SEROLOGICAL OBSERVATIONS IN PATIENTS RECEIVING HU5F9-G4 MONOCLONAL ANTI-CD47 THERAPY

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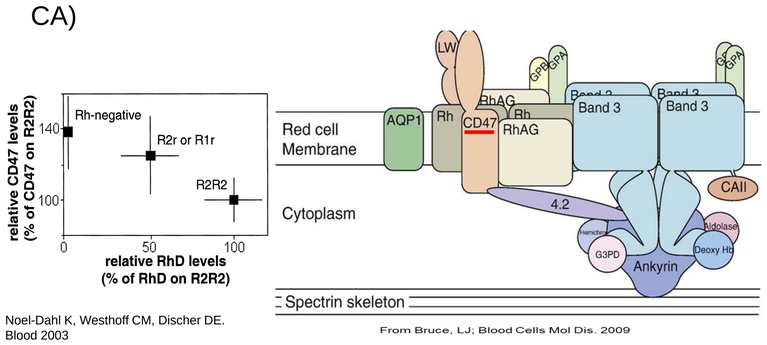
INTRODUCTION

- Interference in blood bank testing with monoclonal antibody (mAb) anti-CD38 therapy and methods to mitigate the interference have been described.[1,2]
- Hu5F9-G4 is a human monoclonal IgG4 antibody targeting CD47 that is in clinical trials to treat hematologic or solid malignancies.
- CD47 is a transmembrane glycoprotein that binds to signal-regulatory protein α (SIRPα) on macrophages and functions to regulate phagocytosis.
- Blocking CD47 on tumor cells is thought to enhance phagocytosis and promote anti-tumor responses.
- CD47 is expressed on platelets and highly expressed on RBCs.
- Expression level of CD47 on RBCs varies depending on Rh phenotype.[3]
  - rr **>** R1r/R2r **>** R2R2
  - Weakest expression on Rh_{null}
- Patients with T-cell lymphoma were enrolled in an IRB-approved phase I escalating dose trial of Hu5F9-G4 monoclonal IgG4 anti-CD47. (Forty Seven Inc. Menlo Park, CA)



Noel-Dahl K, Westhoff CM, Discher DE. Blood 2003

From Bruce, LJ; Blood Cells Mol Dis. 2009

OBJECTIVES

- To investigate possible interference of treatment with Hu5F9-G4 anti-CD47 on blood bank testing.
- Evaluate approaches to mitigate reactivity.

MATERIALS AND METHODS

- **CLINICAL TRIAL**
  - Patients received 1 mg/Kg Hu5F9-G4 on day one of treatment and escalated to 20 mg/Kg IV infusion each week for 4-5 weeks.
- **SEROLOGY**
  - Samples were tested over the course of 1 month treatment.
  - Testing was performed by standard tube methods.
  - Plasma was tested at IS and by indirect antiglobulin test (IAT) with cord cells, R_2R_2, rr, and with D- -, Rh_{mod} and Rh_{null} RBCs.
  - Immucor Gamma-clone anti-IgG (does not detect IgG4) and Ortho BioClone anti-IgG (total IgG) were used in IAT.
  - Panel RBCs were treated with enzymes, DTT or W.A.R.M. (Immucor).
  - For titration studies plasma was diluted in PBS.
  - Adsorption studies used papain treated rr RBCs.
  - Eluates were made using Gamma ELU-KIT II (Immucor).

RESULTS

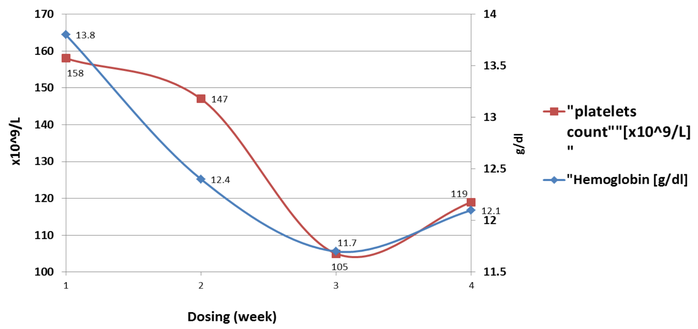
TABLE 1: SUMMARY OF PATIENT NEAT PLASMA TESTING

Panel RBCs	IS	PEG IAT Gamma-clone IgG	Carryover PBS control	PEG IAT Ortho IgG	Enzyme/DTT/ W.A.R.M. treated
R_2R_2	3+	micro	micro	4+	4+
rr	3+ to 4+	micro to 1+	1+	4+	
-D-	0	0	0	4+	
Rh mod	0	0	0	3+	
Rh null	0	0	0	2+	
Cord	3+	micro	2+		
Auto	0	0	0	0	0

- **Plasma testing with Ortho anti-IgG**
  - 3+ to 4+ at IS and 3+ to 4+ in PEG IAT.
  - Reduced reactivity (negative at IS and 2+ to 4+ in PEG IAT) with:
    - -D-, Rh_{mod}, Rh_{null}
  - Auto controls negative at IS and IAT.
- **Plasma testing with Immucor Gamma-clone anti-IgG**
  - Microscopic reactions in PEG IAT due to carry-over.
- **Plasma titrations with R_2R_2 RBCs**
  - Titer of 1 at IS.
  - Titer of ≥ 256 in PEG IAT using Ortho anti-IgG.
- **Plasma testing against enzyme, DTT or W.A.R.M. treated RBCs**
  - Reactivity equivalent to untreated RBCs
- **Plasma adsorptions with papain treated rr RBCs**
  - 4X adsorptions
    - Negative at IS and PEG IAT with rr RBCs using Ortho anti-IgG.
- **Patient RBC testing**
  - Negative or micro reactive in DAT (with any anti-IgG).
  - Spontaneous agglutination observed.
    - Not dispersed by repeated washing with PBS warmed to 37°C.
- **Eluate testing**
  - 3+ to 4+ reactive using Ortho anti-IgG.
  - Negative to micro due to carryover with Gamma-clone anti-IgG.
  - Auto control negative.

CLINICAL OBSERVATIONS

- Hgb drop within 24 hrs of infusion.
  - Average 2-3 g/dL over course of treatment
- Platelet counts fluctuated.
  - Average platelet drop over course ≈ 40,000-80,000
- Neither patient required transfusion over the course of treatment.



SUMMARY OF REACTIVITY

- Hu5F9-G4 anti-CD47 interferes with routine pre-transfusion testing in both antibody screening and ABO typing.

Plasma Testing:

- Due to high levels of CD47 on test RBCs, plasma containing Hu5F9-G4:
  - Causes pan-agglutination at initial spin (mimicking cold-reactive IgM antibodies) with carry-over into IAT.
  - Interferes with reverse ABO typing.
  - Shows strong pan-reactivity (3+ to 4+) with human anti-total IgG but is non-reactive with human anti-IgG that lacks anti-IgG4 component (Immucor/Gamma-clone), although micro reactivity may be observed due to carry-over into IAT.
  - Is strongly reactive with trypsin, ficin/papain, α-chymotrypsin, 0.2M DTT or W.A.R.M. treated cells.

RBC Testing:

- Possible spontaneous agglutination of patient RBCs.
- DAT and auto control tests often very weak or negative.
  - Eluate strong pan-reactive from DAT +w RBCs.
- **Approaches to Mitigate CD47 Interference:**
  - Use of Immucor Gamma-clone anti-IgG for IAT testing because it does not detect IgG4 bound to cells.
  - Plasma allo adsorption with papain treated rr RBCs.
    - Efficacy and number of adsorptions needed is related to circulating drug concentration and date of last infusion.
    - PEG adsorption method invalid due to negative dilution control. Precipitation or complexing of antibody or dilution?

CONCLUSIONS

Strategies different from those used to overcome interference in blood bank testing from Daratumumab, anti-CD38, will be needed for Hu5F9-G4 anti-CD47. Unlike CD38, CD47:

- Cannot be removed from the test RBCs by DTT treatment or any enzyme treatment.
- Is reactive with cord cells.
- CD47 is expressed in high levels compared to CD38, therefore:
  - Reactivity may be robust in IS with back-type and panel cells.
  - DAT and autocontrol can be false negative.
  - Eluate 3+ to 4+ reactive.
- Is IgG4, therefore may be avoided in IAT phase with Gamma-clone anti-human IgG .
- Can be removed by multiple rounds of adsorption on allogenic RBCs.

REFERENCES

1. Chapuy CI, Nicholson RT, Aguad MD, *et al.* Resolving the daratumumab interference with blood compatibility testing. Transfusion 2015;55: 1545-54.
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3. Noel-Dahl K, Westhoff CM, Discher DE. (2003) Fractional attachment of CD47 (IAP) to the erythrocyte cytoskeleton and visual co-localization with Rh protein complexes. Blood 2003; 101:1194-99