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QPCTL is a novel drug target to modify the CD47 immune checkpoint

The 'marker of self' membrane protein CD47 has attracted considerable interest over the last decade as a cancer therapy target, with its inhibition promoting phagocytosis of cancerous cells. At least ten clinical trials (phase I/II) are testing various approaches to block the CD47 'don't eat me' signal. Despite their promise, CD47 inhibitors come with several drawbacks; most notably, destruction of red blood cells and platelets along with the intended target cancer cells. Here at Scenic Biotech, we are using inhibition of the enzyme QPCTL as an alternative route to disrupting CD47. QPCTL is responsible for a post-translational modification of CD47 and QPCTL inhibitors are expected to avoid many of the disadvantages associated with current approaches.

The glycoprotein CD47 is normally expressed on the surface of all cells and acts as a ligand for signal receptor protein α (SIRPα) expressed on the surface of phagocytic cells (e.g., macrophages and dendritic cells, Figure 1). CD47-mediated activation of SIRPα initiates a signal transduction cascade that inhibits phagocytosis. In other words, CD47 acts as a 'marker of self' or a 'don't eat me' signal, preventing the immune system from attacking normal cells.

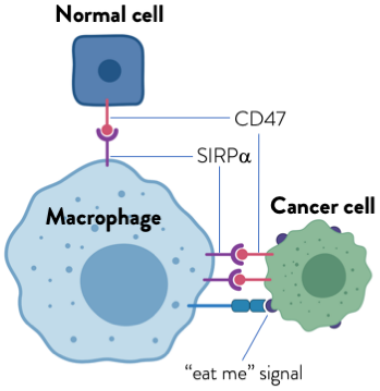

Figure 1. Schematic of the CD47/SIRPα Interaction between normal cells and cancer cells

CD47 overexpression is common in several blood cancers (e.g., acute leukemia) and solid tumors (e.g., colorectal and ovarian cancers), and higher CD47 expression predicts worse clinical outcomes, suggesting that cancer cells use upregulation of this pathway to avoid phagocytosis (Majeti et al, *Cell* 2009; Jaiswal et al, *Cell* 2009). The last decade has, therefore, seen intense interest in identifying ways to block the SIRPα-CD47 interaction and thereby promote phagocytosis of tumor cells. Indeed, not only tumors overexpressing CD47 but also tumors with normal levels of CD47 could potentially be targeted in this way. Blocking SIRPα-CD47 is not expected to induce phagocytosis of normal cells because additional prophagocytic signals are also required, and (in contrast to cancer cells) these are not expressed in normal cells, except in aging red blood cells (RBCs).

Anti-CD47 antibodies have shown promise in preclinical studies, impairing tumor growth, inhibiting metastasis, and leading to tumor regression. This activity is enhanced when combined with cancer targeting antibodies that provide exogenous prophagocytic, or 'eat me', signals (e.g., Chao et al, *Cell* 2010). In addition, phagocytosis of tumor cells promotes processing of tumor antigens and subsequent presentation to T cells, thereby helping to prime cytotoxic T cells. Thus, CD47-targeted therapies may involve both the innate and adaptive immune systems. Currently, several companies have clinical-stage CD47 inhibitors, most of them anti-CD47 antibodies, which are often combined with a second component that creates a tumor-specific 'eat me' signal (e.g., Advani et al, *NEJM* 2018).

However, since almost all cells express CD47, healthy cells act as 'antigen sinks' by soaking up anti-CD47 antibodies, meaning that high doses are needed to target tumors. Apart from obvious practical and economic issues, these high doses, when intravenously injected, suppress the 'don't eat me' signal on blood cells, prompting macrophages to engulf them. Indeed, early

clinical studies have shown dose-limiting hematological toxicity to be a problem, with excessive elimination of (aging) RBCs in particular. Consistent with this, CD47 knockout in mice leads to anemia (RBC loss). Although various strategies might overcome this issue – e.g., using an initial 'priming' dose of anti-CD47 antibodies to clear aging RBCs and stimulate production of new RBCs – it is desirable to find alternative ways of targeting the SIRPα-CD47 axis.

Using the genetics technology that is proprietary to Scenic (i.e., Cell-Seq), Dutch researchers recently identified QPCTL (glutaminyl-peptide cyclotransferase-like protein) as a potential target that may overcome some of the shortcomings of anti-CD47 antibodies (Logtenberg et al, *Nat Med* 2019). QPCTL is a Golgi-resident enzyme that catalyzes the cyclization of N-terminal glutamine and glutamic acid residues on target proteins into a pyroglutamate residue (pGlu). CD47 has an N-terminal pGlu thought to be important for SIRPα binding. Indeed, inhibition or deletion of QPCTL disrupts the SIRPα-CD47 interaction and leads to increased phagocytosis of target cells *in vitro*. Furthermore, interfering with QPCTL expression increases neutrophil-mediated killing of tumor cells in mice.

Targeting QPCTL has several potential advantages over therapies targeting CD47. Importantly, QPCTL inhibitors are not expected to cause hemolysis (destruction of RBCs) or thrombocytopenia (loss of platelets). Already pyroglutamylated CD47 is expected to retain binding to SIRPα in the presence of QPCTL inhibitors and, since RBCs and platelets lack protein synthesis, pGlu-CD47 cannot be replaced by non-pyroglutamylated CD47 in these cells. Consistent with the idea that QPCTL inhibitors are unlikely to have severe adverse side effects, QPCTL knockout mice are viable and healthy (Becker et al, *Biol Chem* 2016). Furthermore, given that QPCTL is an enzyme, QPCTL inhibitors would not suffer from the 'antigen sink' problem of anti-CD47 therapy. Finally, small molecule inhibitors of QPCTL would have advantages over CD47 antibodies in terms of delivery and bioavailability. In particular, this would be beneficial for treating solid tumors.

QPCTL inhibitors may also have applications beyond cancer; for example, atherosclerosis is associated with upregulation of CD47, and anti-CD47 antibodies promote phagocytosis of diseased vascular cells and cellular debris in mouse models of atherosclerosis (Kojima et al, *Nature* 2016). Furthermore, blocking the SIRPα-CD47 axis has a potential use in eliminating host hematopoietic stem cells to enable subsequent donor stem cell engraftment in patients with various diseases of the blood system (Chhabra et al, *Sci Transl Med* 2016). For such an application, QPCTL inhibitors might be advantageous in that they are much shorter-lived than anti-CD47 antibodies, ensuring that the newly engrafted donor stem cells are not also subjected to phagocytosis.

Here at Scenic Biotech, a spin-out company of the Netherlands Cancer Institute and Oxford University, we are capitalizing on our expertise and experience to develop novel QPCTL inhibitors that, either alone or in combination with other therapies, could be used in immune-oncology and beyond.

References

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