

# Suppressing or Enhancing Macrophage Engulfment through the Use of CD47 and Related Peptides

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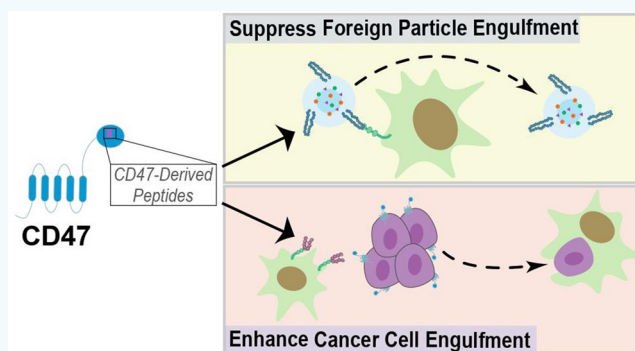
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**ABSTRACT:** Foreign particles and microbes are rapidly cleared by macrophages *in vivo*, although many key aspects of uptake mechanisms remain unclear. “Self” cells express CD47 which functions as an anti-phagocytic ligand for SIRP $\alpha$  on macrophages, particularly when pro-phagocytic ligands such as antibodies are displayed in parallel. Here, we review CD47 and related “Self” peptides as modulators of macrophage uptake. Nanoparticles conjugated with either CD47 or peptides derived from its SIRP $\alpha$  binding site can suppress phagocytic uptake by macrophages *in vitro* and *in vivo*, with similar findings for CD47-displaying viruses. Drugs, dyes, and genes as payloads thus show increased delivery to targeted cells. On the other hand, CD47 expression by cancer cells enables such cells to evade macrophages and immune surveillance. This has motivated development of soluble antagonists to CD47–SIRP $\alpha$ , ranging from blocking antibodies in the clinic to synthetic peptides in preclinical models. CD47 and peptides are thus emerging as dual-use phagocytosis modulators against diseases.



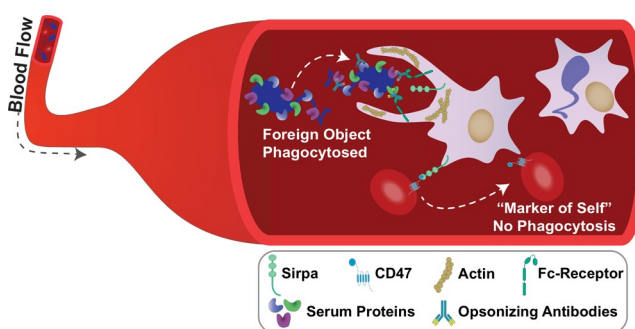
## INTRODUCTION

Phagocytosis is an ancient and basic cellular process that refers to the devouring of a target. Bacteria and fungi are targets of phagocytosis by amoebae and require little to no discrimination. However, in animals, phagocytes such as macrophages must identify, attack, and preferentially engulf “foreign” targets while avoiding healthy “Self” cells. These innate immune phagocytes are the host’s primary line of defense against various invading microbes, both large and small. Phagocytosis is stimulated by “eat me” signals which initiate actin cytoskeleton remodeling that drives macrophage protrusions to envelop—and subsequently internalize and destroy—a “foreign” target. Relevant factors range from highly specific biomolecular interactors (protein–protein) to less specific surface effectors (charge, adsorbed species, ligand patterns) and physiochemical features (rigidity, shape). Opposing some of these “eat me” pathways are “don’t eat me” signaling molecules that can inhibit macrophage uptake. This brief review focuses on some of the latest advances in modulating macrophage elimination of nanoparticles, viruses, and cancer cells through manipulation of the specific “don’t eat me” CD47–SIRP $\alpha$  axis.

## MACROPHAGE CHECKPOINT, CD47–SIRP $\alpha$

The “Marker of Self” protein, CD47, is a ubiquitously expressed, integral membrane protein that interacts with the macrophage receptor SIRP $\alpha$  to inhibit phagocytic uptake

(Figure 1).<sup>1–3</sup> While an interaction between CD24 and Siglec-10 is potentially another macrophage checkpoint,<sup>4</sup> the CD47–SIRP $\alpha$  interaction is more thoroughly characterized and



**Figure 1.** “Marker of Self” CD47 inhibits phagocytosis by macrophages. Serum proteins, such as IgG in blood, adsorb to “foreign” particles or bind specifically, stimulating phagocytosis. Such uptake is inhibited if SIRP $\alpha$  binds its ligand CD47 that is presented on “Self” cells, including red blood cells.

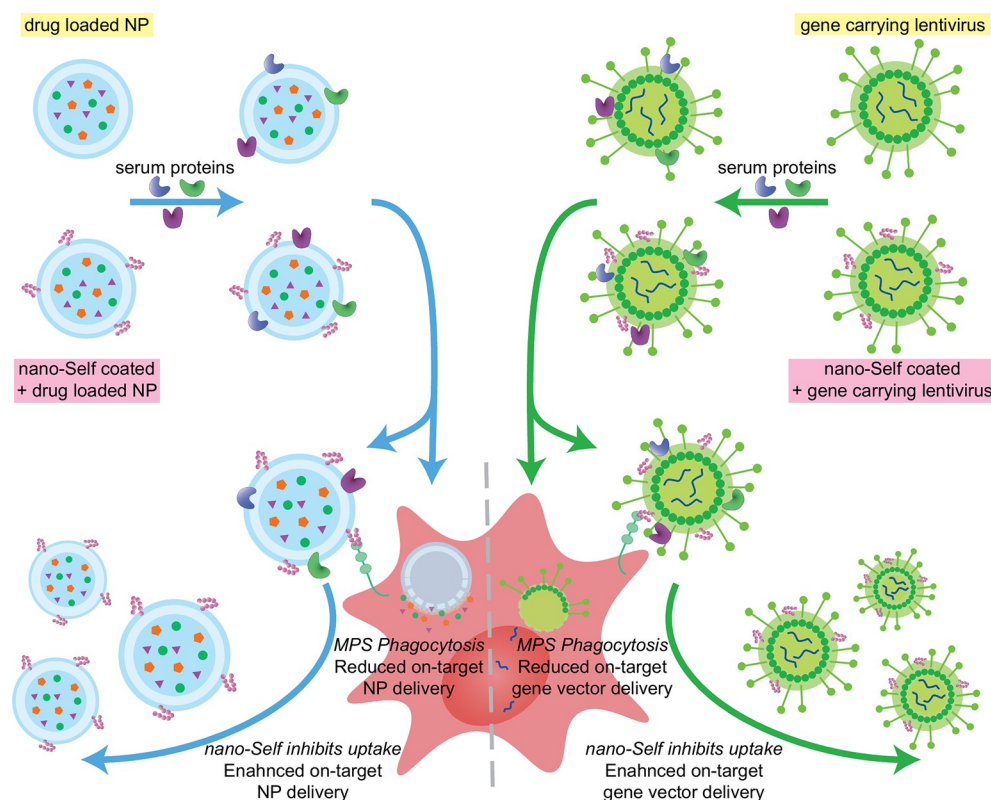
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**Figure 2.** CD47-peptides prolong circulation and increase on-target deliver. Drug-loaded nanoparticles (left) and gene-carrying lentivirus (right) have limited efficacy because of phagocytic uptake. CD47 and related “Self” peptides on nanoparticles and viruses bind SIRP $\alpha$  on macrophages and help recognize the object as “Self”, leading to prolonged circulation and enhanced on-target delivery.

conserved across many higher animals. Inhibition of macrophage uptake involves phosphorylation of SIRP $\alpha$ 's cytoplasmic immunoreceptor tyrosine-based inhibitory motif (ITIM) and activating phosphatases SHP-1 and SHP-2.<sup>5–7</sup> The binding interaction between CD47 and SIRP $\alpha$  tends to be species- and even strain-specific,<sup>8,9</sup> with some notable cross-interactions such as human CD47 binding to NOD mouse and pig SIRP $\alpha$ , and pig CD47 binding to human SIRP $\alpha$ .<sup>9–11</sup> Thus, the inhibitory effect of this receptor–ligand interaction is determined by protein sequence and structure.<sup>12</sup>

The high efficiency by which macrophages remove foreign objects circulating in the body often hinders nanoparticle-based drug delivery, with uptake by pervasive macrophages frustrating delivery to intended targets such as cancer cells. This has led to the idea of using CD47 to make solid particles and viruses more tolerable and increase on-target delivery of nanomedicines and genes. Complementary to such efforts has been the goal of antagonizing the CD47–SIRP $\alpha$  axis to enhance phagocytosis, with antibody-based disruption rapidly emerging as a clinically relevant addition to cancer immunotherapy and new possibilities with small, inhibitory peptide designs.

### ■ NANOPARTICLES DISPLAYING CD47 AND “SELF” PEPTIDE DELAY CLEARANCE TO ENHANCE DELIVERY

Intravenous administration of nanoparticles has the potential advantage of circulating through every tissue and disease site. Unfortunately, such injected nanoparticles are typically cleared in minutes to hours by the mononuclear phagocyte system (MPS), particularly macrophages in the liver and spleen.<sup>13–15</sup> For comparison, fresh red blood cells (RBCs) can circulate for

weeks or longer after infusion but are then also cleared by macrophages, particularly in the spleen.<sup>1</sup> The mechanism(s) by which a macrophage identifies and eliminates a nanoparticle remain unclear. It is known that blood serum proteins physisorb and accumulate on all surfaces to form a “protein corona” that can engage phagocytic receptors—the most notable of which is immunoglobulin-G (IgG) that can bind and activate macrophage Fc-receptors (FcRs).<sup>16–18</sup> The process is often referred to as opsonization and is representative of what has long been described for biomaterials: clean chemistry is invariably “fouled” *in vivo*. PEGylation is a classic approach to prolong nanoparticle circulation and tends to delay protein physisorption to surfaces, but clearance is only delayed.<sup>19–22</sup> Opsonization leads to macrophage interactions, but interactions of cells with materials are further modulated by physical properties, such as rigidity, size, and curvature (shape), which have all proven to be factors that influence nanoparticle clearance by macrophages.<sup>23,24</sup> The limitations of a short circulation half-life of nanoparticles due to macrophage uptake provide an opportunity to modify them in order to make them more like “Self” (Figure 2).

Conjugation of CD47's extracellular domain of ~100 amino acids (via biotinylation) to avidin-coated, cell-sized, polystyrene beads proved sufficient to inhibit the engulfment of the beads when opsonized with anti-avidin IgG.<sup>6</sup> Importantly, CD47 had no effect on beads that lacked IgG. The microbead results encouraged nanobead studies and also motivated synthesis of a related 21-amino-acid “Self” peptide.<sup>25</sup> *In vivo* tests showed CD47 and the “Self” peptide both increased circulation half-life of nanobeads in mice by delaying splenic

macrophage clearance, greatly enhancing tumor imaging and drug delivery to the tumor.<sup>25</sup> Subsequent studies by a separate lab attached the “Self” peptide to nanosheets of graphene oxide, reporting similar results that concluded that the “Self” peptide is more effective than PEGylation.<sup>26</sup> Other laboratories have also shown that functionalizing nanomaterials with recombinant CD47 or “Self” peptides typically prolonged circulation, suppressed clearance, and improved therapeutic responses.<sup>27–30</sup> One additional application attached the “Self” peptide to nanoliposomes and found that they saturate and passivate liver macrophages, unlike control nanoliposomes, thus increasing the circulation and efficacy of subsequently injected nanoparticles.<sup>31</sup> Whether the deposition of serum-opsonizing IgG on these diverse nanoparticles has a role in the results is generally unclear. Nonetheless, the various studies highlight the utility of conjugating CD47 or shorter “Self” peptides to a diverse range of nanomaterials for many types of applications.

### ■ “SELF” ON VIRUSES SUPPRESSES PHAGOCYTOSIS AND ENHANCES GENE DELIVERY

Virus-based gene delivery is in wide clinical use for vaccines (such as Spike protein of SARS-CoV2) and for *ex vivo* engineering of cells (such as CAR T cells). Lentiviral and adeno-associated viral vectors are the most common in efforts to deliver genes to targets intravenously,<sup>32</sup> but macrophages are again stimulated to eliminate these “natural nanoparticles”, possibly resulting in virally induced inflammatory reactions.<sup>33–35</sup> Many groups have tried to suppress MPS-mediated elimination of viruses by conjugating synthetic polymers to minimize opsonization; however, such modifications sterically hinder critical protein interactions for virus binding to the desired target.<sup>36,37</sup>

Lentivirus is generally harvested after exocytosis from a cell line, and so overexpression of the membrane protein CD47 by a suitably engineered cell line can in principle generate CD47 displaying Lentivirus. Two separate studies have indeed generated CD47-Lenti and shown reduced macrophage interactions and improved delivery of genes. The first study delivered red fluorescent protein (RFP) with control or CD47-Lenti to differentiated human macrophage cultures and showed the following: (1) transduction by CD47-Lenti was ~3-fold lower than the control, and (2) SIRP $\alpha$ -expressing A549 lung adenocarcinoma cells were preferentially transduced by CD47-Lenti.<sup>38</sup> The latter suggests that SIRP $\alpha$  serves as a docking receptor for CD47-mediated attachment and infection. Similar results were observed *in vivo*: transgene expression was higher in A549 tumors with CD47-Lenti, while liver and spleen macrophages showed significantly decreased expression relative to controls. Additional experiments, such as antibody-based inhibition of SIRP $\alpha$  interactions, were conducted to validate specificity. A second study used human-CD47 to increase the efficiency of the liver gene transfer by lentivirus.<sup>39</sup> After determining that liver macrophages clear intravenously administered lentivirus, CD47-Lenti increased gene transfer to hepatocytes and decreased transfer to macrophages. The assays were done in both NOD mice, which express SIRP $\alpha$  that binds to human-CD47, and C57BL/6 mice with weaker affinity. Clearance of CD47-Lenti proved greater in the C57BL/6 mice. Safety and efficacy were further demonstrated in nonhuman primates that have higher sequence homology of CD47 and SIRP $\alpha$  to humans. These studies show that displaying CD47 protects a membrane-

enveloped virus such as lentivirus from macrophage elimination, thereby enhancing the efficacy of gene transfer therapies.

Similar to lentivirus, display of the “Self” peptide on the clinically relevant adeno-associated virus vector (AAV) resulted in reduced phagocytic susceptibility of the AAV *in vitro*.<sup>40</sup> Because AAVs do not have a membrane envelope, the “Self” peptide was directly introduced into an AAV2 capsid protein and flanked by glycine-serine linkers to ensure capsid stability and minimize viral titer loss. Such insertion had little to no impact on transduction efficiency but reduced virus uptake up to 10-fold in human macrophages when compared to control AAV2. This difference was again lost upon blocking with anti-SIRP $\alpha$  antibody. AAV is only 20 nm in size, whereas lentivirus is ~100 nm, and since CD47-SIRP $\alpha$  is a specific inhibitor of phagocytic uptake and not endocytosis, the results to date with CD47-conjugated viruses underscore the high efficiency of macrophage phagocytosis of nanoparticles.

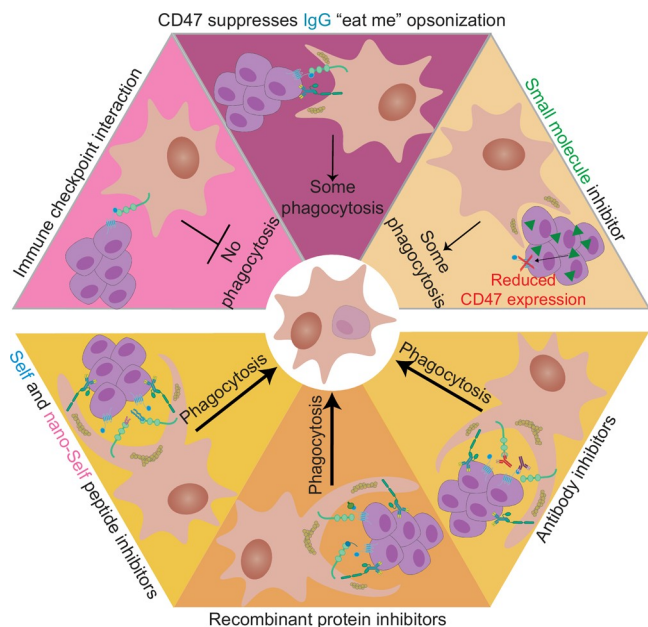
Phagocytosis is often cited in the cell biology literature as relevant only for larger entities (particles, apoptotic cells, or microbes), but early experiments with particles of widely different sizes did not adequately consider particle buoyancy differences and other size effects (per discussion elsewhere<sup>25</sup>). If few small particles settle, then few small particles will be taken up. However, buoyancy is unimportant *in vivo*. Macrophages in the liver and spleen are prominent in the above studies with nanoparticles and viruses because macrophages line the blood vessels in these tissues, facilitating direct and immediate access to intravenously injected particles. Macrophages are nonetheless resident in all tissues and are commonly a major cell presence at disease sites such as tumors or at sites of puncture or injury.<sup>41</sup> Uptake pathways are also important to end points: for example, phagosomes are more oxidative and destructive to cargoes than endosomes. All of these factors have implications for the billions of viral doses being injected as vaccines, such as the DNA delivered by adenovirus in the SARS-CoV2 vaccines from Johnson & Johnson or Oxford AstraZeneca.

### ■ SOLUBLE ANTAGONISTS OF CD47-SIRP $\alpha$ ENHANCE PHAGOCYTOSIS

CD47 is ubiquitously expressed, but not only overexpression of CD47 on ovarian cancer was documented many decades ago, but antibody targeting for tumor imaging before CD47 was also sequenced and eventually shown to inhibit phagocytosis.<sup>42</sup> Antibody targeting of CD47 on other cancers subsequently followed with evidence of a therapeutic window for human tumor xenografts, although it was initially unclear whether the IgG inhibited phagocytosis and/or activated FcR-driven phagocytosis.<sup>43–45</sup> Moreover, the single study<sup>45</sup> of syngeneic mouse tumors in mice treated with anti-mouse CD47 was the subject of a focused reproducibility study that showed no hint of any antitumor effect with anti-CD47 but did show anemia.<sup>46</sup> This latter, negative result for *monotherapy* has largely translated to anticancer efforts in the clinic and seems consistent with the fact that CD47-knockout mice are nearly normal, with minimal defects, and no measurable anemia.<sup>1</sup> This latter observation by an immunology lab raised considerable doubt among hematologists about CD47’s claimed “Marker of Self” function.

Antagonizing the CD47-SIRP $\alpha$  macrophage checkpoint in combination with an “eat me” signal is promising, in contrast to monotherapy, and has prompted an explosion of work on

soluble antagonists. These range from various IgG designs and recombinant proteins in the clinic to small peptides, all of which serve as possible drugs with varying efficacies against numerous liquid and solid malignancies (Figure 3).<sup>12,47–49</sup>



**Figure 3.** Soluble antagonists of SIRP $\alpha$ -CD47 for immunotherapy. Tumor cells express macrophage checkpoint CD47, which inhibits phagocytosis. IgG opsonization alone is insufficient to prompt efficient phagocytosis due to the CD47 “don’t eat” signal, but various strategies can antagonize this inhibition. At least three immunotherapeutic strategies are currently pursued in preclinical and clinical studies: antibodies against CD47 or SIRP $\alpha$ , soluble versions of these proteins as inhibitors, and related “Self” peptide antagonists. Small molecules (green triangles) might eventually be developed to suppress transcription of CD47 but would still require an “eat me” signal.

The most advanced anti-CD47 treatment is a humanized IgG4 monoclonal antibody named magrolimab (or Hu5f9-G4) that binds CD47 and inhibits its binding to SIRP $\alpha$  without soliciting macrophage activation due to weak IgG4 affinity for macrophage FcRs.<sup>50–52</sup> Nonetheless, expression of CD47 on virtually every cell in the body constitutes an “antigen sink” with indiscriminate binding of magrolimab and other CD47-targeting inhibitors resulting in unavoidable on-target binding toxicities, such as anemia and thrombocytopenia.<sup>53,54</sup> Ongoing efforts to address this safety concern include development of nanobodies with strong CD47 binding and antitumor activity but low affinity for human RBCs.<sup>55</sup>

Targeting the SIRP $\alpha$  receptor might prove safer, as its expression is more restricted, although SIRP $\alpha$  expression extends beyond myeloid lineages to cells such as epithelial cells.<sup>56,57</sup> Some studies have indeed suggested that the anti-SIRP $\alpha$  blockade is as effective as anti-CD47 but maintains safe hematological profiles.<sup>58,59</sup> An engineered macrophage approach further demonstrated that a blockade of SIRP $\alpha$  coupled with priming of FcRs with tumor targeting IgG’s is efficacious in shrinking established tumors.<sup>41</sup>

Multivalent 8-amino-acid “nano-Self” antagonists have recently been made based on CD47’s  $\beta$ -hairpin loop that binds SIRP $\alpha$ . Variants of the peptides potently blocked CD47-SIRP $\alpha$  interactions and increased internalization of antibody-opsonized human erythroleukemia cells by human macro-

phages at concentrations as low as 5 nM.<sup>60</sup> Additional observations in the same study included further evidence for CD47 on the macrophage interacting in *cis* with SIRP $\alpha$  on the same cell, conveying an autoinhibition signal in agreement with earlier observations.<sup>61</sup> However, not all studies with soluble CD47 polypeptides added to cultured macrophages have shown increased phagocytosis. Curiously, an early investigation with bacterially expressed human-CD47 protein reported that soluble CD47 reduced *in vitro* phagocytosis by mouse macrophages of colloidal emulsions (for 2 h).<sup>62</sup> Subsequent studies by other groups have shown that the CD47 interaction improves with a post-translational N-terminal modification that is lacking in bacteria,<sup>47</sup> that the particular human–mouse CD47-SIRP $\alpha$  interactions are especially weak, and that IgG opsonization of the target is likely needed to reveal the effect of CD47-SIRP $\alpha$  blockade. All of these remain important considerations for the field going forward.

## CONCLUSIONS

The SIRP $\alpha$ -CD47 axis is an increasingly appealing candidate for a diverse range of delivery and therapy applications. Nanoparticles and viruses that display CD47 or related peptides are recognized as “Self” by macrophages, delaying phagocytosis of these particles, prolonging circulation, and increasing on-target delivery of dyes, drugs, and genes. Further studies are needed to understand pro-phagocytic signals (i.e., opsonization and protein corona formation) on these nanoparticles and viruses. Soluble antagonists of this axis continue to be developed and explored to enhance phagocytosis, particularly of cancer cells, and illustrate the dual uses of developments in this area of research. Challenges remain in limiting off-target effects after systemic injection of antagonists such as anti-CD47 IgG. At least one recent and interesting effort used nanoparticles to both block CD47 and opsonize cancer cells,<sup>63</sup> but of course this requires nanoparticle avoidance of macrophages as well as access to tumor cells. Small size helps with permeation into solid tumors, and a recently synthesized and compact, cyclic version of “nano-Self” was shown to enhance engulfment *in vitro* of mAb-targeted melanoma by primary macrophages, setting the stage for efficacy tests *in vivo*.<sup>64</sup>

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### Notes

The authors declare no competing financial interest.

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## REFERENCES

- (1) Oldenborg, P.; Zheleznyak, A.; Fang, Y.; Lagenaur, C.; Gresham, H.; Lindberg, F. Role of CD47 as a Marker of Self on Red Blood Cells. *Science* **2000**, *288*, 2051–2054.
- (2) Hatherley, D.; Graham, S. C.; Turner, J.; Harlos, K.; Stuart, D. I.; Barclay, A. N. Paired Receptor Specificity Explained by Structures of Signal Regulatory Proteins Alone and Complexed With CD47. *Mol. Cell* **2008**, *31*, 266–277.
- (3) Barclay, A. N. Signal Regulatory Protein Alpha (SIRP $\alpha$ )/CD47 Interaction and Function. *Curr. Opin. Immunol.* **2009**, *21*, 47–52.
- (4) Barkal, A. A.; Brewer, R. E.; Markovic, M.; Kowarsky, M.; Barkal, S. A.; Zaro, B. W.; Krishnan, V.; Hatakeyama, J.; Dorigo, O.; Barkal, L. J.; et al. CD24 Signaling through Macrophage Siglec-10 is a Target for Cancer Immunotherapy. *Nature* **2019**, *572*, 392–396.
- (5) Fujioka, Y.; Matozaki, T.; Noguchi, T.; Iwamatsu, A.; Yamao, T.; Takahashi, N.; Tsuda, M.; Takada, T.; Kasuga, M. A Novel Membrane Glycoprotein, SHPS-1, That Binds the SH2-Domain-Containing Protein Tyrosine Phosphatase SHP-2 in Response to Mitogens and Cell Adhesion. *Mol. Cell. Biol.* **1996**, *16*, 6887–6899.
- (6) Tsai, R. K.; Discher, D. E. Inhibition of “Self” Engulfment Through Deactivation of Myosin-II at the Phagocytic Synapse Between Human Cells. *J. Cell Biol.* **2008**, *180*, 989–1003.
- (7) Johansen, M. L.; Brown, E. J. Dual Regulation of SIRP Alpha Phosphorylation by Integrins and CD47. *J. Biol. Chem.* **2007**, *282*, 24219–24230.
- (8) Subramanian, S.; Boder, E. T.; Discher, D. E. Phylogenetic Divergence of CD47 Interactions with Human Signal Regulatory Protein Alpha Reveals Locus of Species Specificity - Implications for the Binding Site. *J. Biol. Chem.* **2007**, *282*, 1805–1818.
- (9) Subramanian, S.; Parthasarathy, R.; Sen, S.; Boder, E.; Discher, D. Species- and Cell Type-Specific Interactions between CD47 and Human SIRP Alpha. *Blood* **2006**, *107*, 2548–2556.
- (10) Kwong, L. S.; Brown, M. H.; Barclay, A. N.; Hatherley, D. Signal-Regulatory Protein Alpha from the NOD Mouse Binds Human CD47 with an Exceptionally High Affinity - Implications for Engraftment of Human Cells. *Immunology* **2014**, *143*, 61–67.
- (11) Boettcher, A. N.; Cunnick, J. E.; Powell, E. J.; Egner, T. K.; Charley, S. E.; Loving, C. L.; Tuggle, C. K. Porcine Signal Regulatory Protein Alpha Binds to Human CD47 to Inhibit Phagocytosis: Implications for Human Hematopoietic Stem Cell Transplantation into Severe Combined Immunodeficient Pigs. *Xenotransplantation* **2019**, *26*, e12466.
- (12) Jalil, A. R.; Andrechak, J. C.; Discher, D. E. Macrophage Checkpoint Blockade: Results From Initial Clinical Trials, Binding Analyses, and CD47-SIRP $\alpha$  Structure-Function. *Antibody Ther* **2020**, *3*, 80–94.
- (13) Lunov, O.; Syrovets, T.; Loos, C.; Beil, J.; Delacher, M.; Tron, K.; Nienhaus, G. U.; Musyanovych, A.; Mailänder, V.; Landfester, K.; et al. Differential Uptake of Functionalized Polystyrene Nanoparticles by Human Macrophages and a Monocytic Cell Line. *ACS Nano* **2011**, *5*, 1657–1669.
- (14) Lartigue, L.; Wilhelm, C.; Servais, J.; Factor, C.; Dencausse, A.; Bacri, J.; Luciani, N.; Gazeau, F. Nanomagnetic Sensing of Blood Plasma Protein Interactions with Iron Oxide Nanoparticles: Impact on Macrophage Uptake. *ACS Nano* **2012**, *6*, 2665–2678.
- (15) Gustafson, H. H.; Holt-Casper, D.; Grainger, D. W.; Ghandehari, H. Nanoparticle Uptake: The Phagocyte Problem. *Nano today* **2015**, *10*, 487–510.
- (16) Papini, E.; Tavano, R.; Mancin, F. Opsonins and Dysopsonins of Nanoparticles: Facts, Concepts, and Methodological Guidelines. *Front. Immunol.* **2020**, *11*, 2343.
- (17) Binnemars-Postma, K. A.; ten Hoopen, H. W. M.; Storm, G.; Prakash, J. Differential Uptake of Nanoparticles by Human M1 and M2 Polarized Macrophages: Protein Corona as a Critical Determinant. *Nanomedicine* **2016**, *11*, 2889–2902.
- (18) Walkey, C. D.; Olsen, J. B.; Guo, H.; Emili, A.; Chan, W. C. W. Nanoparticle Size and Surface Chemistry Determine Serum Protein Adsorption and Macrophage Uptake. *J. Am. Chem. Soc.* **2012**, *134*, 2139–2147.
- (19) Harris, J. M.; Chess, R. B. Effect of Pegylation on Pharmaceuticals. *Nat. Rev. Drug Discovery* **2003**, *2*, 214–221.
- (20) Guo, S.; Huang, L. Nanoparticles Escaping RES and Endosome: Challenges for siRNA Delivery for Cancer Therapy. *J. Nanomater.* **2011**, *2011*, 742895.
- (21) Hara, E.; Ueda, M.; Makino, A.; Hara, I.; Ozeki, E.; Kimura, S. Factors Influencing in vivo Disposition of Polymeric Micelles on Multiple Administrations. *ACS Med. Chem. Lett.* **2014**, *5*, 873–877.
- (22) Wang, X.; Ishida, T.; Kiwada, H. Anti-PEG IgM Elicited by Injection of Liposomes is Involved in the Enhanced Blood Clearance of a Subsequent Dose of PEGylated Liposomes. *J. Controlled Release* **2007**, *119*, 236–244.
- (23) Sosale, N. G.; Spinler, K. R.; Alvey, C.; Discher, D. E. Macrophage Engulfment of a Cell or Nanoparticle is Regulated by Unavoidable Opsonization, a Species-Specific Marker of Self CD47, and Target Physical Properties. *Curr. Opin. Immunol.* **2015**, *35*, 107–112.
- (24) Sosale, N. G.; Rouhiparkouhi, T.; Bradshaw, A. M.; Dimova, R.; Lipowsky, R.; Discher, D. E. Cell Rigidity and Shape Override CD47's “Self”-Signaling in Phagocytosis by Hyperactivating Myosin-II. *Blood* **2015**, *125*, 542–552.
- (25) Rodriguez, P. L.; Harada, T.; Christian, D. A.; Pantano, D. A.; Tsai, R. K.; Discher, D. E. Minimal “Self” Peptides That Inhibit Phagocytic Clearance and Enhance Delivery of Nanoparticles. *Science* **2013**, *339*, 971–975.
- (26) Shim, G.; Miao, W.; Ko, S.; Park, G. T.; Kim, J. Y.; Kim, M. G.; Kim, Y. B.; Oh, Y. K. Immune-Camouflaged Graphene Oxide Nanosheets for Negative Regulation of Phagocytosis by Macrophages. *J. Mater. Chem. B* **2017**, *5*, 6666–6675.
- (27) Stachelek, S. J.; Finley, M. J.; Alferiev, I. S.; Wang, F.; Tsai, R. K.; Eckells, E. C.; Tomczyk, N.; Connolly, J. M.; Discher, D. E.; Eckmann, D. M.; et al. The Effect of CD47 Modified Polymer Surfaces on Inflammatory Cell Attachment and Activation. *Biomaterials* **2011**, *32*, 4317–4326.
- (28) Qie, Y.; Yuan, H.; Von Roemeling, C. A.; Chen, Y.; Liu, X.; Shih, K. D.; Knight, J. A.; Tun, H. W.; Wharen, R. E.; Jiang, W. Surface Modification of Nanoparticles Enables Selective Evasion of Phagocytic Clearance by Distinct Macrophage Phenotypes. *Sci. Rep.* **2016**, *6*, 26269.
- (29) Pei, W.; Wan, X.; Shahzad, K. A.; Zhang, L.; Song, S.; Jin, X.; Wang, L.; Zhao, C.; Shen, C. Direct Modulation of Myelin-Autoreactive CD4+ and CD8+ T Cells in EAE Mice by a Tolerogenic Nanoparticle Co-Carrying Myelin Peptide-Loaded Major Histocompatibility Complexes, CD47 and Multiple Regulatory Molecules. *Int. J. Nanomed.* **2018**, *13*, 3731–3750.
- (30) Jiang, Z.; Tian, Y.; Shan, D.; Wang, Y.; Gerhard, E.; Xia, J.; Huang, R.; He, Y.; Li, A.; Tang, J.; et al. pH Protective Y1 Receptor Ligand Functionalized Antiphagocytosis BPLP-WPU Micelles for

Enhanced Tumor Imaging and Therapy with Prolonged Survival Time. *Biomaterials* **2018**, *170*, 70–81.

(31) Tang, Y.; Wang, X.; Li, J.; Nie, Y.; Liao, G.; Yu, Y.; Li, C. Overcoming the Reticuloendothelial System Barrier to Drug Delivery with a “Don’t-Eat-Us” Strategy. *ACS Nano* **2019**, *13*, 13015–13026.

(32) Wilson, R. C.; Gilbert, L. A. The Promise and Challenge of In Vivo Delivery for Genome Therapeutics. *ACS chemical biology* **2018**, *13*, 376–382.

(33) Tao, N.; Gao, G.; Parr, M.; Johnston, J.; Baradet, T.; Wilson, J. M.; Barsoum, J.; Fawell, S. E. Sequestration of Adenoviral Vector by Kupffer Cells Leads to a Nonlinear Dose Response of Transduction in Liver. *Molecular therapy* **2001**, *3*, 28–35.

(34) Gordon, S. Phagocytosis: An Immunobiologic Process. *Immunity* **2016**, *44*, 463–475.

(35) Brown, B. D.; Sitia, G.; Annoni, A.; Hauben, E.; Sergi, L. S.; Zingale, A.; Roncarolo, M. G.; Guidotti, L. G.; Naldini, L. In Vivo Administration of Lentiviral Vectors Triggers a Type I Interferon Response that Restricts Hepatocyte Gene Transfer and Promotes Vector Clearance. *Blood* **2007**, *109*, 2797–2805.

(36) Hong, R. L.; Huang, C. J.; Tseng, Y. L.; Pang, V. F.; Chen, S. T.; Liu, J. J.; Chang, F. H. Direct comparison of Liposomal Doxorubicin with or without Polyethylene Glycol Coating in C-26 Tumor-Bearing Mice: Is Surface Coating with Polyethylene Glycol Beneficial? *Clinical Cancer Res.* **1999**, *5*, 3645–3652.

(37) Hwang, B.; Schaffer, D. V. Engineering a Serum-Resistant and Thermostable Vesicular Stomatitis Virus G Glycoprotein for Pseudotyping Retroviral and Lentiviral Vectors. *Gene therapy* **2013**, *20*, 807–815.

(38) Sosale, N. G.; Ivanovska, I. I.; Tsai, R. K.; Swift, J.; Hsu, J. W.; Alvey, C. M.; Zoltick, P. W.; Discher, D. E. “Marker of Self” CD47 on Lentiviral Vectors Decreases Macrophage-Mediated Clearance and Increases Delivery to SIRPA-Expressing Lung Carcinoma Tumors. *Molecular Therapy-Methods & Clinical Development* **2016**, *3*, 16080.

(39) Milani, M.; Annoni, A.; Moalli, F.; Liu, T.; Cesana, D.; Calabria, A.; Bartolaccini, S.; Biffi, M.; Russo, F.; Visigalli, I.; et al. Phagocytosis-Shielded Lentiviral Vectors Improve Liver Gene Therapy in Nonhuman Primates. *Sci. Transl. Med.* **2019**, *11*, eaav7325.

(40) Robinson, T. M.; Chen, M. Y.; Lam, M. T.; Ykema, M. R.; Suh, J. Display of Self-Peptide on Adeno-Associated Virus Capsid Decreases Phagocytic Uptake in Vitro. *ACS Synth. Biol.* **2020**, *9*, 2246–2251.

(41) Alvey, C. M.; Spinler, K. R.; Irianto, J.; Pfeifer, C. R.; Hayes, B.; Xia, Y.; Cho, S.; Dingal, P. C. P. D.; Hsu, J.; Smith, L.; et al. SIRPα-Inhibited, Marrow-Derived Macrophages Engorge, Accumulate, and Differentiate in Antibody-Targeted Regression of Solid Tumors. *Curr. Biol.* **2017**, *27*, 2065–2077.

(42) Campbell, I.; Freemonth, P.; Foulkes, W.; Trowsdale, J. An Ovarian Tumor-Marker with Homology to Vaccinia Virus Contains an Igv-Like Region and Multiple Transmembrane Domains. *Cancer Res.* **1992**, *52*, 5416–5420.

(43) Majeti, R.; Chao, M. P.; Alizadeh, A. A.; Pang, W. W.; Jaiswal, S.; Gibbs, K. D., Jr.; van Rooijen, N.; Weissman, I. L. CD47 Is an Adverse Prognostic Factor and Therapeutic Antibody Target on Human Acute Myeloid Leukemia Stem. *Cells. Cell* **2009**, *138*, 286–299.

(44) Matlung, H. L.; Szilagyi, K.; Barclay, N. A.; van den Berg, T. K. The CD47-SIRP Alpha Signaling Axis as an Innate Immune Checkpoint in Cancer. *Immunol. Rev.* **2017**, *276*, 145–164.

(45) Willingham, S. B.; Volkmer, J.; Gentles, A. J.; Sahoo, D.; Dalerba, P.; Mitra, S. S.; Wang, J.; Contreras-Trujillo, H.; Martin, R.; Cohen, J. D.; et al. The CD47-Signal Regulatory Protein Alpha (SIRPα) Interaction is a Therapeutic Target for Human Solid Tumors. *Proc. Natl. Acad. Sci. U. S. A.* **2012**, *109*, 6662–6667.

(46) Horrigan, S. K Replication Study: The CD47-Signal Regulatory Protein Alpha (SIRPα) Interaction is a Therapeutic Target for Human Solid Tumors. *eLife* **2017**, *6*, e18173.

(47) Ho, C. C. M.; Guo, N.; Sockolosky, J. T.; Ring, A. M.; Weiskopf, K.; Oezkan, E.; Mori, Y.; Weissman, I. L.; Garcia, K. C. “Velcro” Engineering of High Affinity CD47 Ectodomain as Signal

Regulatory Protein Alpha (SIRP Alpha) Antagonists that Enhance Antibody-Dependent Cellular Phagocytosis. *J. Biol. Chem.* **2015**, *290*, 12650–12663.

(48) Weiskopf, K.; Ring, A. M.; Ho, C. C. M.; Volkmer, J.; Levin, A. M.; Volkmer, A. K.; Oezkan, E.; Fernhoff, N. B.; van de Rijn, M.; Weissman, I. L.; et al. Engineered SIRP Alpha Variants as Immunotherapeutic Adjuvants to Anticancer Antibodies. *Science* **2013**, *341*, 88–91.

(49) Lin, Y.; Yan, X.; Yang, F.; Yang, X.; Jiang, X.; Zhao, X.; Zhu, B.; Liu, L.; Qin, H.; Liang, Y.; et al. Soluble Extracellular Domains of Human SIRP Alpha and CD47 Expressed in Escherichia Coli Enhances the Phagocytosis of Leukemia Cells by Macrophages in Vitro. *Protein Expr. Purif.* **2012**, *85*, 109–116.

(50) Liu, J.; Wang, L.; Zhao, F.; Tseng, S.; Narayanan, C.; Shura, L.; Willingham, S.; Howard, M.; Prohaska, S.; Volkmer, J.; et al. Pre-Clinical Development of a Humanized Anti-CD47 Antibody with Anti-Cancer Therapeutic Potential. *PLoS One* **2015**, *10*, e0137345.

(51) Sikic, B. I.; Lakhani, N.; Patnaik, A.; Shah, S. A.; Chandana, S. R.; Rasco, D.; Colevas, A. D.; O’Rourke, T.; Narayanan, S.; Papadopoulos, K.; et al. First-in-Human, First-in-Class Phase I Trial of the Anti-CD47 Antibody Hu5F9-G4 in Patients with Advanced Cancers. *JCO* **2019**, *37*, 946–953.

(52) Sallman, D.; Asch, A.; Al Malki, M.; Lee, D.; Donnellan, W.; Marcucci, G.; Kambhampati, S.; Daver, N.; Garcia-Manero, G.; Komrokji, R.; et al. The First-in-Class Anti-CD47 Antibody Magrolimab (SF9) in Combination with Azacitidine Is Effective in MDS and AML Patients: Ongoing Phase 1b Results. *Blood* **2019**, *134*, 569–569.

(53) Huang, Y.; Ma, Y.; Gao, P.; Yao, Z. Targeting CD47: The Achievements and Concerns of Current Studies on Cancer Immunotherapy. *Journal of Thoracic Disease* **2017**, *9*, E168–E174.

(54) Zhang, X.; Fan, J.; Ju, D. Insights into CD47/SIRPα Axis-Targeting Tumor Immunotherapy. *Antibody Therapeutics* **2018**, *1*, 37.

(55) Ma, L.; Zhu, M.; Gai, J.; Li, G.; Chang, Q.; Qiao, P.; Cao, L.; Chen, W.; Zhang, S.; Wan, Y. Preclinical Development of a Novel CD47 Nanobody with Less Toxicity and Enhanced Anti-Cancer Therapeutic Potential. *J. Nanobiotechnol.* **2020**, *18*, 12.

(56) Veillette, A.; Thibadeau, E.; Latour, S. High Expression of Inhibitory Receptor SHPS-1 and Its Association with Protein-Tyrosine Phosphatase SHP-1 in Macrophages. *J. Biol. Chem.* **1998**, *273*, 22719–22728.

(57) Seiffert, M.; Cant, C.; Chen, Z.; Rappold, I.; Brugger, W.; Kanz, L.; Brown, E. J.; Ullrich, A.; Bühring, H. Human Signal-Regulatory Protein Is Expressed on Normal, but not on Subsets of Leukemic Myeloid Cells and Mediates Cellular Adhesion Involving Its Counterreceptor CD47. *Blood* **1999**, *94*, 3633–3643.

(58) Yanagita, T.; Murata, Y.; Tanaka, D.; Motegi, S.; Arai, E.; Daniwijaya, E. W.; Hazama, D.; Washio, K.; Saito, Y.; Kotani, T.; et al. Anti-SIRPα Antibodies as a Potential New Tool for Cancer Immunotherapy. *JCI Insight* **2017**, *2*, e89140.

(59) Voets, E.; Paradé, M.; Lutje Hulsik, D.; Spijkers, S.; Janssen, W.; Rens, J.; Reinieren-Beeren, I.; van den Tillart, G.; van Duijnhoven, S.; Driessen, L.; et al. Functional Characterization of the Selective Pan-Allele Anti-SIRPα Antibody ADU-1805 that Blocks the SIRPα-CD47 Innate Immune Checkpoint. *J. Immunotherapy Cancer* **2019**, *7*, 340.

(60) Jalil, A. R.; Hayes, B. H.; Andrechak, J. C.; Xia, Y. T.; Chenoweth, D. M.; Discher, D. E. Multivalent, Soluble Nano-Self Peptides Increase Phagocytosis of Antibody-Opsonized Targets while Suppressing “Self” Signaling. *ACS Nano* **2020**, *14*, 15083–15093.

(61) Hayes, B. H.; Tsai, R. K.; Dooling, L. J.; Kadu, S.; Lee, J. Y.; Pantano, D.; Rodriguez, P. L.; Subramanian, S.; Shin, J.; Discher, D. E. Macrophages Eat More After Disruption of Cis Interactions between CD47 and the Checkpoint Receptor SIRPα. *J. Cell. Sci.* **2020**, *1*.

(62) Hsu, Y.; Acuña, M.; Tahara, S. M.; Peng, C. Reduced Phagocytosis of Colloidal Carriers Using Soluble CD47. *Pharm. Res.* **2003**, *20*, 1539–1542.

(63) Zhang, Y.; Luo, J.; Zhang, J.; Miao, W.; Wu, J.; Huang, H.; Tong, Q.; Shen, S.; Leong, K. W.; Du, J.; et al. Nanoparticle-Enabled

Dual Modulation of Phagocytic Signals to Improve Macrophage-Mediated Cancer Immunotherapy. *Small* **2020**, *16*, 2004240.

(64) Jalil, A. R.; Andrechak, J. C.; Hayes, B. H.; Chenoweth, D. M.; Discher, D. E. Human CD47-Derived Cyclic Peptides Enhance Engulfment of mAb-Targeted Melanoma by Primary Macrophages. *Bioconjug Chem.* **2022**, DOI: 10.1021/acs.bioconjchem.2c00020.

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