In situ sprayed bioresponsive immunotherapeutic gel for post-surgical cancer treatment

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Cancer recurrence after surgical resection remains a significant cause of treatment failure. Here, we have developed an in situ formed immunotherapeutic bioresponsive gel that controls both local tumour recurrence after surgery and development of distant tumours. Briefly, calcium carbonate nanoparticles pre-loaded with the anti-CD47 antibody are encapsulated in the fibrin gel and scavenge H+ in the surgical wound, allowing polarization of tumour-associated macrophages to the M1-like phenotype. The released anti-CD47 antibody blocks the ‘don’t eat me’ signal in cancer cells, thereby increasing phagocytosis of cancer cells by macrophages. Macrophages can promote effective antigen presentation and initiate T cell mediated immune responses that control tumour growth. Our findings indicate that the immunotherapeutic fibrin gel ‘awakens’ the host innate and adaptive immune systems to inhibit both local tumour recurrence post surgery and potential metastatic spread.

Despite improvements in surgical techniques, local residual tumour micro-infiltration and circulating tumour cells continue to cause tumour recurrence after resection1–3. Furthermore, perioperative trauma-associated inflammation can promote tumour recurrence by accelerating local growth or tumour spread4–7. Chemotherapy and radiotherapy are often used after surgery to prevent both local and metastatic tumour recurrence, but these therapies often cause toxicities8. More recently, immunotherapy has been considered to inhibit cancer recurrence and metastasis effectively9–13.

Macrophages are cellular components of the innate immunity that phagocytose foreign substances and present them to T lymphocytes (T cells) without requiring specific ‘self-signal’ proteins expressed on the cell surface14–19. However, cancer cells can escape macrophage recognition via upregulation of the integrin-associated protein (IAP)—CD47—which provides the antiphagocytic ‘don’t eat me’ signal20–22. Blocking the interaction of CD47 with its ligand, signal regulatory protein-α (SIRPα), which is expressed on macrophages, dendritic cells and neutrophils23, activates phagocytic cells and promotes their capacity to phagocytize cancer cells. At least three CD47 antagonists are currently being tested in clinical studies, but the occurrence of anaemia and thrombocytopenia caused by systemic administration of these antagonists remains a concern21–24. Efforts to avoid these severe side effects are essential to make CD47-blocking immunotherapies clinically applicable.

Tumour-associated macrophages (TAMs) account for a substantial fraction of tumour-infiltrating immune cells35,36. TAMs differentiate into separate functional phenotypes, denoted classically activated macrophages (the M1-like phenotype) and alternatively activated macrophages (the M2-like phenotype)37. The M1-like TAMs overexpress major histocompatibility complex class I and class II molecules, which play critical roles in antigen presentation. Conversely, M2-like TAMs exert pro-tumorigenic activities28,29 and respond to different tumour determining factors, in particular low pH values30. Infiltration of M2-like TAMs frequently correlates with tumour invasion, metastases, angiogenesis, T cell suppression and poor clinical outcome31–33. Thus, targeting M2-like TAMs could be critical to alter the tumour microenvironment (TME) into a more permissive environment for the function of immune cells.

In this Article we report a sprayed bioresponsive immunotherapeutic fibrin gel that has been engineered to inhibit local tumour recurrence after surgery and development of distant tumours. The fibrin gel is a US Food and Drug Administration approved material, and is formed by the interaction of fibrinogen and thrombin. We leverage the unique merits of fibrin gel here, including excellent biocompatibility, a convenient sprayable administration method for post-surgical treatment, and the ability to promote wound healing by creating a temporary shield to connect and protect injured tissue34. Biocompatible CaCO3 nanoparticles are incorporated into the fibrin gel to serve as a release reservoir of immunomodulatory therapeutics as well as a proton scavenger to modulate the acidity of the tumour environment35,36. The fibrinogen solution containing anti-CD47 antibody-loaded CaCO3 nanoparticles (aCD47@CaCO3) and thrombin solution can be quickly sprayed and mixed within the tumour resection cavity after surgery to form an immunotherapeutic fibrin gel in situ (Fig. 1a). We demonstrate that CaCO3 nanoparticles can gradually dissolve and release the encapsulated aCD47 in the acidic and inflamed TME, thus promoting the activation of M1-type TAMs, inducing macrophage phagocytosis of cancer cells via blockade of the CD47 and SIRPα interaction as well as boosting

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**Fig. 1 | Schematic and characterization of the in situ formed immunotherapeutic fibrin gel.**

**a.** Schematic showing the in situ sprayed bioresponsive fibrin gel containing aCD47@CaCO₃ nanoparticles within the post-surgery tumour bed. aCD47@CaCO₃ nanoparticles encapsulated in fibrin scavenge H⁺ in the surgical wound site and release aCD47, thus promoting both polarization of TAMs to an M1-like phenotype and blockade of the ‘don’t eat me’ signal in cancer cells.

**b.** Transmission electron microscopy (TEM) image of aCD47@CaCO₃ nanoparticles. Scale bar, 100 nm. Experiments were repeated three times; a representative image is shown.

**c.** Representative scanning TEM images of aCD47@CaCO₃ nanoparticles showing the calcium (green), oxygen (red) and gadolinium labelled aCD47 (blue). Experiments were repeated three times.

**d.** Average hydrodynamic size of aCD47@CaCO₃ nanoparticles determined by dynamic light scattering.

**e.** Representative cryo-scanning electron microscope (SEM) images of fibrin gel loaded with aCD47@CaCO₃ nanoparticles. Scale bars, 1 μm. Experiments were repeated three times.

**f.** Representative fluorescent images of a cryosection of fibrin gel, in which fibrinogen was labelled with FITC, CaCO₃ nanoparticles with Cy5.5 (Cy5.5-labelled PEG-b-P(Glu) copolymers), and aCD47 was stained with rhodamine-conjugated anti-rat IgG antibody. Scale bar, 10 μm. Experiments were repeated three times.

**g.** Cumulative release profiles of aCD47 from fibrin in solutions at different pH values. Data are presented as mean ± s.e.m. (n = 3).

**h.** Fluorescence IVIS imaging depicting the in vivo retention of aCD47–Cy5.5, 6 days after delivery of antibodies in different formulations. Experiments were repeated three times.

**i.** Quantification of the in vivo retention profile of aCD47–Cy5.5. FL, fluorescence; a.u., arbitrary unit. Data are presented as mean ± s.e.m. (n = 3). Statistical significance was calculated via one-way analysis of variance (ANOVA) with a Tukey post-hoc test. *P < 0.05.
antitumour T cell responses, while reducing the toxic effects associated with the systemic administration of aCD47.

**In situ formation of fibrin gel by spray**
aCD47@CaCO₃ nanoparticles with a loading capacity of 5% and encapsulation efficiency of 50% were prepared by precipitation of Ca²⁺ and CO₃²⁻ in a solution containing poly(ethylene glycol)-b-poly(glutamic acid) (PEG-b-P(Glu)) block copolymers according to the methods in a previous study³⁷. Monodisperse aCD47@CaCO₃ nanoparticles with a diameter of ~100 nm were obtained, the size of which was controlled by the P(Glu) block through the interaction between carboxyl and Ca²⁺, as well as a PEG shell to avoid Ca²⁺-carbonate precipitation.

**Fig. 2 | Incorporation of CaCO₃@fibrin for relieving immunosuppressive TME.** B16F10 tumours were collected from mice 5 days after treatment. a, b, Representative flow cytometric analysis images (a) and the relative quantification of M2-like macrophages (CD206⁺) and M1-like macrophages (CD80⁺) gating on F4/80⁺CD11b⁺CD45⁺ cells (b). Data are presented as mean ± s.e.m. (n = 4). c, Secretion levels of IL-10 and IL-12p70 in different tumours. Data are presented as mean ± s.e.m. (n = 4). d, Representative flow cytometric analysis images (left) and relative quantification (right) of M2-like macrophages (CD206hi in CD11b⁺) and M1-like macrophages (CD80hi) in tumour tissue. Data are presented as mean ± s.e.m. (n = 4). e, f, Representative flow cytometric analysis images (left) and relative quantification (right) of CD45⁺ cells gating on CD3⁺ (e) and CD4⁺ (f) T cells in the tumour. Data are presented as mean ± s.e.m. (n = 4). g, Representative flow cytometric analysis images (left) and relative quantification (right) of CD4⁺Foxp3⁺ T cells gates on CD3⁺CD4⁺ cells. Data are presented as mean ± s.e.m. (n = 4). h, HIF1-α protein expression levels in B16F10 tumours analysed by western blotting. Experiments were repeated three times. i, Systemic IFN-γ and TNF-α levels before and after CaCO₃@fibrin treatment (n = 4). j, Representative flow cytometric analysis images (left) and absolute quantification (right) of CD8⁺ and CD4⁺ T cells in the tumour. Data are presented as mean ± s.e.m. (n = 4).

Statistical significance was calculated via one-way ANOVA with a Tukey post-hoc test (b, c, d–f, j) or Student’s t-test (b, j). *P < 0.05; **P < 0.01; ***P < 0.001.
Further aggregation and agglomeration (Fig. 1b–d). Elemental mapping images (Fig. 1c) indicated the homogeneous distribution of aCD47 (gadolinium-chelated) in the CaCO3 nanoparticles. Fibrin gel was quickly formed by simultaneously spraying and mixing equal volumes of solutions containing either fibrinogen with size-optimized aCD47@CaCO3 nanoparticles or thrombin, which was validated by a rheology test (Supplementary Fig. 1). Ca2+ associated with CaCO3 nanoparticles in the solution facilitates the formation of fibrin gel. The mixture of the two solutions resulted in a rapid increase in the elastic modulus (G'). The morphology of the fibrin gel containing aCD47@CaCO3 nanoparticles was observed using cryo-scanning electron microscopy (cryo-SEM) imaging (Fig. 1c,f). To further substantiate the distribution of aCD47@CaCO3 nanoparticles in the hydrogel, fluorescein (FITC)-labelled fibrinogen and Cy5.5-labelled CaCO3 nanoparticles were used for hydrogel formation. Frozen sections were stained with rhodamine-conjugated donkey anti-rat IgG antibody to detect the aCD47. As demonstrated by confocal imaging, the Cy5.5 signals from CaCO3 exhibited a uniform distribution pattern in the gel and were co-localized with aCD47, further indicating the encapsulation of aCD47 in the CaCO3 nanoparticles (Fig. 1g). The biodegradation behaviour of the Cy5.5-labelled fibrin gel was monitored using a fluorescence in vivo imaging system (IVIS). Three weeks after spraying, the fluorescence signal...
from the gel was undetectable, indicating degradation of the gel (Supplementary Fig. 2).

The CaCO3 nanoparticles dissolved and released the encapsulated aCD47 by reacting with H+ in the acidic buffer (Supplementary Figs. 3 and 4). Meanwhile, CaCO3 nanoparticles elevated the pH values of the acidic solution as a proton scavenger (Supplementary Fig. 2). Encapsulation of aCD47@CaCO3 nanoparticles in the hydrogel allowed a gradual release of aCD47 (Fig. 1h). To evaluate the release behaviour in vivo, aCD47 was either dispersed in phosphate buffered saline (PBS), encapsulated in CaCO3 nanoparticles or in CaCO3@fibrin gel, and then injected or sprayed into the tumour resection cavity. The Cy5.5-labelled aCD47 signal was monitored in tumour sections, in which a remarkable fluorescence signal associated with the antibodies was observed away from the nanoparticles’ signal, indicating degradation of the CaCO3 nanoparticles and sustained release of antibodies from the nanoparticles (Supplementary Fig. 7). Although the sustained release of therapeutics from the gel–nanoparticle reservoir is advantageous compared with free cargo, the release kinetics for this application could be further optimized to maximize the therapeutic effects and minimize toxicities.

Immune response induced by CaCO3 and aCD47

Glycolytic metabolism of cancer cells in the hypoxic TME leads to the production of lactate and H+ (pH 6.5–6.8), both of which impair the functions of immune cells. Local acidification (pH 6.0–7.0) is also observed in inflamed and injured tissues. Considering the ability of CaCO3 to scavenge H+, we examined the immune effects caused by spreading of the CaCO3@fibrin gel (1 mg CaCO3 per mouse) within the tumour resection cavity. We observed a reduction of M2-like TAMs (CD206hiCD11b+F4/80+) and an increase of M1-like TAMs (CD80hiCD11b+F4/80+) (Fig. 2a,b). This polarization was further confirmed by the reduced level of IL-10 (the predominant cytokine secreted by M2 macrophages) and the increased production of lactate and IL-12.
level of interleukin-12 (IL-12, the predominant cytokine secreted by M1 macrophages) in the TME (Fig. 2c). The observed polarization of macrophages to the M1 phenotype could be ascribed to modulation of the acidity of the TME, which usually favours M2-like macrophage polarization. Compared to small molecules that promote M1-like macrophage polarization, the CaCO3 scavenging of H+ within the inflamed tumour resection provides a simple approach. A correlation was observed between the concentration of CaCO3 nanoparticles and macrophage polarization, as assessed by flow cytometry (Supplementary Fig. 8). After CaCO3@fibrin treatment, reduction of myeloid-derived suppressor cells (MDSCs) and regulatory T cells (Tregs) and reduced expression of hypoxia-inducible factor 1-α (HIF1-α) in the TME were also observed (Fig. 2d–g and Supplementary Fig. 9). Remarkably, CaCO3@fibrin treatment caused an increase in tumour-infiltrating lymphocytes (TILs, CD3+), especially cytotoxic T lymphocytes (CTLs,
Fig. 6 | Local treatment of aCD47@CaCO₃@fibrin for systemic antitumour immune response. a, Schematic illustrating aCD47@CaCO₃@fibrin therapy in a mouse model of incomplete tumour resection and distant tumour. Tumours on the right side were designated as ‘primary tumours’ with aCD47@CaCO₃@fibrin treatment, and those on the left side were designated as ‘distant tumours’ without any treatment. b, In vivo bioluminescence imaging of B16F10 tumours in response to local aCD47@CaCO₃@fibrin treatment. Images associated with day 10 were taken before surgery. Experiments were repeated three times. c, Growth curves for left and right tumours in untreated and treated mice. Data are presented as mean ± s.e.m. (n = 8). d, Representative mouse photographs at day 22. Blue arrows indicate tumours. Experiments were repeated three times. e, Representative flow cytometric analysis images (left) and relative quantification (right) of M1-like macrophages (CD80⁺) gating on F4/80⁺CD11b⁺CD45⁺ cells. Data are presented as mean ± s.e.m. (n = 5). f, Representative flow cytometric analysis images (left) and relative quantification (right) of CD103⁺ dendritic cells gating on CD45⁺CD11c⁺ cells. Data are presented as mean ± s.e.m. (n = 5). g, Representative flow cytometric analysis of T cell infiltration gating on CD3⁺ cells within the tumour (left) and the absolute percentage (right) of CD8⁺ T cells in the tumour in different groups. Data are presented as mean ± s.e.m. (n = 5). Experiments were repeated three times. Statistical significance was calculated via one-way ANOVA with a Tukey post-hoc test. * P < 0.05; ** P < 0.01; *** P < 0.001.
CD3+CD8+), within the tumour (Fig. 2i), as well as an increase in interferon-γ (IFN-γ) and tumour necrosis factor-α (TNF-α) in the plasma (Fig. 2h). Meanwhile, the expression of programmed death-1 (PD-1) protein and programmed death-ligand 1 (PD-L1) on immune cells and cancer cells, respectively, remained nearly unchanged or slightly decreased (Supplementary Fig. 10).

CD47 blockade increased the phagocytosis of cancer cells by macrophages in vitro, as shown in the confocal imaging and flow cytometry results (Fig. 3a,b and Supplementary Fig. 11). When aCD47 was loaded into the fibrin gel (50 μg aCD47 per mouse) and sprayed at the tumour resection site, an increased frequency of Ly6G+Ly6C− macrophages within the resection tumour cavity was observed, but not for Ly6G+Ly6Cdim neutrophils (Fig. 3c,d and Supplementary Fig. 12). An increase in CD11c+ dendritic cells was also recorded, and these cells showed expression of CD80, CD86 and CD103, denoting their maturation status43 (Fig. 3e,f). Thus, CD47 blockade enhances phagocytosis of cancer cells by both macrophages and dendritic cells, activating innate immune systems.

**Immunotherapeutic gel for inhibition of tumour recurrence**

To validate the therapeutic effects of aCD47@CaCO3@fibrin, we used an incomplete tumour resection model. The in situ formed fibrin gels containing IgG@CaCO3, aCD47 or aCD47@CaCO3 (1 mg CaCO3 per mouse; 50 μg aCD47 per mouse) were sprayed into the tumour resection cavity. Tumour growth was then monitored by bioluminescence signals from B16F10 cancer cells (Fig. 4a). Mice treated with the aCD47@CaCO3@fibrin showed improved control of tumour regrowth as four out of eight mice had no detectable tumour (Fig. 4b,c). Fifty per cent of the mice after aCD47@CaCO3@fibrin treatment survived for at least 60 days (Fig. 4d), and the body weights of mice were not impacted by the treatment (Fig. 4e). Moreover, histology analysis of major organs collected from mice 30 days after aCD47@CaCO3@fibrin treatment, together with the complete blood panel test and serum biochemistry assay conducted at 1, 7 and 14 days after treatment, indicated that local delivery of aCD47 did not induce significant side effects to mice (Supplementary Figs. 13 and 14).

Residual tumours were harvested and analysed using flow cytometry and immunofluorescence staining 5 days after surgery. The proportion of both M1- and M2-type macrophages in the residual tumour was increased after aCD47 blockade, together with an increased polarization of M1-phenotype TAMs after CaCO3 treatment (Fig. 5a and Supplementary Fig. 15). Similarly, the absolute number of TILs (CD3+ cells) was increased in the residual tumour after aCD47@CaCO3@fibrin treatment (Fig. 5b). Furthermore, the percentage of CD8+ T cells was significantly increased in the tumours after aCD47@CaCO3@fibrin treatment as compared to controls (Fig. 5bc). Proliferation of CD4+ T cells was also increased, while the percentage of Tregs (CD4+Foxp3+ T cells) was decreased (Fig. 5d and Supplementary Fig. 16). Immunofluorescence staining visually indicated the marked increase in macrophages and CD8+ T cells in the residual tumours after aCD47@CaCO3@fibrin therapy (Fig. 5e and Supplementary Fig. 17). Secretion of cytokines including IFN-γ, IL-6 and IL-12p70 further confirmed the effective innate and adaptive immune responses induced by aCD47@CaCO3@fibrin treatment (Fig. 5f).

**Immunotherapeutic gel for treating distant tumours**

With confirmation that aCD47@CaCO3@fibrin activates locally innate and adaptive immunity, we investigated whether local treatment with aCD47@CaCO3@fibrin triggers systemic immune responses. B16F10 cancer cells were inoculated in the opposite flank of the primary tumour to mimic tumour metastasis. Primary tumours were then partially resected, and fibrin gel containing aCD47@CaCO3 nanoparticles (1 mg CaCO3 per mouse; 50 μg aCD47 per mouse) was sprayed into the tumour resection cavity (Fig. 6a). Local tumour recurrence was inhibited by aCD47@CaCO3@fibrin treatment, and tumour growth at the opposite site was also reduced (Fig. 6b–d). Consistent with these results, the numbers of M1-like TAMs and CD103+ dendritic cells were significantly increased in tumours sprayed with aCD47@CaCO3@fibrin (Fig. 6e,f), and CD8+ T cells were increased in both treated and distal tumours (Fig. 6g and Supplementary Fig. 15). The increased CD8+ T cells in the distal tumour can be attributed to local cross-presentation of tumour antigens by macrophages and dendritic cells that trigger systemic antitumour immunity. Activation of the immune system was further confirmed by the peritumoral injection of aCD47@CaCO3@fibrin by a dual-syringe administration method (Supplementary Fig. 19). aCD47@CaCO3@fibrin-mediated treatment effectively inhibited the growth of both local and distant tumours.

We further evaluated the combination efficacy of anti-PD-1 (aPD1) and aCD47 co-delivered by the sprayed gel44–47. Similarly, the primary tumours were partially resected and the surgical sites were sprayed with different fibrin gels containing aCD47@CaCO3 nanoparticles (50 μg aCD47 per mouse), aPD1@CaCO3 nanoparticles (50 μg aPD1 per mouse) or aCD47&aPD1@CaCO3 nanoparticles (25 μg aCD47 per mouse; 25 μg aPD1 per mouse). Encouragingly, a synergistic effect was achieved with aCD47&aPD1@CaCO3@fibrin treatment in inhibiting tumour recurrence after surgery and distant tumours, as shown in the bioluminescence imaging and growth profiles of local and distant tumours (Supplementary Fig. 20).

**Conclusion**

In summary, we have developed a simple post-surgical cancer immunotherapy strategy by spraying in situ formed therapeutic gel at the tumour resection site, which could facilitate reversal of the immunosuppressive TME and induce systemic immunological responses that inhibit both local recurrence and systemic development. CaCO3 nanoparticles embedded in the gel matrix could help release therapeutics in a controlled manner and modulate the acidic and inflamed tumour resection environment by scavenging H+, thereby promoting antitumour immune responses. Furthermore, the locally released aCD47 from CaCO3 nanoparticles blocked the ‘don’t eat me’ signal associated with cancer cells, allowing cancer cell removal by macrophages. CD47 blockade also triggered the T cell mediated destruction of cancer cells due to the enhanced presentation of tumour-specific antigen by macrophages and dendritic cells. These results encourage the potential clinical translation of this method upon tumour resection. To this end, future evaluations in large animal models are expected to optimize drug dosage, particle amount and treatment frequency.

**Online content**

Any methods, additional references, Nature Research reporting summaries, source data, statements of data availability and associated accession codes are available at https://doi.org/10.1038/s41565-018-0319-4.

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**References**


Methods

Materials, cell lines and animals. All chemicals were purchased from Sigma-Aldrich and used without any purification. Murine thrombin and fibrinogen were purchased from Molecular Innovations. aCD47 and aPD1 were purchased from Biolegend (cat. no. 127518, Clone, miapc31; cat. no. 135233, Clone, 29E1A12). The murine melanoma cell line B16F10 was purchased from UNC tissue culture facility. B16F10-luc cells were obtained from L. Huang at UNC. Cells were cultured in Dulbecco's modified Eagle medium (Gibco, Invitrogen) containing 10% fetal bovine serum (Invitrogen) and 100 U ml−1 penicillin (Invitrogen) in an incubator at 37 °C in 5% CO2. Fibroblasts (CRL-1651; 6–10 weeks) were purchased from the Jackson Lab. All mouse studies were carried out following protocols approved by the Institutional Animal Care and Use Committee at the University of North Carolina at Chapel Hill and North Carolina State University and complied with all relevant ethical regulations.

Preparation and characterization of aCD47@CaCO3. aCD47@CaCO3 nanoparticles were prepared by chemical precipitation. Typically, 1 ml of Tris-HCl buffer (1 mM, pH 7.6) containing 100 mM CaCl2 was mixed with 1 ml HEPES saline buffer (50 mM, pH 7.1, NaCl 140 mM) containing 100 μg aCD47, 10 mg PEG-b-P(Glu) block copolymers (synthesized as previously described) and 10 mM Na2CO3. The mixture was stirred for 12 h at 4 °C. Excess ions, copolymers and antibodies were removed by centrifugation at 14,800 r.p.m. for 5 min. The size distribution was measured by dynamic light scattering and morphology was evaluated by TEM (JEOL 2000FX). The elemental analysis of aCD47@CaCO3 (aCD47 was chelated gadolinium as previously described) was characterized using analytical TEM (Titan). The amount of aCD47 encapsulated in CaCO3 was measured by enzyme-linked immunosorbent assay (ELISA) (rat IgG total ELISA kit, eBioscience, cat. no. 88-50490-22). The encapsulation efficiency (EE) and loading capacity (LC) of CaCO3 nanoparticles were calculated using the following formula: EE = (A − B)/A and LC = (A − B)/C, where A is the free amount of antibody, B is the free non-entrapped antibody and C is the total weight of particles.

Formation and characterization of aCD47@CaCO3@fibrin. Fibrin gels were obtained by spraying equal volumes of fibrinogen (10 mg ml−1) containing aCD47@CaCO3 and thrombin (10 NIHU ml−1). For the control group without CaCO3 nanoparticles, CaCl2 was incorporated with the same amount of free Ca2+ compared with the solution with CaCO3 nanoparticles. The morphology of aCD47@CaCO3@fibrin was characterized by cryo-SEM (JEOL, 7600F, Gatan Alto). Encapsulation of aCD47@CaCO3 in fibrin was further characterized by a confocal microscopy (Zeiss LSM 710).

Release of aCD47 in vitro. The release of aCD47 was studied at 37 °C in PBS at various pH values (pH 6.5 and 7.4). Released aCD47 was measured using a rat IgG total ELISA kit.

Release of aCD47 in vivo. To study the in vivo release of aCD47, Cy5.5-labelled free aCD47 or aCD47@CaCO3 dispersed in PBS was injected at the tumour resection site. To study the in vivo release of aCD47, Cy5.5-labelled free aCD47@CaCO3 dispersed in PBS was injected at the tumour resection site. To study the in vivo release of aCD47, Cy5.5-labelled free aCD47@CaCO3 dispersed in PBS was injected at the tumour resection site. To study the in vivo release of aCD47, Cy5.5-labelled free aCD47@CaCO3 dispersed in PBS was injected at the tumour resection site.

Cytokine detection. The intratumour and plasma levels of IL-10 (Invitrogen, cat. no. BMS215-2), IL-12p70 (Invitrogen, cat. no. BMS6004TEN), IL-6 (Invitrogen, cat. no. BMS6030-2), IFN-γ (Invitrogen, cat. no. BMS6081INST) and TNF-α (Invitrogen, cat. no. BMS6081-3) were measured with ELISA kits according to the manufacturer's instructions. For detection of the local (tumour) concentration of cytokines, 5 days after spraying the fibrin gels, the tumour tissue was harvested and then homogenized in cold PBS buffer in the presence of digestive enzymes, forming single cell suspensions. For the plasma levels, serum samples were isolated from mice after different treatments and diluted for analysis.

Flow cytometry. Tumours collected from mice were divided into small pieces and homogenized in cold staining buffer to form single cell suspensions in the presence of digestive enzyme. Cells were stained with fluorescence-labelled antibodies CD45 (Biolegend, cat. no. 103108, clone 30-F11), CD11b (Biolegend, cat. no. 101208, clone M1/70), CD206 (Biolegend, cat. no. 141716, clone C066/C2), F4/80 (Biolegend, cat. no. 123116, clone BM8), CD80 (Biolegend, cat. no. 104722, clone 10-16A1), Gr-1 (Biolegend, cat. no. 108412, clone RB6-8C5), CD3 (Biolegend, cat. no. 102004, clone 17A2), CD4 (Biolegend, cat. no. 104112, clone GK1.5), CD8 (Biolegend, cat. no. 140408, clone 53-5.8), Foxp3 (Biolegend, cat. no. 126044, clone G5-126.1), CD11c (Biolegend, cat. no. 117310, clone N418), CD66 (Biolegend, cat. no. 105008, clone GL-1), CD103 (Biolegend, cat. no. 121406, clone 2E7), Ly6C (Biolegend, cat. no. 128016, clone HK1.4) and Ly6G (Biolegend, cat. no. 127608, clone IA8) following the manufacturer's instructions. All antibodies were diluted 200 times. The stained cells were measured on a CytoFLEX flow cytometer (Beckman) and analysed by FlowJo software (version 10.0.7, TreeStar). The numbers presented in the flow cytometry analysis images are percentage based.

Immunofluorescence staining. Tumours were collected from the mice and snap-frozen in optimal cutting temperature medium. Tumour sections were cut using a cryostat, mounted on slides and stained with different primary antibodies: CD4 (Abcam, cat. no. ab138685), CD8 (Abcam, cat. no. ab22378) and F4/80 (Abcam, cat. no. ab100790) overnight at 4 °C following the manufacturer's instructions. Following the addition of fluorescently labelled secondary antibodies (goat-anti-rat IgG (H & L; Thermo Fisher Scientific, cat. no. A18866) and goat anti-rabbit IgG (H & L; Thermo Fisher Scientific, cat. no. A32733)), the slides were analysed with a confocal laser microscope (Zeiss LSM 710). All antibodies used in the experiments were diluted 200 times.

Western blotting. Equal amounts of protein (measured using a bichinchoninic acid protein assay kit, BCA) were mixed with an equal volume of 2×Laemmli buffer and boiled at 95 °C for 5 min. After gel electrophoresis and protein transformation, anti-HFI1α (28b) antibody at a dilution of 1:1,000 (Santa Cruz, cat. sc-13515) and anti-β-actin antibody (Abcam, cat. ab8226) at a 1:5,000 dilution were used as primary antibodies. The secondary antibody used for these blots was a goat anti-mouse antibody (Novus Biologicals, cat. no. NBPI-75151).

Statistical analysis. All results are presented as the mean ± standard error of the mean (s.e.m.), as indicated. Tukey post-hoc tests and one-way ANOVA were used for multiple comparisons (when more than two groups were compared), and Student's t-test was used for two-group comparisons. Survival benefit was determined using a log-rank test. All statistical analyses were carried out with Prism software package (GraphPad Software, 2007). The threshold for statistical significance was P < 0.05.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

The data that support the plots within this paper and other data from this study are available from the corresponding author upon reasonable request.

References


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<td>X</td>
<td>For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings</td>
</tr>
<tr>
<td>X</td>
<td>For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes</td>
</tr>
<tr>
<td>X</td>
<td>Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated</td>
</tr>
<tr>
<td>X</td>
<td>Clearly defined error bars</td>
</tr>
<tr>
<td>X</td>
<td>State explicitly what error bars represent (e.g. SD, SE, CI)</td>
</tr>
</tbody>
</table>

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

<table>
<thead>
<tr>
<th>Data collection</th>
<th>Zen software, CytExpert software, Excel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Data analysis</td>
<td>All statistical analyses were performed on Graphpad Prism (version 7). All flowcytometry data were analyzed on FlowJo software package (version 10.0.7; TreeStar, USA, 2014). Living image software (Perkin Elmer) was used to analyse bioluminescent and fluorescent images.</td>
</tr>
</tbody>
</table>

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

A list of figures that have associated raw data.
Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

- [ ] Life sciences
- [x] Behavioural & social sciences
- [ ] Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-flat.pdf

**Life sciences study design**

All studies must disclose on these points even when the disclosure is negative.

<table>
<thead>
<tr>
<th>Sample size</th>
<th>The sample size were determined by G-power analysis software correspondingly.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Data exclusions</td>
<td>No data were excluded.</td>
</tr>
<tr>
<td>Replication</td>
<td>Experiments were repeated and experimental findings were reproducible.</td>
</tr>
<tr>
<td>Randomization</td>
<td>Mice were allocated randomly to each treatment group.</td>
</tr>
<tr>
<td>Blinding</td>
<td>No formal blinding was used. Bioluminescence imaging were conducted by an independent operator, who was unaware of the treatment conditions.</td>
</tr>
</tbody>
</table>

**Behavioural & social sciences study design**

All studies must disclose on these points even when the disclosure is negative.

| Study description | Briefly describe the study type including whether data are quantitative, qualitative, or mixed-methods (e.g. qualitative cross-sectional, quantitative experimental, mixed-methods case study). |
| Research sample | State the research sample (e.g. Harvard university undergraduates, villagers in rural India) and provide relevant demographic information (e.g. age, sex) and indicate whether the sample is representative. Provide a rationale for the study sample chosen. For studies involving existing datasets, please describe the dataset and source. |
| Sampling strategy | Describe the sampling procedure (e.g. random, snowball, stratified, convenience). Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient. For qualitative data, please indicate whether data saturation was considered, and what criteria were used to decide that no further sampling was needed. |
| Data collection | Provide details about the data collection procedure, including the instruments or devices used to record the data (e.g. pen and paper, computer, eye tracker, video or audio equipment) whether anyone was present besides the participant(s) and the researcher, and whether the researcher was blind to experimental condition and/or the study hypothesis during data collection. |
| Timing | Indicate the start and stop dates of data collection. If there is a gap between collection periods, state the dates for each sample cohort. |
| Data exclusions | If no data were excluded from the analyses, state so OR if data were excluded, provide the exact number of exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established. |
| Non-participation | State how many participants dropped out/declined participation and the reason(s) given OR provide response rate OR state that no participants dropped out/declined participation. |
| Randomization | If participants were not allocated into experimental groups, state so OR describe how participants were allocated to groups, and if allocation was not random, describe how covariates were controlled. |

**Ecological, evolutionary & environmental sciences study design**

All studies must disclose on these points even when the disclosure is negative.

| Study description | Briefly describe the study. For quantitative data include treatment factors and interactions, design structure (e.g. factorial, nested, hierarchical), nature and number of experimental units and replicates. |
| Research sample | Describe the research sample (e.g. a group of tagged Passer domesticus, all Stenocereus thurberi within Organ Pipe Cactus National Monument), and provide a rationale for the sample choice. When relevant, describe the organism taxa, source, sex, age range and condition. |
**Materials & experimental systems**

<table>
<thead>
<tr>
<th>n/a</th>
<th>Involved in the study</th>
</tr>
</thead>
<tbody>
<tr>
<td>☑️</td>
<td><strong>Unique biological materials</strong></td>
</tr>
<tr>
<td>☑️</td>
<td>Antibodies</td>
</tr>
<tr>
<td>☑️</td>
<td>Eukaryotic cell lines</td>
</tr>
<tr>
<td>☑️</td>
<td>Palaeontology</td>
</tr>
<tr>
<td>☑️</td>
<td>Animals and other organisms</td>
</tr>
<tr>
<td>☑️</td>
<td>Human research participants</td>
</tr>
</tbody>
</table>

**Methods**

<table>
<thead>
<tr>
<th>n/a</th>
<th>Involved in the study</th>
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<tbody>
<tr>
<td>☑️</td>
<td>ChIP-seq</td>
</tr>
<tr>
<td>☑️</td>
<td>Flow cytometry</td>
</tr>
<tr>
<td>☑️</td>
<td>MRI-based neuroimaging</td>
</tr>
</tbody>
</table>

**Field work, collection and transport**

**Field conditions**

Describe the study conditions for field work, providing relevant parameters (e.g. temperature, rainfall).

**Location**

State the location of the sampling or experiment, providing relevant parameters (e.g. latitude and longitude, elevation, water depth).

**Access and import/export**

Describe the efforts you have made to access habitats and to collect and import/export your samples in a responsible manner and in compliance with local, national and international laws, noting any permits that were obtained (give the name of the issuing authority, the date of issue, and any identifying information).

**Disturbance**

Describe any disturbance caused by the study and how it was minimized.

**Reporting for specific materials, systems and methods**

**Unique biological materials**

Policy information about availability of materials

Obtaining unique materials

Describe any restrictions on the availability of unique materials OR confirm that all unique materials used are readily available from the authors or from standard commercial sources (and specify these sources).

**Antibodies**

Antibodies used

The following primary antibodies were used for blocking. They are listed as antigen first, followed by supplier, catalog number and clone/lot number as applicable. All the antibodies were diluted and used following the supplier protocol.

1) Anti-mouse CD47, Biolegend Inc, cat. no. 127518, Clone: miap301;
2) Anti-mouse PD1, Biolegend Inc, cat. no. 135233, Clone: 29F.1A12;
The following primary antibodies were used for western blotting. They are listed as antigen first, followed by supplier, catalog number and clone/lot number as applicable.
1) Anti HIF1-α (28b), Santa Cruz, cat. no. sc-13515;
2) Anti-beta actin, Abcam, cat. no. ab8226;
3) Goat anti-mouse antibody, Novus Biologicals, cat. no. NBP1-75151;

The following primary antibodies were used for immunofluorescence. They are listed as antigen first, followed by supplier, catalog number and clone/lot number as applicable.
1) Rhodamine (TRITC)-conjugated donkey anti-rat IgG antibody, Jackson Immuno Research Labs, cat. no. NC9656482;
2) Anti-mouse CD4, Abcam, cat. no. ab183685;
3) Anti-mouse CD8, Abcam, cat. no. ab22378;
4) Anti-mouse F4/80, Abcam, cat. no. ab100790;

The following primary antibodies were used for flow cytometry. They are listed as antigen first, followed by supplier, catalog number and clone/lot number as applicable.
1) Anti-mouse CD45, Biolegend, cat. no. 103108, Clone: 30-F11;
2) Anti-mouse CD11b, Biolegend, cat. no. 101208, Clone: M1/70;
3) Anti-mouse CD206, Biolegend, cat. no. 141716, Clone:CD68C2;
4) Anti-mouse F4/80, Biolegend, cat. no. 123116, Clone:BMD28;
5) Anti-mouse CD80, Biolegend, cat. no. 104722, Clone:16-10A1;
6) Anti-mouse Gr-1, Biolegend, cat. no. 108412, Clone: R6-6C5;
7) Anti-mouse CD3, Biolegend, cat. no. 100204, Clone: 17A2;
8) Anti-mouse CD4, Biolegend, cat. no. 100412, Clone: GK1.5;
9) Anti-mouse CD8, Biolegend, cat. no. 140408, Clone: 53-6.8;
10) Anti-mouse Foxp3, Biolegend, cat. no. 126404, Clone: MF-14;
11) Anti-mouse CD11c, Biolegend, cat. no. 117310, Clone: N418;
12) Anti-mouse CD86, Biolegend, cat. no. 105008, Clone: GL-1;
13) Anti-mouse CD103, Biolegend, cat. no. 121406, Clone: ZE7;
14) Anti-mouse Ly6C, Biolegend, cat. no. 128016, Clone: HK1.4;
15) Anti-mouse Ly6G, Biolegend, cat. no. 127608, Clone: 1A8;

The following primary antibodies were used for ELISA. They are listed as antigen first, followed by supplier, catalog number and clone/lot number as applicable.
1) Anti-rat IgG, eBioscience, cat. no. 88-50490-22;
2) Anti-mouse IL10, Invitrogen, cat. no. BMS215-2;
3) Anti-mouse IL12p70, Invitrogen, cat. no. BMS6004TEN;
4) Anti-mouse IL 6, Invitrogen, cat. no. BMS603-2;
5) Anti-mouse TNF-α, Invitrogen, cat. no. BMS607-3;
6) Anti-mouse IFN gamma, Invitrogen, cat. no. BMS606INST;

Validation
All antibodies were verified by the supplier and each lot has been quality tested.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)
The murine melanoma cell line B16F10 was purchased from UNC tissue culture facility. B16F10-luc cells were obtained from Dr. Leaf Huang at UNC.

Authentication
The cells lines were authenticated by IDEXX BioResearch and the UNC-tissue culture facility for pathogen testing.

Mycoplasma contamination
All cell lines were tested for mycoplasma contamination. No mycoplasma contamination was found.

Commonly misidentified lines
No commonly misidentified cell lines were used.

Palaeontology

Specimen provenance
Provide provenance information for specimens and describe permits that were obtained for the work (including the name of the issuing authority, the date of issue, and any identifying information).

Specimen deposition
Indicate where the specimens have been deposited to permit free access by other researchers.
Dating methods

If new dates are provided, describe how they were obtained (e.g. collection, storage, sample pretreatment and measurement), where they were obtained (i.e. lab name), the calibration program and the protocol for quality assurance OR state that no new dates are provided.

Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.

Animals and other organisms

Policy information about studies involving animals. ARRIVE guidelines recommended for reporting animal research

Laboratory animals Female C57BL/6 mice (6–10 weeks) were purchased from Jackson Lab.

Wild animals The study did not involve wild animals.

Field-collected samples The study did not involve samples collected from field.

Human research participants

Policy information about studies involving human research participants

Population characteristics Describe the covariate-relevant population characteristics of the human research participants (e.g. age, gender, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."

Recruitment Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.

ChIP-seq

Data deposition

Confirm that both raw and final processed data have been deposited in a public database such as GEO.

Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links For "Initial submission" or "Revised version" documents, provide reviewer access links. For your "Final submission" document, provide a link to the deposited data.

Files in database submission Provide a list of all files available in the database submission.

Genome browser session Provide a link to an anonymized genome browser session for "Initial submission" and "Revised version" documents only, to enable peer review. Write "no longer applicable" for "Final submission" documents.

Methodology

Replicates Describe the experimental replicates, specifying number, type and replicate agreement.

Sequencing depth Describe the sequencing depth for each experiment, providing the total number of reads, length of reads and whether they were paired- or single-end.

Antibodies Describe the antibodies used for the ChIP-seq experiments; as applicable, provide supplier name, catalog number, clone name, and lot number.

Peak calling parameters Specify the command line program and parameters used for read mapping and peak calling, including the CHIP, control and index files used.

Data quality Describe the methods used to ensure data quality in full detail, including how many peaks are at FDR 5% and above 5-fold enrichment.

Software Describe the software used to collect and analyze the ChIP-seq data. For custom code that has been deposited into a community repository, provide accession details.
Flow Cytometry

Plots

- Confirm that:
  - The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
  - The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
  - All plots are contour plots with outliers or pseudocolor plots.
  - A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation  For tissue sample, the tissue was first mechanically disrupted from mice and divided into small pieces and homogenized in cold staining buffer to form single cell suspensions in the presence of digestive enzyme.

Instrument  CytoFLEX flow cytometer (Beckman)

Software  FlowJo software package (version 10.0.7; TreeStar, USA, 2014)

Cell population abundance  No sorting was performed.

Gating strategy  Generally, cells was first gated on FSC/SSC. Singlet cells were usually gated using FSC-H and FSC-A. Surface antigen gating was performed on the live cell population.

- Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.

Magnetic resonance imaging

Experimental design

Design type  Indicate task or resting state; event-related or block design.

Design specifications  Specify the number of blocks, trials or experimental units per session and/or subject, and specify the length of each trial or block (if trials are blocked) and interval between trials.

Behavioral performance measures  State number and/or type of variables recorded (e.g. correct button press, response time) and what statistics were used to establish that the subjects were performing the task as expected (e.g. mean, range, and/or standard deviation across subjects).

Acquisition

Imaging type(s)  Specify: functional, structural, diffusion, perfusion.

Field strength  Specify in Tesla

Sequence & imaging parameters  Specify the pulse sequence type (gradient echo, spin echo, etc.), imaging type (EPI, spiral, etc.), field of view, matrix size, slice thickness, orientation and TE/TR/flip angle.

Area of acquisition  State whether a whole brain scan was used OR define the area of acquisition, describing how the region was determined.

Diffusion MRI  □ Used  □ Not used

Preprocessing

Preprocessing software  Provide detail on software version and revision number and on specific parameters (model/functions, brain extraction, segmentation, smoothing kernel size, etc.).

Normalization  If data were normalized/standardized, describe the approach(es): specify linear or non-linear and define image types used for transformation OR indicate that data were not normalized and explain rationale for lack of normalization.

Normalization template  Describe the template used for normalization/transformation, specifying subject space or group standardized space (e.g. original Talairach, MNI305, ICBM152) OR indicate that the data were not normalized.

Noise and artifact removal  Describe your procedure(s) for artifact and structured noise removal, specifying motion parameters, tissue signals and physiological signals (heart rate, respiration).

Volume censoring  Define your software and/or method and criteria for volume censoring, and state the extent of such censoring.
Statistical modeling & inference

Model type and settings
Specify type (mass univariate, multivariate, RSA, predictive, etc.) and describe essential details of the model at the first and second levels (e.g. fixed, random or mixed effects; drift or auto-correlation).

Effect(s) tested
Define precise effect in terms of the task or stimulus conditions instead of psychological concepts and indicate whether ANOVA or factorial designs were used.

Specify type of analysis: □ Whole brain □ ROI-based □ Both

Statistic type for inference
(See Eklund et al. 2016)
Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods.

Correction
Describe the type of correction and how it is obtained for multiple comparisons (e.g. FWE, FDR, permutation or Monte Carlo).

Models & analysis
n/a Involved in the study

□ □ Functional and/or effective connectivity
□ □ Graph analysis
□ □ Multivariate modeling or predictive analysis

Functional and/or effective connectivity
Report the measures of dependence used and the model details (e.g. Pearson correlation, partial correlation, mutual information).

Graph analysis
Report the dependent variable and connectivity measure, specifying weighted graph or binarized graph, subject- or group-level, and the global and/or node summaries used (e.g. clustering coefficient, efficiency, etc.).

Multivariate modeling and predictive analysis
Specify independent variables, features extraction and dimension reduction, model, training and evaluation metrics.