

Elimination of Anti-CD47 Drug Interference in Serological Testing by Antigen Masking

Background

CD47 is a self-marker on cells that is overexpressed in several malignancies. Its interaction with SIRP α serves as a “don't eat me” signal to macrophages. Blocking this signal transduction to increase the rate of phagocytosis of cancer cells is one of the rationales behind the development of therapeutic anti-CD47 drugs such as the monoclonal antibody magrolimab. However, magrolimab induces strong agglutination of red blood cells (RBCs) not only in the indirect antiglobulin test (IAT) but also in the neutral test, impairing the determination of irregular antibodies and serological typing.¹

Even though all trials with magrolimab have been discontinued, there are still a number of other anti-CD47 drugs under clinical investigation. It is unclear to which degree these interfere with serological testing.

Methods

We tested the anti-CD47 drugs ontorpacept (TTI-621), ligufalimab (AK 117), letaplimab (IBI-188) and lempzoparlimab (TJ011133/TJC4) (see Table 1, all obtained from MedChemExpress) in the IAT, in the neutral test, and in the Capture Assay in comparison with magrolimab. Furthermore, we tested if MagroEx (imusyn), an optimized soluble SIRP α variant under development, is able to eliminate the interference by antigen masking (see Fig. 1).

Table 1. Overview over the tested Anti-CD47 Drug Candidates

Given are the international nonproprietary name, the drug candidate number, the clinical trial status, the type of the drug and the binding domain.

Anti-CD47 Drug	Clinical Trials	Type	Binding Domain
Magrolimab (Hu5F9-G4)	Abandoned	IgG4 antibody	anti-CD47
Ligufalimab (AK117)	Phase II	IgG4 antibody	anti-CD47
Lempzoparlimab (TJC4)	Phase III	IgG4 antibody	anti-CD47
Letaplimab (IBI188)	Phase I + II	IgG4 antibody	anti-CD47
Ontorpacept (TTI-621)	Phase II	IgG1 fusion protein	SIRP α

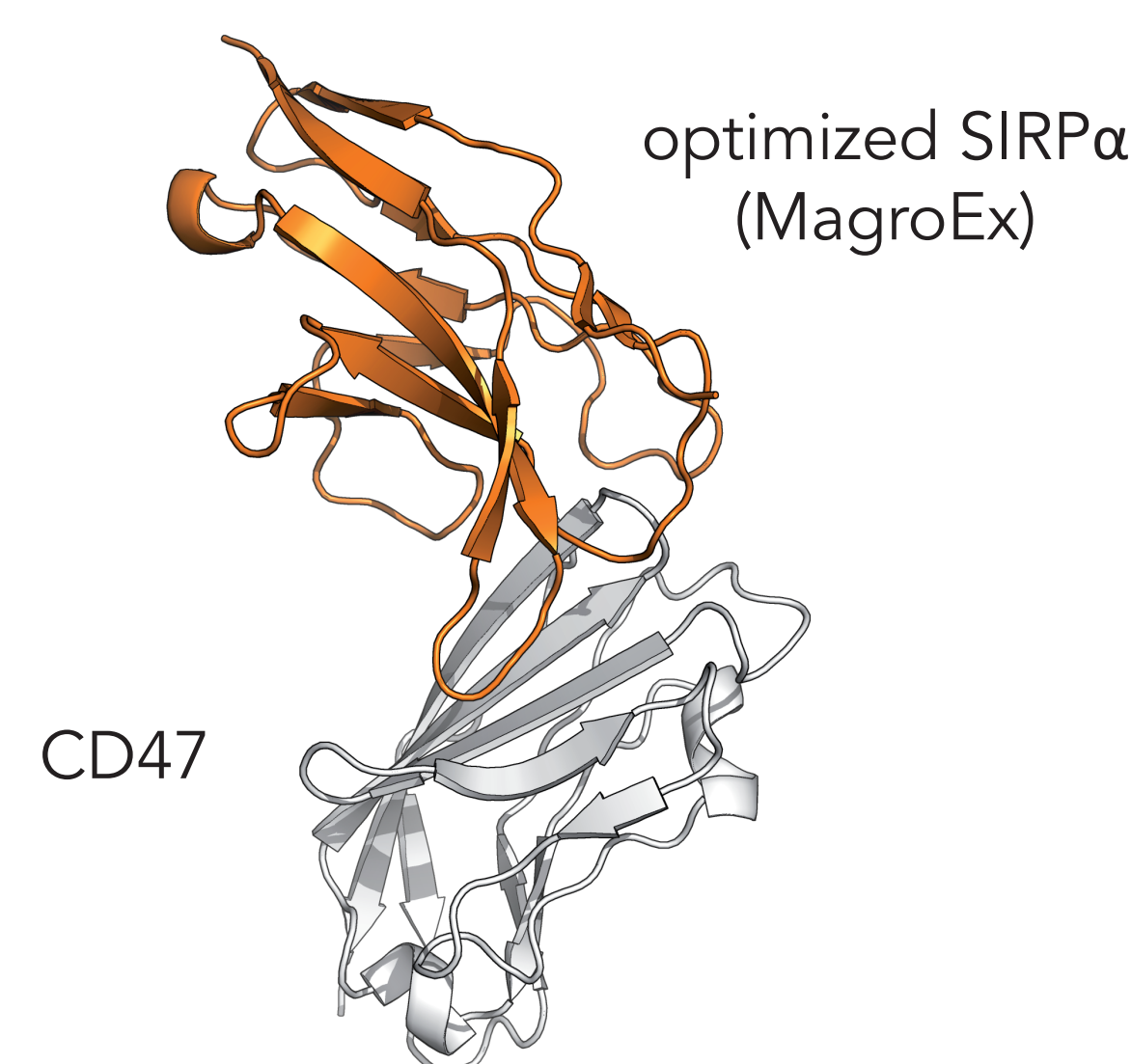


Figure 1. Principle of Antigen Masking with SIRP α

MagroEx, an optimized soluble SIRP α variant, here shown in orange, binds to the extracellular domain of CD47 (white). This masks CD47 on the surface of the red blood cells (RBC) and prevents anti-CD47 drugs from binding.

Results

With the exception of the SIRP α IgG1 Fc fusion protein ontorpacept, all anti-CD47 drugs tested were able to cross-link the RBCs without addition of anti-human globulin (see Table 2). In the IAT, all of the tested anti-CD47 drugs induced agglutination (see Table 2) and all but ontorpacept resulted in positive reactions in the Capture Assay (Fig. 2). MagroEx completely mitigated the interference in the neutral card and in the Capture Assay, and at least partially mitigated the interference in the IAT by antigen masking (Fig. 3).

Table 2. Minimal Interfering Concentrations

The indicated anti-CD47 drugs were titrated on either Bio-Rad LISS/Coombs or neutral (NaCl) cards. Given are the lowest concentrations of the drugs that still induced agglutination of RBCs and the ratio of these concentrations between the two card types. NI: no interference, N/A: not available

Anti-CD47 Drug	LISS/Coombs Card	Neutral Card	Factor Neutral/LISS
Magrolimab	2.5 - 5 ng/ml	50 ng/ml	~10
Ligufalimab	10 - 20 ng/ml	4000 - 15000 ng/ml	200 - 1500
Lempzoparlimab	12.5 - 25 ng/ml	250 ng/ml	~10
Letaplimab	5 - 10 ng/ml	50 ng/ml	~10
Ontorpacept	156 - 312 ng/ml	NI	N/A

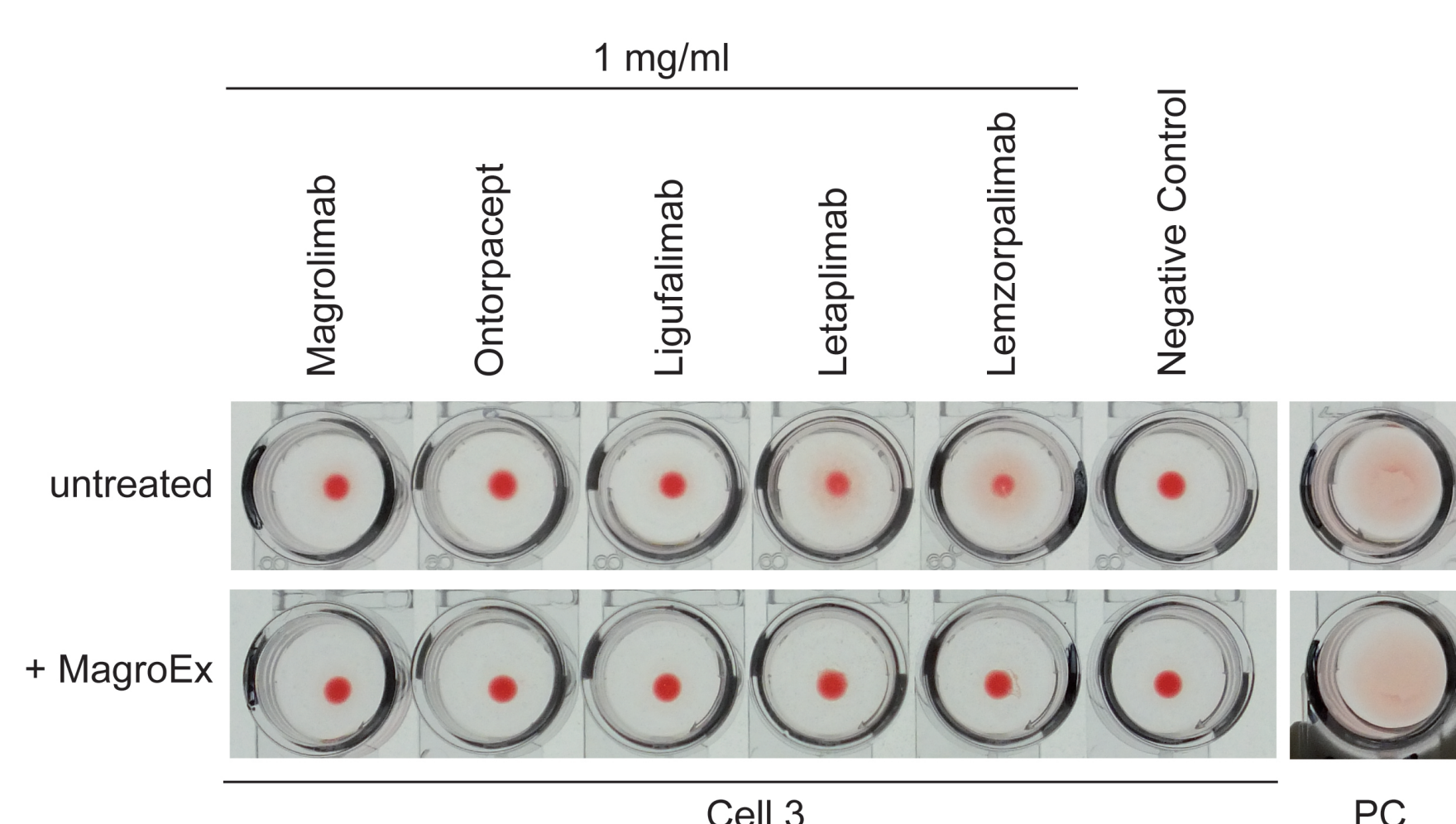


Figure 2. All tested anti-CD47 drugs but ontorpacept interfere with the Capture Assay. Treatment of the plates with MagroEx completely eliminates this interference. EDTA-Plasma was spiked with 1 mg/ml of the indicated anti-CD47 drug candidates.

References

1 Velliquette, R. W., Aeschlimann, J., Kirkegaard, J., Shakarian, G., Lomas-Francis, C., & Westhoff, C. M. (2019). Monoclonal anti-CD47 interference in red cell and platelet testing. *Transfusion*, 59(2), 730–737. <https://doi.org/10.1111/trf.15033>

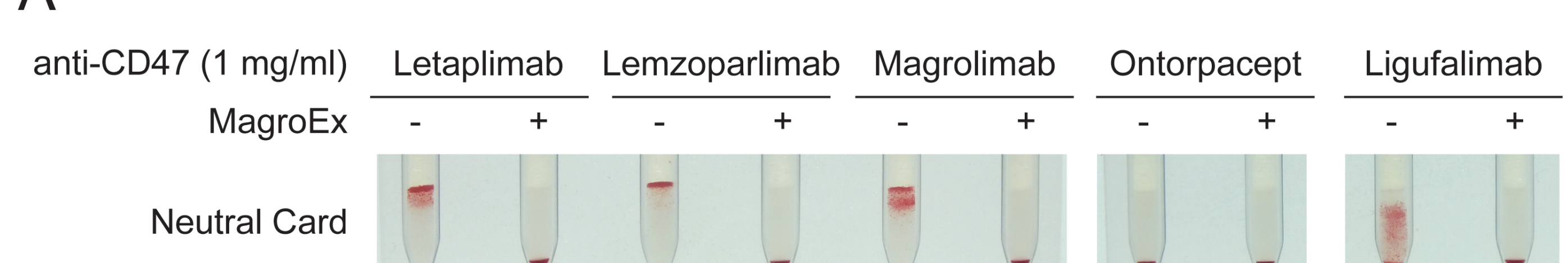
Acknowledgements

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^a imusyn GmbH & Co. KG, Feodor-Lynen-Str. 5, 30625 Hannover, Germany

^b corresponding author: schneeweiss.clemens@imusyn.de

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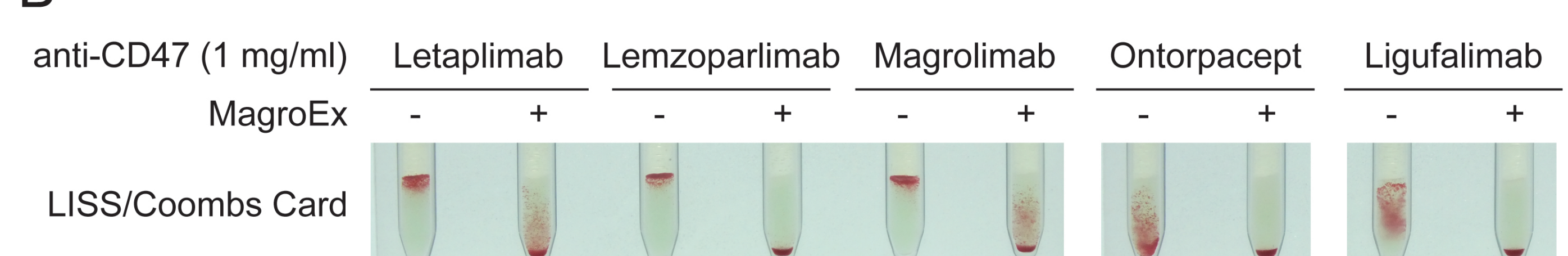


Figure 3. MagroEx eliminates anti-CD47 drug interference completely in (A) the neutral card and partially in (B) the LISS/Coombs card. EDTA-Plasma was spiked with 1 mg/ml of the indicated anti-CD47 drug candidates.

Conclusion

Anti-CD47 drugs will become a major problem in serological testing if they are approved for anti-cancer therapy. Antigen masking with SIRP α has proven to be an effective and easy method to mitigate their interference.

Conflict of Interest

CPH and CS are employees of imusyn GmbH & Co. KG. CS is a designated inventor of the technology presented here.