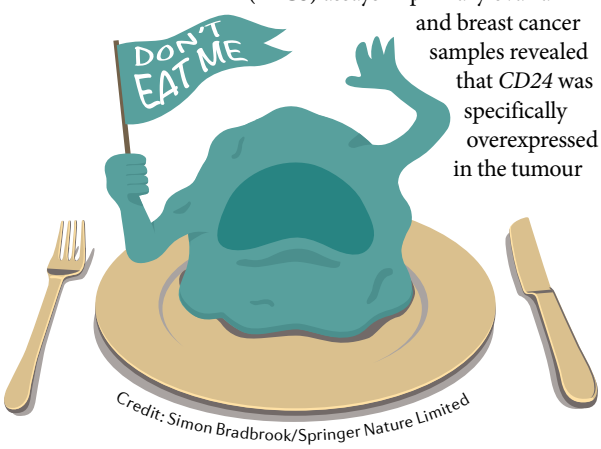
TUMOUR IMMUNOLOGY

CD24 — a novel 'don't eat me' signal

> CD24 is a potent 'don't eat me' signal that modulates the antitumour innate immune response

Emerging data indicate a role for innate immune checkpoints in immune evasion, whereby tumours can escape macrophage-mediated phagocytosis through expression of anti-phagocytic signals. A new study now reports that one such 'don't eat me' signal, CD24, orchestrates a novel innate immune checkpoint through interaction with the inhibitory receptor sialic acid-binding Ig-like lectin 10 (Siglec-10) on tumour-associated macrophages (TAMs).

Using RNA sequencing (RNA-seq) data from the TARGET and TCGA databases, Barkal et al. first observed *CD24* upregulation across almost all tumour types analysed relative to normal tissues, which was particularly strong when compared with expression of known innate immune checkpoints such as *CD47*. Importantly, the largest increases in *CD24* expression were in triple-negative breast cancer (TNBC) and ovarian cancer, and stratification by *CD24* expression revealed improvements in relapse-free survival and overall survival in patients with *CD24*-low ovarian cancer and breast cancer, respectively. Subsequently, single-cell RNA-seq data and fluorescence-activated cell sorting (FACS) assays in primary ovarian and breast cancer samples revealed that *CD24* was specifically overexpressed in the tumour compartment, whereas *SIGLEC10* was expressed in a substantial fraction of TAMs, suggesting the possibility of a CD24–Siglec-10 interaction.



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Next, the role of CD24–Siglec-10 interactions in regulating macrophage-mediated antitumour immune responses was examined. In a co-culture model coupled to a flow-cytometry-based phagocytosis assay, stable genetic ablation of *CD24* (ΔCD24) potentiated the phagocytosis of MCF-7 human breast cancer cells by Siglec-10$^+$ M2-like macrophages. Moreover, live-cell microscopy-based phagocytosis assays illustrated that ΔCD24 MCF-7 cells were more readily engulfed into the phagolysosomes of co-cultured macrophages than wild-type MCF-7 cells. In addition, *SIGLEC10* knockout in donor-derived macrophages markedly exacerbated their phagocytic ability towards wild-type MCF-7 cells, further illustrating that the CD24–Siglec-10 interaction exerts an anti-phagocytic effect.

The therapeutic relevance of the findings was then investigated. Using live-cell microscopy-based phagocytosis assays, anti-CD24 antibody treatment was shown to markedly increase engulfment of wild-type MCF-7 cells into the phagolysosome of co-cultured macrophages. Subsequent FACS-based assays confirmed this enhanced phagocytic response upon CD24 blockade in primary TNBCs and ovarian cancers and cell lines, which, interestingly, was greater than the pro-phagocytic effect seen upon anti-CD47 antibody treatment. Crucially, genetic deletion of *SIGLEC10* in macrophages greatly reduced the pro-phagocytosis response to CD24 blockade, suggesting the anti-CD24 antibody functions primarily by specifically disrupting the CD24–Siglec-10 interaction.

In addition to MCF-7 cells, CD24 expression was positively correlated with the pro-phagocytic response to CD24 blockade across a panel of CD24$^+$ pancreatic adenocarcinoma, pancreatic neuroendocrine tumour and small-cell lung cancer cell lines, and no effect on the phagocytosis was noted in other CD24$^-$ cell lines, indicating that CD24 is a potent 'don't eat me' signal that modulates the antitumour innate immune response.

Finally, to assess the anti-phagocytic role of CD24 in vivo, GFP-luciferase$^+$ MCF-7 cells (wild-type or ΔCD24) were engrafted into NOD SCID gamma (NSG) mice. At 21–28 days, bioluminescence assays revealed a markedly lower tumour burden in the ΔCD24 group than the wild-type group. Importantly, TAM depletion significantly abrogated the aforementioned reduction in tumour burden in mice with ΔCD24, but not wild-type, tumours, suggesting that the antitumour effects were attributable to TAM-mediated phagocytosis. Indeed, mice engrafted with ΔCD24 tumours had a substantial survival advantage over those with wild-type tumours. In support of the therapeutic promise of CD24 blockade, anti-CD24 antibody treatment led to major decreases in tumour growth in mice with established MCF-7 wild-type tumours.

Overall, the findings identify the CD24–Siglec-10 interaction as a novel innate immune checkpoint that modulates antitumour immunity, highlighting the promise of CD24 blockade as an immunotherapy strategy.

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ORIGINAL ARTICLE Barkal, A. A. et al. CD24 signalling through macrophage Siglec-10 is a target for cancer immunotherapy. *Nature* https://doi.org/10.1038/s41586-019-1456-0 (2019)