

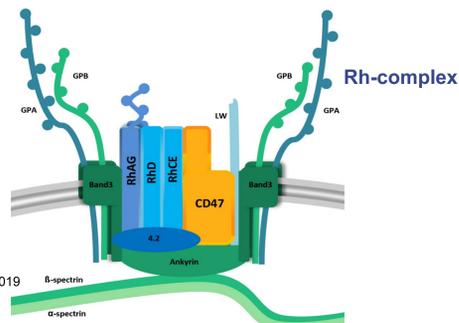


Serological Interference In Patients Receiving SRF231 Anti-CD47 Immunotherapy

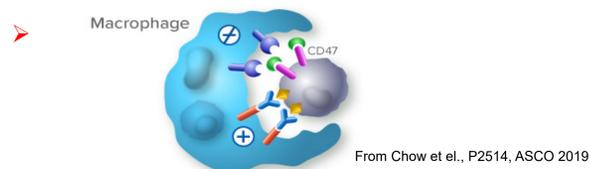
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INTRODUCTION

- CD47 is highly expressed on RBCs as a member of the Rh-complex in the membrane.



- When CD47 binds to SIRPα on phagocytic cells it delivers a “don’t eat me” signal.
- Some cancer cells express higher levels of CD47 as a mechanism to evade immune surveillance.
- Blocking CD47 on tumor cells is thought to enhance phagocytosis and promote anti-tumor responses.



- SRF231 (Surface Oncology, Cambridge MA) is a fully human anti-CD47 isotype IgG4 and has been shown to bind CD47 with high affinity.
- SRF231 is in clinical trials as a single agent or with combinational therapies to treat patients with advanced solid malignancies or lymphoma.
- Expression level of CD47 on RBCs varies with Rh phenotype.¹
 - RhD– (rr) > RhD+ (R₁r) / RhD+ (R₂r) > RhD+ (R₂R₂)
 - Weakest expression on Rh_{null}
- Anti-CD47 can bind to RBCs *in-vivo* and cause anemia² and can interfere with serologic testing *in-vitro*.³
- Although SRF231 did not induce direct hemagglutination of RBCs *in vitro* (Peluso et.al. P272 SITC 2019), interference in pre-transfusion testing is reported here.

OBJECTIVES

- To investigate interference of SRF231 in blood bank testing.
- Evaluate approaches to mitigate reactivity.

MATERIALS AND METHODS

➤ CLINICAL

- Two patients who were receiving SRF231 monotherapy.

➤ SEROLOGY

- Testing was performed by tube methods with and without enhancement media or by column agglutination technology (CAT, Ortho).
- Patient plasma samples were tested at room temperature (RT) and by indirect antiglobulin test (IAT) against cord cells, R₁R₁, R₂R₂, rr, and with -D- and Rh_{null} RBCs.
- Immucor Gamma-clone anti-IgG (does not detect IgG4) and Ortho BioClone anti-IgG (total IgG) were used in IAT.
- For adsorptions, RBCs were treated with papain.
- Patient RBCs were treated with EDTA-Glycine Acid (EGA)
- For titration studies, the plasma and eluate of patient 1 were diluted in PBS.
- Adsorption studies used papain treated rr RBCs.
- Eluates were made using Gamma ELU-KIT II (Immucor).

RESULTS

➤ Plasma Testing

- In tube testing against RBCs with common Rh types plasma was:
 - Non-reactive at RT.
- **Variable reactivity in the IAT using Immucor anti-IgG:**
 - Neg by LISS.
 - Neg or micro+ by PEG.
 - Micro+ to 1+ by papain.
 - Neg auto controls.
- **Strong 3+ to 4+ by all methods using Ortho anti-IgG:**
 - -D- or Rh_{null} reacted weaker.
 - -D- 2+
 - Rh_{null} 1+
- Moderately reactive with autologous controls.
- The titer of patient 1’s sample was 16 in SAL IAT against RhD+ (R₂R₂) RBCs.

➤ Adsorption Studies

- 3X papain-treated RhD– (rr) allo-adsorptions removed the plasma SRF231 reactivity.

RESULTS (CONT)

➤ RBC Direct Antiglobulin Test

- Negative with Immucor IgG.
- 3+ to 4+ with Ortho IgG.
- Negative with anti-C3.
- EGA removed the IgG coating the patient RBCs.

➤ Eluate Testing

- Reacted 3+ to 4+ in IAT (tube or CAT with Ortho anti-IgG) with all cells tested and 2+ with Rh_{null}.
- Reacted micro+ with all RBCs using Immucor anti-IgG.
- Negative last washes were obtained.
- Eluate titer of patient 1’s sample was 128 against R₂R₂ RBCs.

CONCLUSIONS

- SRF231 monotherapy reported here was shown to interfere with RBC antibody detection and potentially crossmatch testing in the IAT. Interference was dependent on the anti-IgG used for testing.
- SRF231 is IgG4 and use of Immucor Gamma-clone anti-IgG can avoid interference in the IAT although some very weak reactivity may be observed.
- Interference was not observed in direct testing at RT or 37°C or in ABO typing, in contrast to monoclonal anti-CD47 Hu5F9-G4 previously reported.³
- Multiple RBC allo-adsorptions (n=3) were successful in removing SRF231 interference.
- SRF231 therapy was associated with patient RBCs 2+–3+ reactive in the DAT using Ortho anti-IgG, but RBCs with a negative DAT observed with Immucor anti-IgG.
- SRF231 anti-CD47 reported here, like other anti-CD47 such as Magrolimab (formerly Hu5F9-G4) and CD47 antagonists like ALX148, interferes in pre-transfusion testing. and requires individual and unique strategies to mitigate.

REFERENCES

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2. Brierly CK, Staves J, Roberts C, et al. The effects of monoclonal anti-CD47 on RBCs, compatibility testing, and transfusion requirements in refractory acute myeloid leukemia. *Transfusion* 2019;59:2248-54.
3. Velliquette RW, Aeschlimann J, Kirkegaard J, et al. Monoclonal anti-CD47 interference in red cell and platelet testing. *Transfusion* 2019;59: 730-7.