Cell-based Reporter Bioassays for Development of Fc-functional and Fc-silent SIRPα/CD47 Checkpoint Inhibitors

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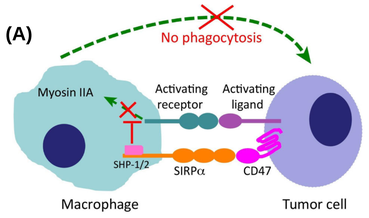
Abstract #3163

1. Introduction



(C)

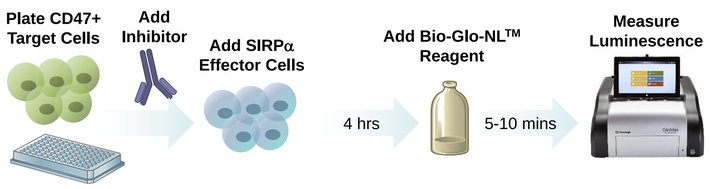
Agent	Structure	Binds to		
		CD47	SIRPα	FcR
Anti-CD47 antibody - intact		+	-	+
Anti-CD47 antibody - F(ab')$_2$		+	-	-
Anti-CD47 antibody – F(ab)		+	-	-
Anti-SIRPα antibody - intact		-	+	+
Anti-SIRPα antibody - F(ab')$_2$		-	+	-
Anti-SIRPα antibody – F(ab)		-	+	-
Soluble SIRPα-Fc		+	-	+
Monomeric SIRPα		+	-	-
Anti-CD47 nanobody		+	-	-
Anti-CD47 single chain variable fragment		+	-	-

SIRPα/CD47 Immune Checkpoint.
(A) CD47 overexpressed on tumor cells engages the myeloid receptor SIRPα and delivers a "don't eat me" signal that inhibits phagocytosis. **(B)** SIRPα/CD47 blockade promotes phagocytosis of tumor cells driven by pro-phagocytic ligands and/or FcγR engagement by Fc-functional antibodies (ADCP). **(C)** SIRPα/CD47 inhibitors vary in molecular structure and mechanism-of-action but can be broadly categorized as Fc-silent vs. Fc-functional.

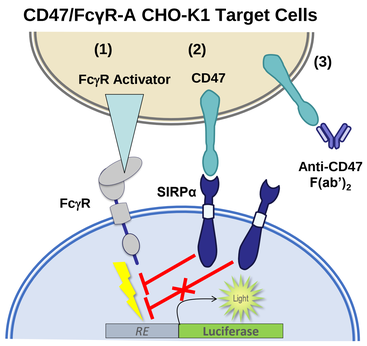
Figures from Veillette & Chen. 2018. Trends Immunol.

2. SIRPα/CD47 Blockade Bioassays: Design & Workflow

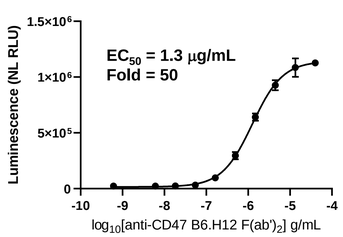
- We have developed a pair of reporter-based bioassays for measuring the activity of *Fc-silent* or *Fc-functional* SIRPα/CD47 inhibitors
- These SIRPα/CD47 Blockade Bioassays follow a simple, add-mix-read format



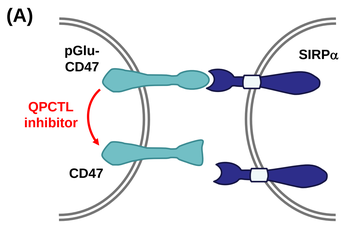
3. SIRPα/CD47 Blockade Bioassay Measures the Potency of Fc-silent SIRPα/CD47 Blocking Antibody

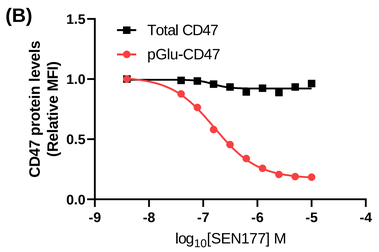


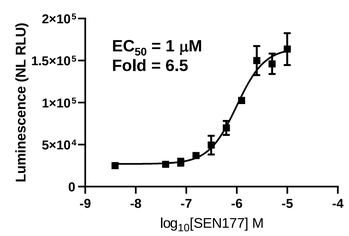
Luciferase activity indicating monocyte activation is:
(1) induced by FcγR-A protein that engages FcγRs
(2) inhibited by co-engagement of SIRPα with CD47
(3) restored by CD47 blockade using anti-CD47 F(ab´)$_2$ fragment of blocking clone B6.H12



4. SIRPα/CD47 Blockade Bioassay Measures the Activity of Small Molecule SIRPα/CD47 Inhibitors



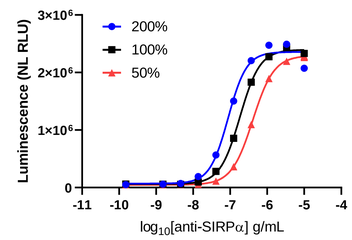




(A) SIRPα/CD47 interaction requires CD47 modification (pGlu-CD47) by glutaminyl-peptide cyclotransferase-like protein (QPCTL). **(B and C)** Pre-treatment of CD47/FcγR-A CHO-K1 Target Cells with QPCTL inhibitor SEN177 results in: **(B)** specific loss of pGlu-CD47 and **(C)** recovery of FcγR-mediated luciferase activity in the SIRPα/CD47 Blockade Bioassay.

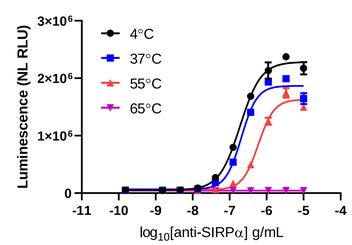
5. SIRPα/CD47 Blockade Bioassay can Measure Relative Potency and is Stability Indicating

(A) Relative potency of anti-SIRPα blocking Ab



Expected Relative Potency	Measured Relative Potency
50%	45.1%
200%	208%

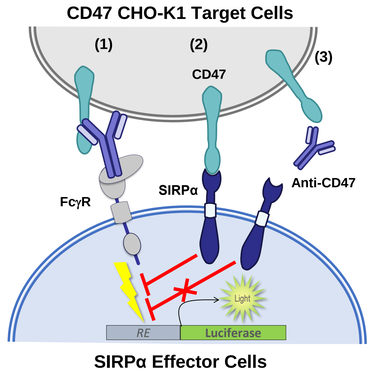
(B) Stability of anti-SIRPα blocking Ab



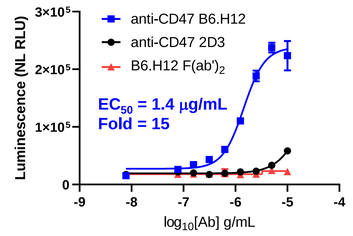
Temperature	EC_{50} (µg/mL)
4°C	0.19
37°C	0.21
55°C	0.59
65°C	No assay response

SIRPα/CD47 Blockade Bioassays were performed using anti-SIRPα blocking Ab (clone SE5A5): **(A)** Simulated potency series of anti-SIRPα Ab and measured relative potency compared to 100% reference. **(B)** Anti-SIRPα Ab was incubated at the indicated temperatures for 24 h prior to analysis in the SIRPα/CD47 Blockade Bioassay.

6. SIRPα/CD47 Blockade Bioassay, Fc-dependent Measures Potency of Fc-functional CD47 Blocking Abs

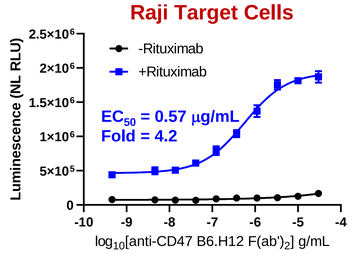


Luciferase activity (i.e. monocyte activation) is:
(1) induced via FcγR engagement by Fc-functional anti-CD47 Abs
(2) inhibited by SIRPα/CD47 co-engagement
(3) enhanced when anti-CD47 has blocking function



FcγR-mediated luciferase activity is observed in the SIRPα/CD47 Blockade Bioassay, Fc-dependent with full-length anti-CD47 blocking Ab (clone B6.H12, mouse IgG1 isotype), but not with B6.H12 F(ab')$_2$ fragment or a non-blocking anti-CD47 Ab (clone 2D3, mouse IgG1 isotype).

7. SIRPα/CD47 Blockade Bioassays Enable Testing of Drug Combinations using CD47+ Cancer Cells



SIRPα/CD47 Blockade Bioassay, Fc-dependent was performed using SIRPα Effector Cells and Raji target cells (human B-cell lymphoma, CD47+/CD20+). Anti-CD47 F(ab')$_2$ fragment was added at increasing concentrations in the presence or absence of anti-CD20 Ab (rituximab, EC_{100}). As expected, the anti-CD47 F(ab')$_2$ fragment enhanced rituximab-mediated luciferase activity. No response was observed with anti-CD47 F(ab')$_2$ fragment alone.

8. Conclusions

We have developed a pair of cell-based reporter gene assays for measuring biological activity of diverse SIRPα/CD47 inhibitors:

1. **SIRPα/CD47 Blockade Bioassay**
   - Suitable for Fc-silent CD47 blocking Abs, SIRPα blocking Abs, and small molecule inhibitors
2. **SIRPα/CD47 Blockade Bioassay, Fc-dependent**
   - Suitable for Fc-functional CD47 blocking Abs, drug combinations, and bispecific Abs

SIRPα/CD47 Blockade Bioassays offer a simple, high-throughput platform for drug development, lot release, and stability studies.

SIRPα/CD47 Blockade Bioassays can be performed using fresh cell cultures or thaw-and-use cells that eliminate the need for cell propagation.

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