0	0	+	0	+	+	+	+	0	0	0	+	+	0	0	0	+	0	+	+	0	+	+	+	4+	4+	4+	0	4+
3	R2R2	+	0	+	+	0	0	+	0	+	+	0	+	0	+	0	+	0	0	+	+	0	+	0	0	+	+	4+	4+	4+	0	4+
4	R2R2	+	0	+	+	0	+	0	0	+	+	+	0	0	+	0	+	0	0	+	0	+	0	+	0	+	+	4+	4+	4+	0	4+
5	Ro	+	0	0	+	+	0	+	0	0	+	0	+	+	+	0	+	0	0	+	0	0	+	+	0	+	+	4+	4+	4+	0	4+
6	rr	0	0	0	+	+	+	0	+	0	+	0	0	0	+	0	+	0	0	+	0	+	+	0	+	0	+	4+	4+	4+	0	4+
7	rr	0	0	0	+	+	+	0	+	0	+	0	+	0	+	0	+	0	0	+	+	0	0	+	0	+	+	4+	4+	4+	0	4+
8	rr	0	0	0	+	+	+	+	+	+	w	+	0	0	+	+	0	0	0	+	+	+	0	+	0	+	+	4+	4+	4+	0	4+
Auto			3+ MF	1+ MF	4+	4+																						0	0	0	0	0

Eluate made as patient was transfused

ABO	IS
Anti-A	4+
Anti-B	0
Anti-D	4+
4X alloadsorbed plasma	
A1 cells	0
A2 cells	0
B cells	4+
Interpretation	A Pos

- 4x differential adsorption (patient had been transfused)
- Plasma reactivity removed when tested with any anti-IgG
- ABO discrepancy resolved by testing alloadsorbed plasma

RBC Treatments
No Help

What can we do to investigate further?

Eluate made as patient was transfused

		Rh-hr					MNS				P1	Lewis		Luth.		Kell			Duffy		Kidd		Domb				PLASMA			ELUATE	
		D	C	E	c	e	M	N	S	s	P1	Le a	Le b	Lu a	Lu b	K	k	Kpa	Js a	Js b	Fy a	Fy b	Jk a	Jk b	Do a	Do b	Xg	Trypsin	α-chymo	DTT	PEG IAT	
																												IAT IgG (no anti-IgG4)			IgG (no IgG4)	Total IgG
1	R_1R_1	+	+	0	0	+	0	+	0	+	0	0	+	0	+	0	+	0	0	+	+	0	0	+	0	+	+	4+	4+	4+	0	4+
2	R_1R_1	+	+	0	0	+	0	+	+	+	+	0	0	0	+	+	0	0	0	+	0	+	+	0	+	+	+	4+	4+	4+	0	4+
3	R_2R_2	+	0	+	+	0	0	+	0	+	+	0	+	0	+	0	+	0	0	+	+	0	+	0	0	+	+	4+	4+	4+	0	4+
4	R_2R_2	+	0	+	+	0	+	0	0	+	+	+	0	0	+	0	+	0	0	+	0	+	0	+	0	+	+	4+	4+	4+	0	4+
5	R_o	+	0	0	+	+	0	+	0	0	+	0	+	+	+	0	+	0	0	+	0	0	+	+	0	+	+	4+	4+	4+	0	4+
6	rr	0	0	0	+	+	+	0	+	0	+	0	0	0	+	0	+	0	0	+	0	+	+	0	+	0	+	4+	4+	4+	0	4+
7	rr	0	0	0	+	+	+	0	+	0	+	0	+	0	+	0	+	0	0	+	+	0	0	+	0	+	+	4+	4+	4+	0	4+
8	rr	0	0	0	+	+	+	+	+	+	w	+	0	0	+	+	0	0	0	+	+	+	0	+	0	+	+	4+	4+	4+	0	4+
Auto			3+ MF		1+ MF	4+	4+																					0	0	0	0	0

ABO	IS
Anti-A	4+
Anti-B	0
Anti-D	4+
4X alloadsorbed plasma	
A1 cells	0
A2 cells	0
B cells	4+
Interpretation	A Pos

- 4x differential adsorption (patient had been transfused)
- Plasma reactivity removed when tested with any anti-IgG
- ABO discrepancy resolved by testing alloadsorbed plasma

RBC Treatments
No Help

Call to hospital blood bank in the morning:
Any chance the patient is on CD47 therapy?

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What can we do to investigate further?

Eluate made as patient was transfused

		Rh-hr					MNS				P1	Lewis		Luth.		Kell			Duffy		Kidd		Domb				PLASMA			ELUATE	
		D	C	E	c	e	M	N	S	s	P1	Le a	Le b	Lu a	Lu b	K	k	Kpa	Js a	Js b	Fy a	Fy b	Jk a	Jk b	Do a	Do b	Xg a	Trypsin	α-chymo	DTT	PEG IAT	
																												IAT IgG (no anti-IgG4)			IgG (no IgG4)	Total IgG
1	R1R1	+	+	0	0	+	0	+	0	+	0	0	+	0	+	0	+	0	0	+	+	0	0	+	0	+	+	4+	4+	4+	0	4+
2	R1R1	+	+	0	0	+	0	+	+	+	+	0	0	0	+	+	0	0	0	+	0	+	+	0	+	+	+	4+	4+	4+	0	4+
3	R2R2	+	0	+	+	0	0	+	0	+	+	0	+	0	+	0	+	0	0	+	+	0	+	0	0	+	+	4+	4+	4+	0	4+
4	R2R2	+	0	+	+	0	+	0	0	+	+	+	0	0	+	0	+	0	0	+	0	+	0	+	0	+	+	4+	4+	4+	0	4+
5	Ro	+	0	0	+	+	0	+	0	0	+	0	+	+	+	0	+	0	0	+	0	0	+	+	0	+	+	4+	4+	4+	0	4+
6	rr	0	0	0	+	+	+	0	+	0	+	0	0	0	+	0	+	0	0	+	0	+	+	0	+	0	+	4+	4+	4+	0	4+
7	rr	0	0	0	+	+	+	0	+	0	+	0	+	0	+	0	+	0	0	+	+	0	0	+	0	+	+	4+	4+	4+	0	4+
8	rr	0	0	0	+	+	+	+	+	+	w	+	0	0	+	+	0	0	0	+	+	+	0	+	0	+	+	4+	4+	4+	0	4+
Auto			3+ MF		1+ MF	4+	4+																					0	0	0	0	0

ABO	IS
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RBC Treatments No Help

Follow-up samples from the patient:

- At times, spontaneous agglutination observed
- Not dispersed by repeated washing with PBS warmed to 37°C or any other strategies

Call to hospital blood bank in the morning:
Any chance the patient is on CD47 therapy?

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Learning Points from This Case

- Patient was receiving magrolimab CD47 therapy
- Careful analysis of results can provide clues
  - Different reactivity with different antiglobulin reagents or test method?
  - RBC treatments?
  - ABO discrepancy
  - What doesn't fit with any "assumptions"?
- **Critical: communication**
  - Ensure communication between patients, clinical teams, and laboratory staff
  - Avoid unnecessary delays in providing blood components for transfusion
  - Lack of communication may have an adverse impact on patient care (and other team members)
  - Remember: one size does not fit all
    - Know which CD47 therapeutic the patient is on

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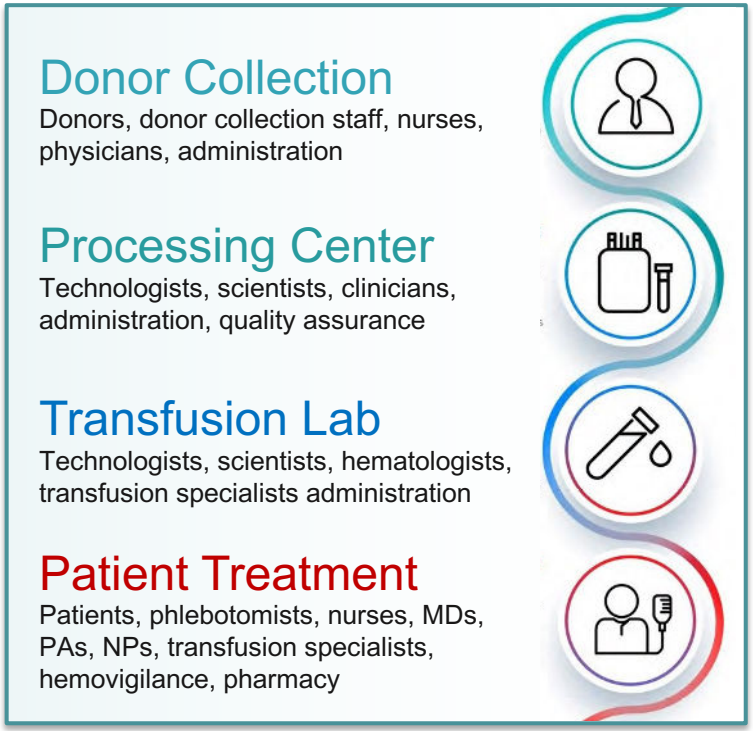
Multidisciplinary Teams in the Blood Transfusion Chain

- Safe and effective transfusions rely on a multidisciplinary team
- Each team member—at every stage of the transfusion chain—ultimately contribute to enhanced patient care
- Improving communication and understanding of one another's role and scope of practice is important to improve outcomes
- The patient should always be considered the key member of the multidisciplinary health care team



Moss R, et al. *Transfusion Today.* 2021:129. Galel SA, Richards CA; *Am J Clin Pathol.* 1997;107(4 Suppl 1):S43–S49.

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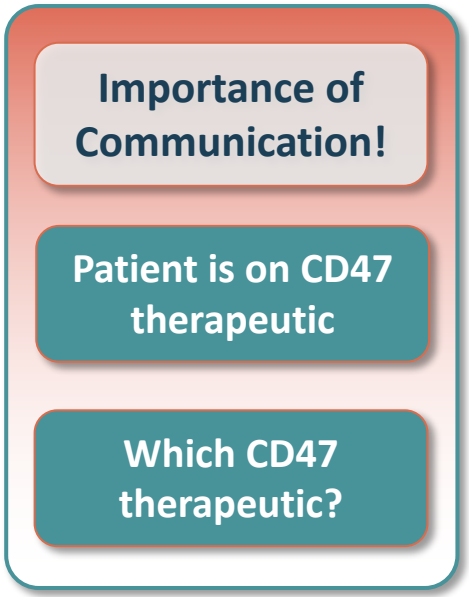
What Do You Need to Know When a Sample from a Patient on a CD47 Therapeutic Arrives?

- **Which CD47 therapeutic?**
  - Drug name may be different than name of agent during early phases of clinical trials (example: magrolimab = Hu5F9-G4)
  - Has serologic interference been reported?
  - Is the agent IgG4?
- **What AHG reagents does your facility use?**
  - Is anti-IgG that lacks reactivity with IgG4 available?
- **What mitigation strategies work for the particular agent?**
  - Use of AHG lacking reactivity with IgG4? Multiple adsorptions?
- **Obtain patient phenotype/genotype**
  - Informs which antibodies patient can make
  - Needed to provide phenotype-matched units



Velliquette RW, et al. *Transfusion*. 2019;59:730–737. Reyland L, et al. *Transfus Med*. 2020;30(2):157–160.

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Transfusion
Further Considerations

- **ABO discrepancy not resolved**
  - Transfuse group O RBCs, group AB plasma
- **Crossmatches (XMs)**
  - IAT XM with AHG lacking reactivity with IgG4
  - Electronic XM if all clinically significant antibodies to common antigens have been ruled out
  - Immediate-spin (IS) XM may be possible if agent doesn't interfere in IS phase
- **If patient has clinically significant antibody**
  - Provide antigen negative units

Consider transfusing phenotype-matched donor units

Velliquette RW, et al. *Transfusion.* 2019;59:730–737. Tan M, et al. *Intern Med J.* 2022;52:2165–2171. Brierley CK, et al. *Transfusion.* 2019;59:2248–2254.

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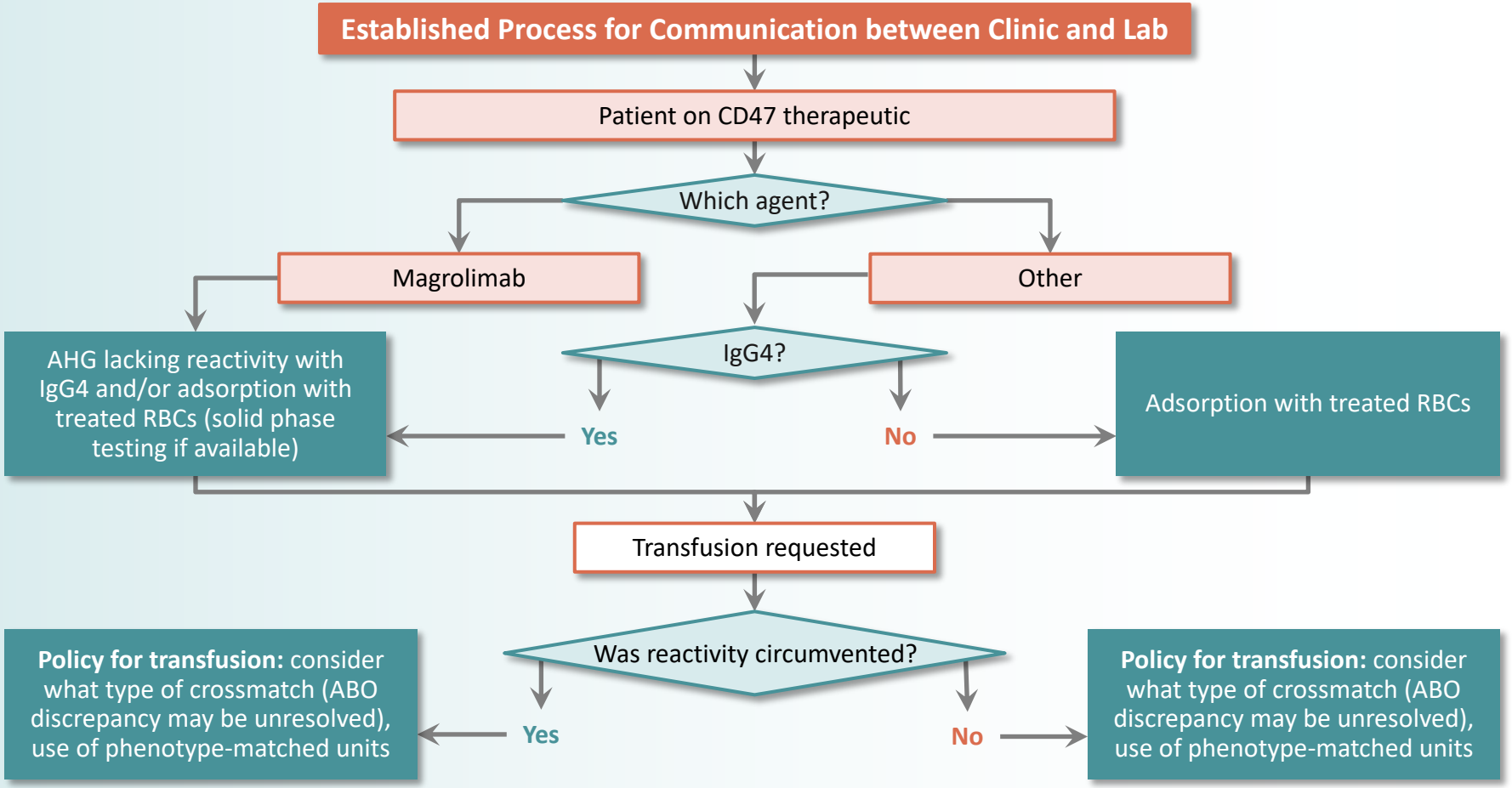
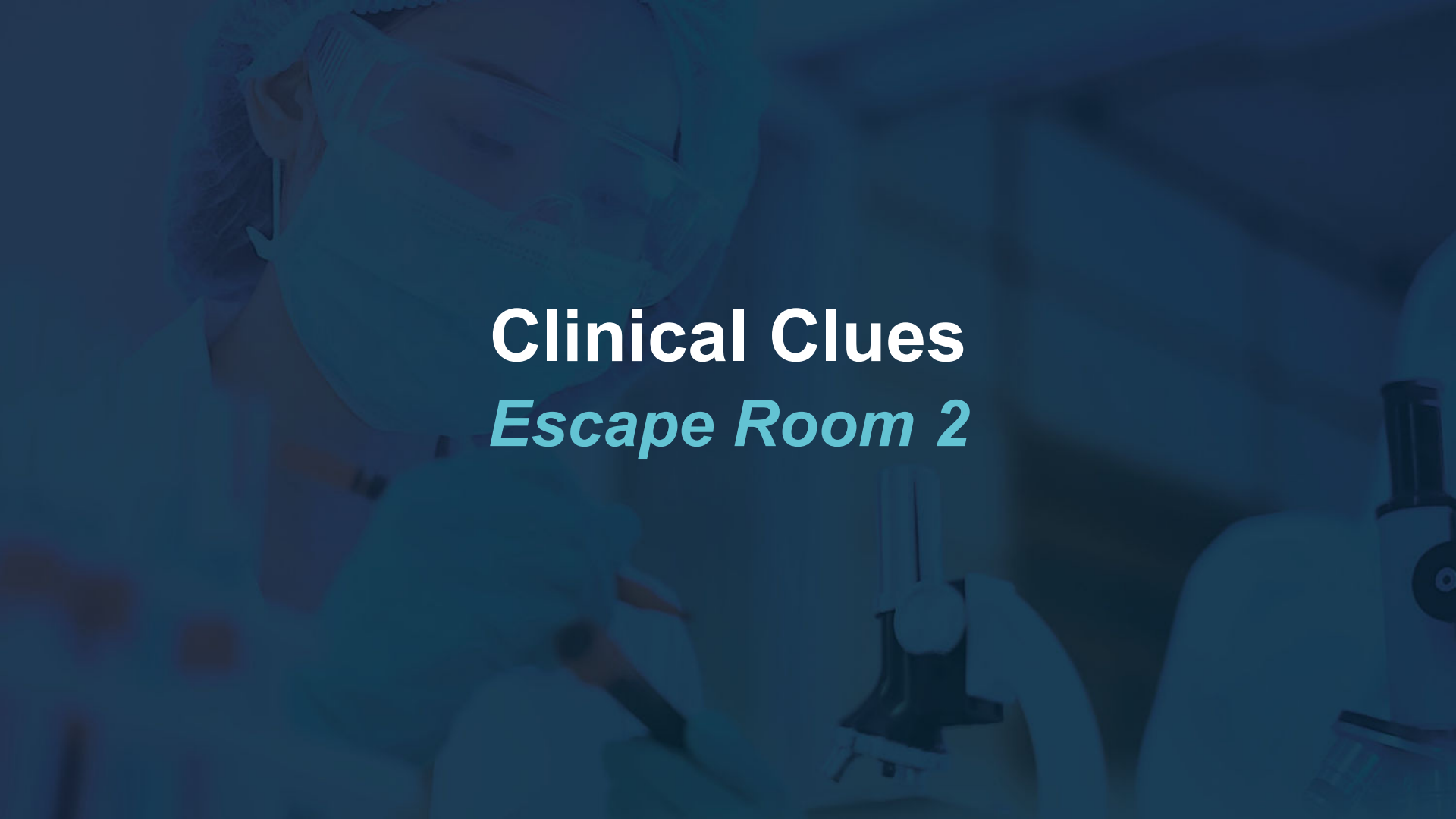
Laboratory Processes—Patients on a CD47 Therapeutic



Courtesy of Lynsi Rahorst, MHPE, MLS(ASCP)SBB^{CM}



Clinical Clues

Escape Room 2

SMART Goals
Specific, Measurable, Attainable, Relevant, Timely

Put information into action! Consider the following goals; then set a time frame that fits with your work environment and a reasonable improvement target that aligns with your patient population.

- **Develop, update, and/or implement** a reminder system to alert team members of receipt of blood samples from patients treated with a CD47 therapeutic to **improve compliance with mitigation protocols.**
- **Improve** rate of selection of anti-IgG that does not react with IgG4 to **mitigate interference when testing blood** from patients on magrolimab.
- **Establish** regular multidisciplinary team meetings to review updates in CD47 therapeutics and communication strategies with all team members—clinical and technical—to **improve mitigation protocol use.**


QUESTIONS
&
ANSWERS

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Deciphering the Clinical Clues

Updates to Protocols and Procedures for Anti-CD47 Agents in Clinical Laboratories

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