Therapeutic targeting of macrophages enhances chemotherapy efficacy by unleashing type I interferon response

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Recent studies have revealed a role for macrophages and neutrophils in limiting chemotherapy efficacy; however, the mechanisms underlying the therapeutic benefit of myeloid-targeting agents in combination with chemotherapy are incompletely understood. Here, we show that targeting tumour-associated macrophages by colony-stimulating factor-1 receptor (CSF-1R) blockade in the K14cre;Cdhl−/−;Trp53−/− transgenic mouse model for breast cancer stimulates intratumoural type I interferon (IFN) signalling, which enhances the antitumour efficacy of platinum-based chemotherapeutics. Notably, anti-CSF-1R treatment also increased intratumoural expression of type I IFN-stimulated genes in patients with cancer, confirming that CSF-1R blockade is a powerful strategy to trigger an intratumoural type I IFN response. By inducing an inflamed, type I IFN-enriched tumour microenvironment and by further targeting immunosuppressive neutrophils during cisplatin therapy, antitumour immunity was activated in this poorly immunogenic breast cancer mouse model. These data illustrate the importance of breaching multiple layers of immunosuppression during cytotoxic therapy to successfully engage antitumour immunity in breast cancer.

Poor chemotherapy response is a major obstacle to successful cancer treatment. There is a growing appreciation for the influential role of the immune system on the success of cytotoxic anticancer therapy. Although the adaptive immune system contributes to the therapeutic benefit of certain chemotherapeutic drugs in immunogenic tumour models, it frequently fails to be unleashed by these same agents in less immunogenic transgenic mouse tumour models, suggesting the involvement of immunosuppressive mechanisms. Indeed, macrophages and neutrophils are frequently the most abundant immune cells in tumours, and clinical studies have reported a correlation between these myeloid cells and poor chemotherapy efficacy. Experimental animal studies confirm a causal relationship between tumour-associated myeloid cells and poor chemotherapy response. For example, inhibition of macrophages in mammary tumour-bearing MMTV-PyMT mice increases paclitaxel efficacy via activation of antitumour immunity. Notably, macrophage-targeting and neutrophil-targeting agents are currently under clinical evaluation. Although promising, the aforementioned preclinical studies only show a transient therapeutic effect of combined myeloid cell targeting and chemotherapy. A deeper understanding of the mechanisms of action is needed to facilitate the rational design of therapeutic combination strategies that convert ‘cold’ non-T cell-inflamed tumours into ‘hot’ inflamed tumours, thus engaging durable antitumour immunity in otherwise poorly immunogenic tumours.

By combining in vivo intervention experiments and mechanistic studies in the K14cre;Cdhl−/−;Trp53−/− (KEP) mouse model for spontaneous mammary tumorigenesis with validation studies in tumour biopsies of patients treated with anti-colony-stimulating factor-1 receptor (anti-CSF-1R), here, we demonstrate that CSF-1R inhibition synergizes with platinum-based chemotherapy by unleashing an intratumoural type I interferon (IFN) response. Besides this anti-CSF-1R-mediated conversion of the tumour microenvironment into a type I IFN-enriched milieu, it takes breaching of an additional layer of immunosuppression to engage antitumour immunity during cytotoxic therapy.

Results

CSF-1R blockade does not affect mammary tumour growth or metastasis in KEP mice. We set out to assess the role of CSF-1–CSF-1R signalling, which is vital for macrophages, in tumour progression in the KEP model, which spontaneously develops mammary tumours resembling human invasive lobular carcinomas (ILCs) at 6–8 months of age. Similar to human ILCs, KEP tumours...
are strongly infiltrated by macrophages (Supplementary Fig. 1a,b). In the MMTV-PyMT breast cancer model it has been reported that two distinct macrophage populations reside within the TME: CD11b+MHCII+CD206− mammary tissue macrophages and CD11b+MHCII−CD206+ tumour-associated macrophages (TAMs)26, in mammary tumours of KEP mice, all F4/80+ macrophages express high levels of CD11b, low levels of CD206 and only a proportion of these cells expresses major histocompatibility complex class II (MHCII) (Supplementary Fig. 1c). These differences in intratumoural macrophage phenotypes between mouse tumour models underscore the complexity of macrophage plasticity in different tumour contexts. In line with the macrophage influx, CSF-1 protein levels are increased in KEP tumours versus healthy mammary glands of age-matched wild-type littermates (Fig. 1a). Both cancer cells and host cells in KEP tumours express Csf1 mRNA, whereas Csf1 mRNA is barely detectable in healthy mammary glands (Fig. 1b). CSF-1R is highly expressed on TAMs and to a lesser extent on infiltrating monocytes and neutrophils (Supplementary Fig. 1d), but not on other tumour-associated immune cells or CD45− cells (Supplementary Fig. 1d).

To determine whether intratumoural macrophage accumulation depends on CSF-1–CSF-1R signalling and whether macrophages influence tumour outgrowth and dissemination, we treated tumour-bearing KEP mice with a chimeric mouse IgGl antagonistic antibody (2G2) that binds to mouse CSF-1R with high affinity (dissociation constant \(K_d\) = 0.2 nM) or with a control antibody26. CSF-1R blockade strongly reduced the TAM population (Fig. 1c,d) and, as a result, also the total CD45+ population (Supplementary Fig. 1e). Treatment with anti-CSF-1R alone did not influence tumour-specific survival (Fig. 1f) or spontaneous metastasis formation (Supplementary Fig. 1f). We also investigated the therapeutic activity of anti-CSF-1R in the KEP-based model of spontaneous breast cancer metastasis27. In this model, after orthotopic transplantation of a KEP-derived tumour piece followed by surgical removal of the outgrown tumour, mice develop overt multi-organ metastatic disease. Anti-CSF-1R was started either after a palpable mammary tumour had developed (continuous setting) or after mastectomy (adjuvant setting) and continued until the development of metastatic disease (Supplementary Fig. 1g). Regardless of the treatment schedule, metastasis-specific survival and metastatic burden in the lungs were similar between control and anti-CSF-1R groups (Supplementary Fig. 1h,i). Thus, anti-CSF-1R monotherapy fails to affect outgrowth and dissemination of KEP mammary tumours.

CSF-1R blockade in tumour-bearing KEP mice enhances the anticancer efficacy of platinum-based chemotherapy. We next tested the anticancer efficacy of anti-CSF-1R in combination with two conventional chemotherapeutics with a different mode of action: cisplatin, a platinum-based anticancer drug that crosslinks DNA and induces apoptosis, and docetaxel, an antimitotic agent that interferes with cell division through stabilization of microtubules. Successful blockade of the CSF-1R pathway during treatment of tumour-bearing KEP mice with chemotherapy and anti-CSF-1R was confirmed by the reduction in the number of TAMs (Fig. 1c,d and Supplementary Fig. 2a,b). Interestingly, anti-CSF-1R synergized with cisplatin, resulting in prolonged survival compared to cisplatin + control antibody-treated mice (Fig. 1f). By contrast, no therapeutic synergy was observed in docetaxel + anti-CSF-1R-treated mice (Supplementary Fig. 2c). The therapeutic synergy observed upon cisplatin + anti-CSF-1R was associated with more necrosis in KEP tumours (Fig. 1g) but not with more cleaved caspase 3+ apoptotic cells (Supplementary Fig. 2d). Perhaps other mechanisms of cell death are involved or the timing of our analysis was suboptimal for this parameter. Furthermore, anti-CSF-1R monotherapy—and to a lesser extent, the combination with cisplatin—decreased the number of BrdU+–proliferating cells (Fig. 1h). No significant changes in the number and pericyte coverage of CD31+ microvessels, the amount of intratumoural DNA double-strand breaks and intratumoural cisplatin-adduct formation were observed at the time-point analysed (Supplementary Fig. 2e–h). As expected, none of these parameters was changed in the docetaxel setting (Supplementary Fig. 2i–m).

To assess whether the anti-CSF-1R-mediated therapeutic synergy was unique to cisplatin or could be extended to drugs with a similar mechanism of action, we tested another platinum-containing drug, oxaliplatin, and also found that the survival benefit of oxaliplatin was improved by combined CSF-1R blockade (Fig. 1i and Supplementary Fig. 2n). These data demonstrate that anti-CSF-1R acts synergistically with platinum-based chemotherapeutic drugs to extend the survival of mammary tumour-bearing KEP mice.

CSF-1R inhibition alters the innate immune landscape of KEP tumours. Macrophages are key orchestrators of the inflammatory TME28. Thus, we set out to assess the effect of anti-CSF-1R on the innate immune landscape of KEP tumours. Despite the strong reduction of CD11b+Csf1+TAMs on anti-CSF-1R, up to 20% of the intratumoural CD45+ immune cells still expresses the macrophage marker F4/80 (Fig. 1d). Detailed analysis of this surviving CD11b+Csf1+ population revealed that an increased proportion of these cells expresses the inflammatory monocyte marker Ly6C compared to CD11b+Csf1− cells in control antibody-treated tumours (Fig. 2a and Supplementary Fig. 3a). Moreover, the surviving CD11b+Csf1+ cells in cisplatin + anti-CSF-1R-treated mice express elevated levels of the co-stimulatory molecules CD80 and CD86, slightly elevated MHCII levels, decreased levels of the chemokine receptors C-C chemokine receptor type 2 (CCR2) and CX3C chemokine receptor 1 (CX3CR1), and increased levels of programmed cell death ligand 1 (PD-L1) compared to intratumoural CD11b+Csf1− cells in cisplatin + control antibody-treated mice (Fig. 2b–g). Furthermore, in the independent orthotopically transplanted K14cre;Trp53F/F (KP) mammary tumour model, intratumoural CD11b+Csf1− myeloid cells remaining after CSF-1R inhibition display an altered phenotype corresponding to that in anti-CSF-1R-treated KEP tumours (Fig. 2h–m). Thus, anti-CSF-1R depletes the majority of CD11b+Csf1+ TAMs, whereas a small population of CD11b+Csf1+ cells with a distinct phenotype survives. To explore whether these surviving cells could derive from circulating monocytes, we transferred tdTomato+ monocytes into control antibody or anti-CSF-1R-treated tumour-bearing KEP mice. After 4d, the transferred monocytes that infiltrated tumours of anti-CSF-1R-treated, and not control antibody-treated, animals partially acquired the phenotype of the surviving intratumoural CD11b+Csf1+ cell population (that is, loss of CX3CR1 and elevated PD-L1 expression) (Supplementary Fig. 3b–d). These findings suggest that the surviving CD11b+Csf1+ cells in anti-CSF-1R-treated tumours may derive from newly recruited circulating monocytes, although other mechanisms cannot be excluded.

Whereas in treatment-naive KEP tumours the macrophage/neutrophil ratio is approximately 3:1, in anti-CSF-1R-treated tumours, either in the presence or absence of cisplatin, this ratio is reversed (Fig. 1d,e). However, the absolute number of intratumoural neutrophils was not increased upon CSF-1R inhibition (Supplementary Fig. 3e). Anti-CSF-1R treatment induced an increase in the number of monocytes and a modest, but not significant, and very variable increase in the number of intratumoural eosinophils and mast cells (Supplementary Fig. 3f–h). Together, these data show that cisplatin + anti-CSF-1R synergy is accompanied by changes in the myeloid immune landscape of tumours. Most notably, anti-CSF-1R treatment resulted in a surviving population of CD11b+Csf1− cells with an altered phenotype. 
Fig. 1 | CSF-1R blockade improves the anticancer efficacy of platinum-based chemotherapeutic drugs in the KEP mouse model for de novo mammary tumorigenesis. a, CSF-1 protein levels in end-stage mammary tumours of KEP mice and mammary glands of age-matched wild-type (WT) mice (n = 5 animals per group) measured by Luminex cytokine array. b, Representative images of RNA in situ hybridization of Csf1 (brown signal) in end-stage KEP tumours and normal mammary glands of age-matched WT mice. c, Representative immunohistochemistry images of F4/80+ macrophages in tumours of time-point-sacrificed KEP mice treated as indicated. Data are representative of five animals per group. Scale bars, 25 μm. d, Proportion of CD11b−F4/80+ macrophages (d) and Ly6G−Ly6C− neutrophils (e) gated on CD45+ cells, as determined by flow cytometry in tumours of end-stage KEP mice treated as indicated (untreated: n = 5 animals; anti-CSF-1R: n = 3 animals; cisplatin: n = 6 animals; cisplatin + anti-CSF-1R: n = 5 animals). f, Kaplan–Meier tumour-specific survival curves of KEP mice treated with control antibody (Ab) (n = 20 animals), anti-CSF-1R (n = 22 animals), cisplatin (CIS) + control Ab (n = 21 animals) or cisplatin + anti-CSF-1R (n = 16 animals). G, Kaplan–Meier survival curves of untreated KEP mice (n = 12 animals) and KEP mice treated with oxaliplatin (OX) + control Ab (n = 10 animals) and oxaliplatin + anti-CSF-1R (n = 12 animals). Oxaliplatin + control Ab versus no treatment: P = 0.0015; oxaliplatin + control Ab versus oxaliplatin + anti-CSF-1R: P = 0.0029 (two-tailed log-rank test). Data presented in a, d, e, g and h are mean ± s.e.m., and statistical analysis was performed using the two-tailed Mann–Whitney test.
Macrophage blockade enhances cisplatin response by unleashing intratumoural type I IFN signalling. To better characterize the phenotype of the anti-CSF-1R-surviving intratumoural CD11b^+ F4/80^+ cells, next-generation RNA sequencing (RNA-seq) analysis was performed on CD11b^+ F4/80^+ cells sorted from cisplatin + control antibody-treated or cisplatin + anti-CSF-1R-treated tumours. Hierarchical clustering of the top 400 variable genes revealed that CD11b^+ F4/80^+ cells from cisplatin + anti-CSF-1R-treated tumours displayed a different transcriptome profile, mainly characterized by a strong enrichment of genes involved in type I IFN signalling and type I IFN production, whereas cell-cycle-associated genes were reduced (Fig. 3a,b). Interestingly, CSF-1R expression levels were lower in the remaining CD11b^+ F4/80^+ cells from cisplatin + anti-CSF-1R-treated tumours (fold change: −2.04; P = 3.63 × 10^−5), perhaps explaining why these cells resisted anti-CSF-1R therapy. In parallel, we also performed RNA-seq on flow-sorted Ly6G^+ Ly6C^int neutrophils isolated from tumours of cisplatin + control antibody-treated and cisplatin + anti-CSF-1R-treated KEP mice. Hierarchical clustering of the top 400 variable genes within this data set revealed that anti-CSF-1R treatment also had a significant effect on the transcriptome profile of tumour-associated neutrophils (Supplementary Fig. 4a). To ensure that these transcriptome alterations in neutrophils are not a direct effect of anti-CSF-1R on neutrophils, but rather an indirect consequence of macrophage targeting, we performed

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**Fig. 2 | Characterization of F4/80^+ cells by flow cytometry in spontaneous KEP tumours and in orthotopically transplanted KP tumours after anti-CSF-1R treatment.** a, Percentage of CD11b^+ F4/80^+ immune cells expressing Ly6C in end-stage KEP tumours (untreated: n = 5 animals; anti-CSF-1R: n = 3 animals; cisplatin: n = 6 animals; cisplatin + anti-CSF-1R: n = 5 animals). b, c, Geometric mean fluorescence intensity (gMFI) of CD80 (b) and CD86 (c) expression on F4/80^+ Siglec F^− cells in KEP tumours (cisplatin + control Ab: n = 4 animals; cisplatin + anti-CSF-1R: n = 6 animals). d, Percentage of MHCII-expressing F4/80^+ Siglec F^− cells (left) and gMFI (right) of MHCII on F4/80^+ Siglec F^− in the KEP tumours (cisplatin + control Ab: n = 5 animals; cisplatin + anti-CSF-1R: n = 6 animals). gMFI was calculated by subtracting the gMFI of the MHCII-negative population from the gMFI of the MHCII-positive population. e–g, gMFI of CCR2 (e), CX3CR1 (f) and PD-L1 (g) expression on F4/80^+ Siglec F^− cells in KEP tumours (CCR2 and CX3CR1: cisplatin + control Ab: n = 4 animals; cisplatin + anti-CSF-1R: n = 6 animals; PD-L1: cisplatin + control Ab: n = 4 animals; cisplatin + anti-CSF-1R: n = 5 animals). h–m, KP tumour pieces were orthotopically transplanted in the mammary fat pad of FVB/N mice. The percentage of CD11b^+ F4/80^+ Siglec F^− immune cells expressing Ly6C in time-point-sacrificed KP tumours (h). gMFI of CD80 (i), CD86 (j), CCR2 (k), CX3CR1 (l) and PD-L1 (m) expression on F4/80^+ Siglec F^− cells in time-point-sacrificed KP tumours (n = 8 animals per group, except CCR2: control Ab: n = 7 animals, anti-CSF-1R: n = 6 animals). The gMFI values presented in b, c, e–g and i–m were determined by subtracting the gMFIs of the fluorescence minus one staining from the gMFI of the full staining. Data presented in a–m are mean ± s.e.m., and statistical analysis was performed using the two-tailed Mann–Whitney test.
gene set enrichment analysis (GSEA) of target genes of early growth factor (EGF2), a transcription factor downstream of CSF-1R signalling. No differences were observed in the expression of EGF2 target genes between neutrophils isolated from anti-CSF-1R-treated and control antibody-treated tumours (Supplementary Fig. 4b), suggesting that neutrophils are not directly influenced by anti-CSF-1R. Interestingly, BiNGO analysis of the top 100 upregulated and downregulated genes and Ingenuity pathway analysis of the differentially expressed genes revealed an enrichment in genes involved in type I IFN signalling in neutrophils isolated from
cisplatin + anti-CSF-1R-treated tumours versus cisplatin + control antibody-treated tumours (Supplementary Fig. 4c,d and Supplementary Table 3). These data indicate that the therapeutic benefit of cisplatin + anti-CSF-1R is accompanied by induction of type I IFN-stimulated genes (ISGs) in both intratumoural CD11b+ F4/80+ cells and neutrophils.

We hypothesized that the enrichment of ISGs in these intratumoural immune populations was a consequence of increased levels of type I IFNs in KEP tumours upon CSF-1R blockade. Indeed, by using primers hybridizing to all Ifna genes, mRNA expression of Ifna, but not Ifnb, was increased in tumours of cisplatin + anti-CSF-1R-treated mice compared to cisplatin + control antibody-treated mice (Fig. 3c). In line with this, the mRNA levels of various intracellular pattern recognition receptors, such as Tlr3, RIG1 and Ifih1, whose signals induce type I IFN production, were upregulated in cisplatin + anti-CSF-1R-treated tumours compared to cisplatin + control antibody treatment (Fig. 3d). Notably, the increase in type I IFN expression upon anti-CSF-1R was independent of chemotherapy treatment, as a similar intratumoural increase in Ifna expression was observed upon anti-CSF-1R alone (Fig. 3e) or with docetaxel + anti-CSF-1R (Supplementary Fig. 5a). We also confirmed the increased expression of Ifna—and of two ISGs, Isg15 and Oas1a—upon anti-CSF-1R treatment in the independent KP-based tumour transplantation model and in inoculated MC38 colorectal adenocarcinoma tumours (Fig. 3f,g and Supplementary Fig. 5b,c). Together, these data demonstrate that anti-CSF-1R induces type I IFN in the TME.

To pursue the cellular source of type I IFN, we flow-sorted different cell populations from cisplatin and cisplatin + anti-CSF-1R-treated KEP tumours (Supplementary Fig. 5d) and compared Ifna and Ifnb transcript levels. Plasmacytoid dendritic cells are known for their ability to produce type I IFN; however, as very few plasmacytoid dendritic cells—less than 0.1% of the total intratumoural immune population—are present in KEP tumours (Supplementary Fig. 5e,f), we could not recover RNA of sufficient quality. Likewise, we did not obtain RNA of sufficient quality from sorted CD31+ endothelial cells. Only the CD11b+F4/80+ immune cell population displayed elevated Ifna expression levels upon CSF-1R blockade (Fig. 3h and Supplementary Fig. 5g,h). In line with these in vivo findings, in vitro treatment of bone marrow-derived macrophages (BMDMs) with anti-CSF-1R modestly induces Ifna levels after 24 h of culture (Supplementary Fig. 5i). These analyses suggest that the surviving population of intratumoural CD11b+F4/80+ cells is an important source of IFN-α in cisplatin + anti-CSF-1R-treated KEP tumours.

To dissect the functional significance of type I IFN signalling in the therapeutic benefit of cisplatin + anti-CSF-1R therapy, we blocked the IFN-α/β receptor subunit 1 (IFNAR1) in KEP mice. Whereas blockade of type I IFN signalling did not influence the anticancer efficacy of cisplatin, anti-IFNAR1 treatment completely abrogated the synergistic effect of cisplatin + anti-CSF-1R treatment (Fig. 4a). These findings reveal that therapeutic targeting of macrophages with anti-CSF-1R in tumour-bearing KEP mice unleashes intratumoural type I IFN signalling, which enhances the therapeutic efficacy of cisplatin.

**Emactuzumab treatment induces intratumoural type I ISGs in patients with cancer.** To validate our preclinical findings that CSF-1R blockade unleashes intratumoural type I IFN signalling in patients, we compared ISG expression levels in pre-treatment and on-treatment tumour biopsies from patients with advanced solid tumours treated with emactuzumab (RG7155), a humanized anti-human CSF-1R monoclonal antibody (NCT01494688)31,32. Gene expression profiling was performed on tumour biopsies taken before the start of treatment and after 4 weeks of emactuzumab therapy. We assessed the expression level of a set of 28 ISGs that was selected based on the RNA-seq results from our KEP mouse model (Fig. 3a, Supplementary Fig. 4a.c,d and Supplementary Table 3). The intratumoural expression of all 28 selected ISGs was increased in emactuzumab on-treatment biopsies versus pre-treatment biopsies, of which 11 ISGs were significantly upregulated (Fig. 4b,c). Thus, in line with our preclinical studies, these clinical findings indicate that CSF-1R blockade is a powerful strategy to augment intratumoural type I IFN signalling.

**Combined CSF-1R inhibition and neutrophil depletion engages antitumour immunity that further improves the therapeutic benefit of cisplatin.** Type I IFNs are emerging as key regulators of cancer growth and therapy response34,42. Type I IFNs can affect cancer biology via different mechanisms, including the induction of anti-proliferative and pro-apoptotic effects on IFNAR+ cancer cells35,36. Moreover, exposure of a cell line derived from a spontaneous KEP mammary tumour to recombinant IFN-α1 results in a dose-dependent decrease in colony-forming ability, also in combination with cisplatin, suggesting that type I IFNs have a direct inhibitory effect on KEP cancer cells (Fig. 5). Because type I IFNs are also key orchestrators of antitumour immunity33–35, we hypothesized that the anti-CSF-1R-induced type I IFN-enriched TME may foster antitumour CDB+ T cell activity. However, we observed fewer numbers of tumour-infiltrating CD4+ or CD8+ T cells in cisplatin + anti-CSF-1R-treated tumours than in cisplatin + control antibody-treated tumours (Supplementary Fig. 6a–c), and the CD8/ regulatory T cell ratio was not affected (Fig. 6a). More natural killer (NK) cells were infiltrating the cisplatin + anti-CSF-1R-treated KEP tumours; however, the number of granzyme B+ cells was not affected compared to cisplatin + control antibody treatment (Fig. 6b,c and Supplementary Fig. 6d). We previously reported that cisplatin efficacy is independent of the adaptive immune system1, and, in line with the lack of more intratumoural granzyme B+ cells and T cells, here, we also show that antibody-mediated depletion of CD8+ T cells does not reduce the therapeutic efficacy of cisplatin + anti-CSF-1R therapy (Fig. 6d). In addition, genetic ablation of the entire adaptive immune system by crossing KEP mice with Rag1−/− mice did not affect therapeutic synergy (Fig. 6g). These data indicate that the anti-CSF-1R-mediated conversion of the TME into a type I IFN-enriched milieu in cisplatin-treated mice is not sufficient to successfully engage an endogenous antitumour T cell response.

We next hypothesized that it may be necessary to breach an additional layer of immunosuppression before antitumour immunity can be unleashed. The most abundant immune cell population in cisplatin + anti-CSF-1R-treated KEP tumours is neutrophils (Fig. 1c) and we have previously reported that KEP tumour-educated neutrophils are very immunosuppressive44. To address whether neutrophils impede antitumour immunity in cisplatin + anti-CSF-1R-treated mice, we treated tumour-bearing KEP mice with the neutrophil-specific anti-Ly6G antibody (clone 1A8). Immunohistochemistry for S100A9 confirmed a reduction in the number of neutrophils in the lungs and to a lesser extent in the tumour (Supplementary Fig. 6e,f). Cisplatin + anti-CSF-1R + anti-Ly6G treatment significantly improved tumour control and prolonged the survival of KEP mice compared to cisplatin + anti-CSF-1R therapy (Fig. 6e). Whereas cisplatin + anti-CSF-1R temporarily stabilizes tumour outgrowth, we observed tumour shrinkage in six out of ten mice treated with cisplatin + anti-CSF-1R + anti-Ly6G, and the mammary tumours of two of these mice regressed completely during treatment (Supplementary Fig. 6g,h). Anti-Ly6G treatment alone failed to influence primary tumour growth in KEP mice as previously shown46, neither did the combination of anti-CSF-1R + anti-Ly6G (Supplementary Fig. 6i) nor did anti-Ly6G alter the efficacy of cisplatin (Fig. 6e). Further characterization of cisplatin + anti-CSF-1R + anti-Ly6G-treated KEP tumours showed a significant reduction in the number of BrdU+—proliferating cells and γ-H2AX+...
DNA-damaged cells (Supplementary Fig. 6j,k). No statistically significant differences were observed in the number of apoptotic cells, CD31+ vessels and cisplatin adducts (Supplementary Fig. 6l–n). Interestingly, the CD8/regulatory T cell ratio, the absolute number of NK cells and the absolute and relative number of granzyme B+ immune cells were increased in cisplatin + anti-CSF-1R + anti-Ly6G-treated tumours compared to cisplatin + control antibody therapy (Fig. 6a–c and Supplementary Fig. 6d). Importantly, the additional therapeutic benefit obtained by anti-Ly6G treatment was partially lost after antibody-mediated depletion of CD8+ T cells or...
cathepsins or secretion of lysophospholipids that interfere with the DNA damage response. These macrophage-mediated chemotherapeutic resistance mechanisms are dependent on the production of soluble mediators from TAMs. Our study reveals a conceptually different mechanism of how therapeutic targeting of macrophages fosters engagement of antitumour immunity in the anticancer effect of cisplatin in this poorly immunogenic mouse tumour model.

Discussion

There is a growing realization that immune-mediated mechanisms influence the responsiveness of tumours to chemotherapy. Notably, macrophages actively interfere with the therapeutic efficacy of chemotherapy via several mechanisms in mouse tumour models, including suppression of antitumour immunity through IL-10 secretion, secretion of chemoprotective proteases such as cathepsins or secretion of lysophospholipids that interfere with the DNA damage response. These macrophage-mediated chemotherapy resistance mechanisms are dependent on the production of soluble mediators from TAMs. Our study reveals a conceptually different mechanism of how therapeutic targeting of macrophages improves chemotherapy efficacy. Through in vivo mechanistic studies in the KEP transgenic mouse model for breast cancer, we demonstrate that macrophage inhibition with anti-CSF-1R induces intratumoural type I IFN signalling, which acts synergistically with cisplatin to inhibit tumour outgrowth and extend survival.

There is a growing interest in the effect of type I IFNs on cancer behaviour and response to immune checkpoint inhibitors, radiotherapy and chemotherapy. Besides being associated with an improved prognosis, an intratumoural IFN signature in patients with breast cancer has been correlated with improved chemotherapy response, and preclinical studies reported that type I IFN enhanced chemotherapy efficacy. However, IFN-related gene signatures have also been correlated with chemotherapy resistance, consistent with a pleomorphic and still poorly understood role of type I IFN signalling in the tumour context. Importantly, impaired type I IFN signalling is a prominent feature of immune dysfunction in patients with cancer. Our study reveals that anti-CSF-1R represents a powerful approach to induce intratumoural IFN signalling and to sensitize tumours to cisplatin. Notably, we find that anti-CSF-1R treatment in patients with cancer also results in increased intratumoural expression of ISGs, confirming our findings that anti-CSF-1R unleashes type I IFN response in tumours.

Our study shows that anti-CSF-1R depletes the majority of F4/80+ TAMs; however, a small intratumoural CD11b+F4/80+ population with a distinct phenotype survives. Interestingly, these surviving cells express lower levels of Csf1r and significantly higher Ifna mRNA levels than the CD11b+F4/80+ cells in untreated tumours, probably accounting for the increased Ifna levels in the tumours. A shift in macrophage phenotype was also observed in pancreatic cancer and glioblastoma models upon interference with the CSF-1–CSF-1R pathway. Similar to our model, targeting CSF-1 in the pancreatic cancer models on the one hand depleted TAMs and on the other hand reprogrammed the remaining macrophages to an antitumour phenotype. Interestingly, type I IFN was also found to...
Fig. 6 Neutrophil inhibition engages antitumour immunity and further improves cisplatin + anti-CSF-1R efficacy. a, CD8⁺ T cell/Foxp3⁺ T cell ratio based on immunohistochemistry staining in the tumour of time-point-sacrificed KEP mice (cisplatin + control Ab and cisplatin + anti-CSF-1R: n = 7 animals; cisplatin + anti-CSF-1R + anti-Ly6G: n = 6 animals). b, c, Quantification of NKp46⁺ cells (b) and granzyme B⁺ cells (c) in viable areas of mammary tumours of time-point-sacrificed KEP mice treated with cisplatin + control Ab (NKp46: n = 5 animals; granzyme B: n = 15 animals), cisplatin + anti-CSF-1R (NKp46: n = 5 animals; granzyme B: n = 15 animals), cisplatin + anti-CSF-1R + anti-Ly6G (NKp46: n = 5 animals; granzyme B: n = 7 animals). The values represent the average number of positive cells per FOV quantified by counting five high-power microscopic fields per tumour. Representative granzyme B immunohistochemistry stainings are shown (d). Scale bars, 50 μm. d, Kaplan–Meier tumour-specific survival curves of KEP mice treated with cisplatin + control Ab (n = 6 animals), cisplatin + anti-CSF-1R (n = 16 animals) and cisplatin + anti-CSF-1R + anti-Ly6G (n = 14 animals). Cisplatin + anti-CSF-1R + anti-Ly6G treated mice versus cisplatin + anti-CSF-1R-treated mice: P = 0.3728 (two-tailed log-rank test). e, Kaplan–Meier tumour-specific survival curves of KEP mice treated with cisplatin + control Ab, cisplatin + anti-CSF-1R (same groups as in Fig. 1f), cisplatin + anti-Ly6G (n = 17 animals), cisplatin + anti-CSF-1R + anti-Ly6G (n = 16 animals), or cisplatin + anti-CSF-1R + anti-CSF-1R + anti-Ly6G + anti-CD8 (n = 17 animals). Cisplatin + anti-CSF-1R-treated mice versus cisplatin + anti-CSF-1R + anti-Ly6G-treated mice: P = 0.0885; cisplatin + anti-CSF-1R-treated mice versus cisplatin + anti-CSF-1R + anti-Ly6G + anti-CD8-treated mice: P = 0.1104 (two-tailed log-rank test). f, Kaplan–Meier tumour-specific survival curves of KEP mice treated with cisplatin + anti-CSF-1R (n = 16 animals, same curve as in d), cisplatin + anti-CSF-1R + anti-Ly6G (n = 17 animals of which 4 mice were treated with cisplatin + anti-CSF-1R + anti-Ly6G + IgG2a; no differences were observed between cisplatin + anti-CSF-1R + anti-Ly6G and cisplatin + anti-CSF-1R + anti-Ly6G + IgG2a, or cisplatin + anti-CSF-1R + anti-Ly6G + anti-NK11 (n = 12 animals). Cisplatin + anti-CSF-1R-treated mice versus cisplatin + anti-CSF-1R + anti-Ly6G-treated mice: P = 0.0226; cisplatin + anti-CSF-1R-treated mice versus cisplatin + anti-CSF-1R + anti-Ly6G + anti-NK11-treated mice: P = 0.4073 (two-tailed log-rank test). g, Kaplan–Meier tumour-specific survival curves comparing cisplatin + anti-CSF-1R and cisplatin + anti-CSF-1R + anti-Ly6G treatment in KEP (same as in f) and KEP/Rag1⁻/⁻ mice. Cisplatin + anti-CSF-1R in KEP/Rag1⁻/⁻ (n = 12 animals) and cisplatin + anti-CSF-1R + anti-Ly6G in KEP/Rag1⁻/⁻ (n = 11 animals). Cisplatin + anti-CSF-1R + anti-Ly6G-treated KEP/Rag1⁻/⁻ mice versus cisplatin + anti-CSF-1R-treated KEP/Rag1⁻/⁻ mice: P = 0.9597 (two-tailed log-rank test). Data presented in a–c are mean ± s.e.m., and statistical analysis was performed using the two-tailed Mann–Whitney test.
be increased in these macrophages; however, its effect was not functionally pursued in this study. These data, combined with our observation that IFN-α is also upregulated in anti-CSF-1R-treated MC38 colon adenocarcinoma tumours, indicate that anti-CSF-1R-mediated induction of type I IFNs is not limited to breast cancer, but extends to other cancer types.

Type I IFNs can directly affect cancer cells by inducing apoptosis or blocking proliferation, or indirectly by stimulating antitumour immune responses or inhibiting angiogenesis. In line with the observed in vivo reduction of proliferating tumour cells upon anti-CSF-1R therapy, our in vitro studies indicate that IFN-α can directly suppress KEp cancer cells. We did not observe an effect of CSF-1R inhibition on the number of intratumoural blood vessels or their pericyte coverage, excluding an angiogenesis effect. Despite a key role for type I IFNs in dictating antitumour immunity, the increase in the number of intratumoural type I IFNs was not sufficient to induce effective antitumour T cell responses. In line with the immunosuppressive phenotype of tumour-educated neutrophils in KEp mice and in other models, the additional ablation of neutrophils stimulated antitumour immunity. It may be surprising that we observed a therapeutic benefit of depletion of neutrophils with an IFN gene signature in cisplatin + anti-CSF-1R-treated KEp mice, whereas some studies have suggested that type I IFNs can induce antitumour properties in neutrophils. However, in line with our data, a type I IFN transcriptional signature in neutrophils in malaria-infected hosts and in patients with active tuberculosis correlated with tissue damage and disease pathogenesis, suggesting that, in these settings, type I IFN signalling in neutrophils may contribute to their harmful actions. In addition, although type I IFNs are often considered to exert antitumour functions, several studies on chronic viral infections show negative-feedback mechanisms when persistently present in the environment by, for example, generating an immunosuppressive milieu. Perhaps in our study a similar mechanism is involved, explaining why the cytotoxic activity of platinum-based chemotherapy is enhanced by type I IFNs, but at the same time, involved, explaining why the cytotoxic activity of platinum-based chemotherapy response during cisplatin treatment.

Necessary for cisplatin in vivo data demonstrate that, although the release of type I IFNs is in the context of chemotherapy was not completely resolved. Our online content

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Interestingly, the platinum-based drugs cisplatin and oxaliplatin synergized with anti-CSF-1R treatment, whereas docetaxel did not, despite the induction of IFN-α in the docetaxel setting. It will be important to mechanistically understand how the type of chemotherapy dictates its ability to act in synergy with type I IFN signalling. These insights will facilitate the development of optimal combination therapies of CSF-1R-targeting drugs or other type I IFN-inducing agents, including STING (stimulator of IFN genes) agonists, with chemotherapeutic agents. To maximize the therapeutic benefit of cytotoxic therapy in poorly immunogenic tumour types, it will be critical to simultaneously target neutrophil-dependent immunosuppression.
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Methods

Mice. The generation and characterization of KEPI mice have been previously described\(^1\) and are commercially available via Taconic Biosciences. KEPI mice were back-crossed onto the FVB/N background, and genotyping was performed by PCR analysis of the tail-tip DNA as described\(^2\). KEPI mice were crossed with Rag1\(^{-/-}\) mice (FVB/N, a gift from L. Coussens, Oregon Health & Science University, Portland) to generate KEPI,Rag1\(^{-/-}\) mice\(^1\). Female KEPI and KEPI,Rag1\(^{-/-}\) mice were monitored twice weekly for the spontaneous onset of mammary tumour formation by palpation starting at 4 months of age. Donor tumours from KEPI and KEPI,Rag1\(^{-/-}\) mice were collected in ice-cold PBS, cut into small pieces and resuspended in DMEM F12 containing 30% FCS and 10% dimethyl sulfoxide and stored at -150°C. The perpendicular tumour diameters of mammary tumours were measured twice a week using a caliper. Age-matched wild-type littermates were described above. Mice were sacrificed 5 d after the second treatment\(^2\).

Histology, immunohistochemistry, immunofluorescence and RNA in situ

Antibody treatment continued until recipient mice developed clinical signs of metastatic disease. Tumour-bearing recipient mice were treated either with control antibody or with anti-CSF-1R once the mammary tumours were injected intraperitoneally into mice 90 min before being sacrificed. Treatment started at a tumour size of 25 mm\(^2\) and continued until the tumour reached a size of 100 mm\(^2\) in the therapy-responsive phase or at a tumour size of 100 mg in the end-stage (±100 mg) or at 100 mm\(^2\) in the end-stage (±100 mm\(^2\)) disease. A single loading dose of 400 μg of control antibody or 0.5 mg of anti-CSF-1R (Fresenius Kabi) was administered intravenously every 10 days for three cycles. The MTD dose of oxaliplatin (6 mg per kg diluted in NaCl; Fresenius Kabi) was administered intravenously every 10 days for three cycles. The MTD dose of docetaxel (15 mg per kg; Accord Healthcare Limited) was administered intravenously every week for a total of four cycles. The MTD dose of doxetaxel (15 mg per kg; Accord Healthcare Limited) was administered intravenously every week for a total of four cycles. The MTD dose of oxaliplatin (6 mg per kg diluted in NaCl; Fresenius Kab) was administered intravenously every 10 days for three cycles.

Anti-CSF-1R, control antibodies, anti-Ly6G, anti-CD8 and anti-NK1.1 treatment started when mammary tumours reached a size of 25 mm\(^2\); and anti-IFNAR1, ckit, doxetaxel and oxaliplatin treatment started when mammary tumours reached a size of 50 mm\(^2\). For time-point analyses, mice were killed 1 d after the second chemotherapy injection (therapy-responsive phase) or at a tumour size of 100 mm\(^2\) in chemotherapy-naive mice. To assess tumour cell proliferation, BrdU (50 mg per kg) was injected intraperitoneally into mice twice a week. The MTD dose of doxetaxel (15 mg per kg; Accord Healthcare Limited) was administered intravenously every week for a total of four cycles. The MTD dose of oxaliplatin (6 mg per kg diluted in NaCl; Fresenius Kab) was administered intravenously every 10 days for three cycles.

Flow cytometry

KEPI tumours were collected in ice-cold PBS and processed as described\(^3\). Briefly, samples were mechanically chopped using the McIlwain tissue chopper (Mickle Laboratory Engineering) and enzymatically digested with 3 mg ml\(^{-1}\) collagenase type A (Roche) and 25 μg ml\(^{-1}\) DNase I (Sigma) in serum-free IMDM containing 8% FCS, 100 IU ml\(^{-1}\) penicillin, 100 μg ml\(^{-1}\) streptomycin, 0.5% β-mercaptoethanol, 50 mm ml\(^{-1}\) PMA, 1 μM ionomycin and Golgi-Plug (1:1,000; BD Biosciences) for 1 h at 37°C. After fixation for 1 h at 4°C, samples were stained with fluorochrome-conjugated antibodies (Supplementary Table 1). For intracellular staining of granzyme B, single-cell suspensions were stimulated in free medium for 1 h at 37°C in the presence of ionomycin and Golgi-Plug. APC conjugates were acquired on BD LSRII or BD LSRIF. Flow cytometry was performed using FlowJo software version 9.9.6.

Isolation of intratumoural cell populations

Primary mammary tumours were harvested from KEPI mice 1 d after two cycles of chemotherapy (±100 mm\(^2\)) or at end-stage (±225 mm\(^2\)), and single-cell suspensions were generated as described above. Enrichment of CD11b\(^{+}\) cells was performed using magnetic columns (Miltenyi Biotec), as described previously\(^4\). Briefly, single-cell suspensions were stained with anti-CD11b-APC (1:200; clone M1/70, eBioscience) for 20 min and incubated with magnetic anti-APC MicroBeads according to the manufacturer’s instructions (Miltenyi Biotec). CD11b\(^{+}\) cells were isolated with LS columns (Miltenyi Biotec) according to the manufacturer’s instructions. For the isolation of macrophages and neutrophils from tumours through the therapy-responsive phase, the enriched CD11b\(^{+}\) fraction was stained with antibodies against Ly6G-FITC (1:200; clone 1A8, BD Biosciences), F4/80-PE (1:200; clone BM8, eBioscience), and Ly6C-PE (1:400; clone Harding, BD Biosciences). LIVE/DEAD fixable aqua dead cell stain (Thermo Fisher Scientific) was added 1:100 in PBS to exclude dead cells. CD11b/F4/80\(^{+}\) macrophages and F4/80\(^{+}\)Ly6G\(^{+}\)Ly6C\(^{+}\) neutrophils were isolated with the BD FACSARIA II sorter with DIVA software (BD Biosciences). For the isolation of cell populations from end-stage tumours, we separated intratumoral CD11b\(^{+}\) and CD11b\(^{-}\) cells by magnetic cell sorting as described above. Enrichment of the CD11b\(^{+}\) and CD11b\(^{-}\) populations was performed with LS columns (Miltenyi Biotec) as described above. Supplementary Table 1. CD11b\(^{+}\)F4/80\(^{+}\) macrophages, CD11b\(^{+}\)F4/80\(^{-}\)Ly6G\(^{+}\)Ly6C\(^{-}\) monocytes, CD11b\(^{+}\)F4/80\(^{-}\)Ly6G\(^{+}\)Ly6C\(^{+}\) neutrophils, CD11b\(^{-}\)CD45\(^{-}\)CD11c\(^{-}\)
Adoptive transfer of monocytes. Front legs, hind legs and hips were collected from female mTmG mice and the bone marrow was flushed out. Bone marrow cells were incubated with Fc Block (1:50; CD16/CD32, BD Biosciences), stained with anti-Ly6G-APC (1:200; clone 1A8, BioLegend) and, consequently, negative populations were isolated as described previously. The Ly6G- fraction was then stained with fluorochrome-conjugated antibodies (Supplementary Table 1). After gating out lineage- cells (CD3, CD8, CD4, NKp46 and Ter119) and Siglec F-, Cd11b+ and Cd14+ cells were positioned. Subsequently, adoptive transfer was performed into the tail vein of a tumour-bearing KEP with control antibody and control antibody treatments started at a tumour size of 25 mm2, and 1 d after the second antibody injection (1 week apart), monocytes were transferred. KEP mice were sacrificed 4 d later and tumours were isolated for flow cytometry analysis. Antibodies are listed in Supplementary Table 1.

CRISPR-Cas9-mediated gene disruption and colony-forming assay. IFNAR1 was knocked out (KO) from a cell line derived from a spontaneous KEP mammary tumour by transient transfection with a lentiCRISPRv2 (ref. 62) vector containing IFNAR1-specific single guide RNA targeting exon 1 (sgRNA1: 5′-GCTCGCTGTCGTGGGCGCGG-3′). Twenty-four hours after transfection, cells were exposed to puromycin for 48 h. Cells were stained with IFNAR1-PE (1:200; clone MAR1-5A3, eBioscience) and IFNAR1-negative cells were sorted with BD FACSAria FUSION sorter with DIVA software (BD Biosciences). Between 1.5 and 2 106 sorted cells were infused per mouse (80–90% confluency) for 24 h in RPMI containing 8% FCS, 100 IU ml−1 recombinant M-CSF (Peprotech). After differentiation, BMDMs were harvested and seeded in a 24-well plate (400,000 BMDMs per well) and cultured overnight. The next morning, BMDMs were exposed to conditioned medium from a KEP cell cancer line in the presence of 8μg ml−1 of either control antibody or anti-CSF-1R for 24 h. Conditioned medium was obtained by culturing KEP cancer cells (80–90% confluency) for 24 h in RPMI containing 8% FCS, 100 IU ml−1 penicillin and 100 μg ml−1 streptomycin. The RNA of BMDMs was isolated with the Isolate II RNA Mini Kit (Bioline), and quantitative RT–PCR for ifnα was performed as described below.

RNA isolation and quantitative RT–PCR. RNA from sorted cells and tumours of KEP mice was isolated using TRIzol (Invitrogen). Samples were treated with DNase I (Invitrogen) followed by RNA cleanup with the Qiagen RNeasy Mini Kit according to the manufacturer’s recommendation. Isolated RNA was quantified with Nanodrop (Thermo Scientific). Transformation of RNA into cDNA was performed as described previously (Supplementary Table 1). RNA isolation and quantitative RT–PCR for ifnα was performed as described below.

RNA-seq and data analysis. RNA isolation, library construction and deep sequencing. CD11b- and Cd14- immunoselected immune cell populations were isolated as described above from KEP tumours treated with either control antibody, anti-CSF-1R, cisplatin + control antibody or cisplatin + anti-CSF-1R at the therapy-responsive phase (tumour size ≥ 100 mm3). Some of the biological replicates consisted of pools of cells from two to six different mice. Total RNA was extracted using the RNeasy Mini and Micro kit (QIAGEN). RNA sequencing libraries were prepared and sequenced using the Ovation RNA-seq system V2 and Encore Rapid library systems protocols (NuGen), 10 ng RNA was converted into cDNA libraries, subsequently sequenced on a HiSeq 1500 system and demultiplexed using CASAVA v1.8 (Illumina).

Preprocessing of sequenced data. Using default parameters, all reads were aligned against the murine mm10 reference genome by TopHat2 v2.0.11 (ref. 62). The data were imported into Partek Genomics Suite v6.6 (PGS), and the gene and transcript information was deduced before conducting normalization utilizing statistical software R (v3.3.1) and the DESeq2 package (https://www.bioconductor.org/packages/3.3/bioc/html/DESeq2.html). Normalized read counts were floored to a value of at least one thereafter and the data set was trimmed by defining a gene as expressed if the maximum value over all group means was higher than ten.

Identification of differentially expressed genes. Using PGS, a two-way analysis of variance (ANOVA) was performed to compute the top variable genes (treatment versus control) within the data set, as well as differentially expressed genes present in cisplatin + anti-CSF-1R neutrophils (versus cisplatin + control antibody neutrophils). Genes were defined to be differentially expressed when having a fold change ≥ 2 or ≤ 0.5. Based on previous knowledge, hierarchical clustering was performed on the top 400 variable genes within the data set (neutrophils and macrophages, cisplatin + anti-CSF-1R versus cisplatin + control antibody) using default settings in PGS.

Gene Ontology enrichment analysis. Gene Ontology network visualization, Ingenuity pathway analysis and GSEA. To link transcriptional information to previous knowledge, we applied Gene Ontology enrichment analysis on the 100 most upregulated and 100 most downregulated genes (fold change ≥ 1.5, unadjusted P ≤ 0.05) extracted from neutrophils exposed to cisplatin + anti-CSF-1R treatment (versus cisplatin + control antibody-exposed neutrophils). Subsequently, the data were visualized using BioNGO69, EnrichmentMap70 and WntClouding71 plug-ins in CytoScape. In addition, all differentially expressed genes found in neutrophils were analysed with Ingenuity pathway analysis (Qiagen).

GSEA was performed utilizing the BubbleGUM GSEA tool1 to find enriched pathways in macrophages from cisplatin + anti-CSF-1R-treated tumours (versus cisplatin + control antibody macrophages). Pathways interrogated were derived from the reactome gene sets, and all pathways demonstrating a significant enrichment (false discovery rate (FDR) ≤ 0.25) in one condition were shown. Specifically addressing enrichment of EG2R target genes in neutrophils from anti-CSF-1R-treated tumours versus neutrophils from control antibody-treated tumours, GSEA was employed on transcription factor target gene sets using the Gene Set Enrichment Analysis (GSEA) previously published65. The reactome and transcription factor target gene sets were obtained via the online available Molecular Signatures Database (MsigDB) of the Broad Institute (http://software.broadinstitute.org/gsea/msigdb/index.jsp).

No custom codes were used in the manuscript.

Evaluation of expression of ISGs in patient biopsies. The selection of type I ISGs was based on the RNA-seq results from our KEP mouse model upon cisplatin + anti-CSF-1R. The genes belong to the biological processes listed in Supplementary Table 3. We assessed the effect of anti-CSF-1R on these selected genes in human tumours by analysing RNA-seq data of paired biopsy and on-treatment tumour biopsies of patients enrolled in a clinical phase I trial with emactuzumab (RG7155), a humanized anti-human CSF-1R monoclonal antibody. Biopsies were taken from a multicentre, open-label study (ClinicalTrials.gov identifier NCT01494688). Patients received emactuzumab every 2 weeks as intravenous infusion. Tumour biopsies of 31 patients with a broad range of different solid malignancies treated with either emactuzumab alone or in combination with paclitaxel (with an overrepresentation of breast cancer (n = 13) and ovarian cancer (n = 7) samples) were collected. The study was conducted in accordance with the Declaration of Helsinki, current International Conference on Harmonisation of Technical Requirements forRegistration of Pharmaceuticals for Human Use guidelines and all applicable regulatory and ethical requirements. The study is compliant with all relevant ethical regulations regarding research involving human participants. All patients provided written informed consent before study-related procedures were performed. RNA extraction, RNA-seq and data analysis were performed as previously described.

Statistics and reproducibility. Information on study design, sample size, number of biological replicates, number of independent experiments and statistical analysis is reported in the main text and figure legends. The survival curves of cisplatin + control antibody (or cisplatin only)-treated, cisplatin + anti-CSF-1R-treated and cisplatin + anti-CSF-1R + anti-Ly6G-treated mice were compared using the Log-rank (Mantel-Cox) test, with a P-value of 0.05 as the threshold. Survival curves of cisplatin + control antibody (or cisplatin only)-treated, cisplatin + anti-CSF-1R-treated and cisplatin + anti-CSF-1R + anti-Ly6G-treated mice were compared using the Log-rank (Mantel-Cox) test, with a P-value of 0.05 as the threshold. Survival curves of cisplatin + control antibody (or cisplatin only)-treated, cisplatin + anti-CSF-1R-treated and cisplatin + anti-CSF-1R + anti-Ly6G-treated mice were compared using the Log-rank (Mantel-Cox) test, with a P-value of 0.05 as the threshold.
Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability
The RNA-seq data derived from mouse samples that support the findings of this study have been deposited in the Gene Expression Omnibus (GEO) repository under accession number GSE101881. Source data for Figs. 1a,d–i, 2, 3c–h, 4, 5 and 6 and Supplementary Figs. 1a,b,e,f,h,i, 2, 3e–h, 5a–c,f–i and 6a–f–n have been provided as Supplementary Table 4. All other data supporting the findings of this study are available from the corresponding author on reasonable request.

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Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- The statistical test(s) used AND whether they are one- or two-sided
- Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- A description of all covariates tested
- A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. $F$, $t$, $r$) with confidence intervals, effect sizes, degrees of freedom and $P$ value noted
- Give $P$ values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen's $d$, Pearson's $r$), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection

- For Flow cytometry data: data acquisition was performed on BD LSRII flow cytometer using Diva software version 8.0.1 (BD Biosciences).
- For isolation of immune cells: data acquisition was performed on BD FACSARIA II sorter with DIVA software version 8.0.1 (BD Biosciences) and BD FACSARIA FUSION sorter with DIVA software version 8.0.1 (BD Biosciences).
- For RNAseq of murine immune cell populations: according to the Ovation RNA-Seq system V2 and Encore Rapid library systems protocols (NuGen), RNA was converted into cDNA libraries, subsequently sequenced on a HiSeq 1500 system and demultiplexed using CASAVA v1.8 (Illumina).
- For the quantitative assessment of areas in the tumor that had lost viability: H&E slides were digitally processed using the Aperio ScanScope (Aperio, Vista, CA).
- For Luminex cytokine array: data acquisition was performed with Bio-Plex 200 reader, using Bio-Plex Manager 6.0 software (Bio-Rad).
- For immunohistochemistry images were acquired with cellSens Entry software v. 1.5 (Olympus).
- For immunofluorescence of CD31 and alpha-SMA, images were taken with LAS AF software version 2.7.4 (Leica) on Leica SP5 confocal microscope.

Data analysis

- Statistical analyses were performed using GraphPad Prism 7 and 8 (GraphPad Software Inc., La Jolla, CA).
- Flow cytometry data analysis was performed using Flowjo software version 9.9.6.
- For the RNA-Seq analysis of murine samples, TopHat2 v2.0.11, Partek Genomics Suite v6.6, R (v3.3.1), Cytoscape (BiNGO, EnrichmentMap and Word Clouding plug-ins), Ingenuity Pathway analysis (QIAGEN) and BubbleGUM GSEA were used. The reactome and transcription factor target gene sets were obtained via the online available Molecular Signatures Database (MSigDB) of the Broad Institute.
- For Luminex cytokine array: data analysis was performed with Bio-Plex 200 reader, using Bio-Plex Manager 6.0 software (Bio-Rad).
- For the quantitative assessment of areas in the tumor that had lost viability, slides were analyzed with ImageJ (1.47t)

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. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.
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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

The RNA-Seq datasets derived from mouse samples are available in the Gene Expression Omnibus (GEO) repository under accession number GSE101881. (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE101881).

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Life sciences

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Life sciences study design
All studies must disclose on these points even when the disclosure is negative.

Sample size
No statistical method was used to predetermine sample size. Our previous experience with the mouse model allowed us to determine an adequate sample size in each experiment.
For survival curves, at least 10 mice were enrolled in each group.
For immunohistochemistry, flow cytometry and RT-qPCR experiments, >4 biological replicates were used.

Data exclusions
The following criteria were pre-established:
H&E slides of de novo tumors of K14cre;Cdh1F/F;Trp53F/F mice enrolled in intervention studies, were checked at the end of the experiment for the type of tumor. Skin tumors were excluded from the study and from other analyses because the study focus on mammary tumors.
For the count of metastatic nodules in the lungs in the metastasis model, mice that developed overt metastatic disease were included in the analysis and mice that were sacrificed because of local recurrence of the primary tumor were excluded.
For RT-PCR analysis, samples were run in duplicate and if the difference between the Ct values of the duplo was bigger than 1 cycle, samples were considered unreliable and discarded.
The following criteria were not pre-established:
In case of technical flaws during sample processing, collection or data acquisition, selected samples were excluded from specific analyses.

Replication
The in vitro experiments were repeated at least 3 times, as stated in the figure legend, and showed similar results.
For the in vivo intervention studies, we repeated survival curves of cisplatin, cisplatin/anti-CSF-1R and cisplatin/anti-CSF-1R/anti-Ly6G in a different mouse facility of the Netherlands Cancer Institute and showed the same results.

Randomization
The first animal was assigned randomly in the treatment or control group, after which each subsequent animal was placed in the next group.

Blinding
For the in vivo tumor measurements, the investigator did not know the tumor size of the previous measurement. In addition, the analyses in the lab was performed in a blinded fashion. IHC counts were performed by 2 or 3 independent researchers in a blinded fashion.

Reporting for specific materials, systems and methods
We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

| Materials & experimental systems | Methods |
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| [ ] | Clinical data |
| [ ] | Involved in the study |
| [ ] | ChIP-seq |
| [ ] | Flow cytometry |
| [ ] | MRI-based neuroimaging |
Antibodies

Antibodies used

The anti-CSF-1R antibody was provided by Roche Innovation Center Munich. A detailed list of antibodies used for immunohistochemistry, immunofluorescence and flow cytometry (including vendor, catalog number and dilution) is found in Supplementary Table 1-3. In addition, we used:

- CD68 (1:2000, clone KP1, Dako, catalog number: M0814) was performed by NKI-Avl Core Facility Molecular Pathology & Biobanking (CFMPB) on FFPE material of invasive lobular carcinoma breast cancer patients from the RATHER cohort.
- other antibodies for flow cytometry: CD11b-APC (1:200; clone M1/70, eBioscience; catalog #17-0112-82), Ly6G-APC (1:200, clone 1A8, Biolegend, catalog #127614), IFNAR1-PE (1:200, clone MAR1-5A3, eBioscience, catalog#12-5945-82).

For intervention studies: the chimeric (hamster/mouse) anti-CSF-1R antibody (clone 2G2, Roche Innovation Center Munich); control antibody (IgG1, MOPC21, Roche Innovation Center Munich); anti-Ly6G antibody (1A8, BioXCell, catalog# BE0075); anti-IFNAR1 (MAR1-5A3, BioXCell, catalog# BE0241); anti-CD8 (2.43, BioXCell, catalog#: BE0061); anti-NK1.1 (PK136, BioXCell, catalog# BE0036); IgG2a (C1.18.4, BioXCell, catalog# BE0085).

Validation

All antibodies used, except for anti-CSF-1R antibody for intervention studies, are commercially available. Antibody-mediated depletion of immune cells was validated by the use of a non-competitive antibody. Antibodies used for IHC were validated by the Pathology facilities at the NKI and at the Research UK Beatson Institute.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)
KEP cell line was derived from a spontaneous KEP mammary tumor. MC38 tumors were provided by Roche. IFNAR1 was knocked out from the KEP cell line as described in the methods.

Authentication
None of the cell line used were authenticated

Mycoplasma contamination
KEP cell line were tested negative for mycoplasma contamination

Commonly misidentified lines (See iclac register)
no commonly misidentified lines were used

Animals and other organisms

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

Laboratory animals
Female K14cre;Cd1f1F/Trp53F/F (KEP) mice on the FVB/N background were used. KEP mice were crossed with Rag1-/- mice (FVB/N, a gift from L. Coussens) to generate K14cre;Cd1f1F/Trp53F/F;Rag1-/- (KEP;Rag1-/-). Spontaneous tumor development starts at 6-8 months of age. Age-matched WT littermates were used as controls. Female FVB/N mice (age 10-12 weeks) were obtained from Charles River. mTmG mice were back-crossed to the FVB/N background and mTmG female mice (8-24 weeks of age) were used for experiments. K14cre;Trp53F/F female mice develop tumors at 8-10 months of age.

Wild animals
The study does not involve wild animals

Field-collected samples
The study does not involve field-collected samples

Ethics oversight
Animal experiments were approved by the Animal Ethics Committee of the Netherlands Cancer Institute and performed in accordance with institutional, national and European guidelines for Animal Care and Use.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Human research participants

Policy information about studies involving human research participants

Population characteristics
For the immunohistochemistry analysis of CD68 expression, we used FFPE archival material of invasive lobular carcinoma breast cancer patients from the EU FP7 RATHER project (termed ‘RATHER’, http://www.ratherproject.com/) recruited at the NKI. Please refer to Michaut M., et al., Sci Rep, 2016 and Schouten P.C., et al. Mol Oncol, 2015 for patients information. For the assessment of the effect of CSF-1R blockade on selected genes in human tumors we analyzed RNA-Seq data of paired baseline and on-treatment patient samples from a Clinical Phase 1 Trial with emactuzumab (RG7155), a humanized anti-human CSF-1R monoclonal antibody. Biopsies were taken from a multicenter, open-label study (clinicaltrials.gov identifier NCT01494688). Patients had a broad range of different solid malignancies (breast cancer [n=13], ovarian cancer [n=7], CRC [n=3], Head and neck cancer [n=1], Intrahepatic cholangiocarcinoma [n=1], malignant sarcomatous fibrous tumor [n=1], mesothelioma [n=3], pancreatic cancer [n=1] and prostate cancer [n=1] samples) and were treated with either emactuzumab alone or in combination with paclitaxel.

Recruitment
Please note, that we did not recruit patients for this manuscript. We have analyzed archival patient samples that originally were...
Recruitment

collected as part of clinical trials. Immunohistochemistry analysis for CD68 expression was performed on archival FFPE material of invasive lobular carcinoma breast cancer patients from the RATHER cohort enrolled at the NKI. Anonymized archival tissue was used according to national guidelines regarding the use of archival material and with approval of the NKI-AVL translational research board (TRB). For the data from the Clinical Phase 1 Trial with emactuzumab (RG7155) please refer to clinicaltrials.gov identifier NCT01494688 where all inclusion and exclusion criteria can be found. The study was conducted in accordance with the Declaration of Helsinki, current International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guidelines and all applicable regulatory and ethical requirements. All patients provided written informed consent before study-related procedures were performed. In this regular Ph1 cancer patient cohort, certain tumor types like HCC or gastric cancers were excluded and there was a self-selection bias towards patients who had tumor lesion amenable to biopsies. However, these biases are unlikely to impact results, since we compared pre-treatment versus on-treatment samples of the same patient cohort. There is no such covariate-relevant characteristic as we make a pre- and post emac treatment comparison.

Ethics oversight

for samples of the RATHER cohort, the NKI-AVL and Cambridge medical ethical committees approved the study and the use of anonymized archival tissue (Michaut M. et al., Sci Rep, 2016; Schouten P.C. et al., Mol Oncol, 2015). In addition, we obtained approval of the NKI Translational Research Board (TRB) to use the anonymized archival tissues for the current study. For the data from the Clinical Phase 1 trial with emactuzumab (clinicaltrials.gov identifier NCT01494688), the study was conducted in accordance with the Declaration of Helsinki, current International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guidelines and all applicable regulatory and ethical requirements.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

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

KEP tumors were collected in cold PBS and processed as described (Salvagno C. & de Visser K.E., Methods Mol Biol, 2016). Briefly, samples were mechanically chopped using the McIlwain tissue chopper (Mickle Laboratory Engineering) and enzymatically digested with 3 mg/ml collagenase type A (Roche) and 25 μg/ml of DNase I (Sigma) in serum-free medium for 1h at 37°C in a shaking water bath. After washing, cells were stained with fluorochrome-conjugated antibodies (Supplementary Table 2) for 20 min at 4°C in the dark in PBS containing 0.5% BSA. 7AAD (1:20; eBioscience) or Fixable Viability Dye eFluor 780 (1:1000; eBioscience) was added to exclude dead cells. For intracellular staining, single-cell suspensions were stimulated in IMDM containing 8% FCS, 100 μg/ml penicillin, 100 μg/ml streptomycin, 0.5% β-mercaptoethanol, 50 ng/ml PMA, 1 μM ionomycin and Golgi-Plug (1:1000; BD Biosciences) for 3 h at 37°C. Following surface antigen staining, samples were fixed and permeabilized (BD Biosciences) and stained for intracellular proteins. For antibody panels please refer to Supplementary Table 3.

Instrument

Data acquisition was performed on BD LSRII flow cytometer using Diva software version 8.0.1 (BD Biosciences). For isolation of immune cells: data acquisition was performed on BD FACSAria II sorter with DIVA software version 8.0.1 (BD Biosciences) and BD FACSARIA FUSION sorter with DIVA software version 8.0.1 (BD Biosciences).

Software

The software used to collect data was Diva software version 8.0.1 (BD Biosciences) and data analysis was performed using FlowJo software version 9.9.6.

Cell population abundance

The purity of sorted population was higher than 92% for macrophages, 97% for tumor cells, 88% for monocytes, neutrophils and lymphocytes. For RNAseq analysis, the purity of sorted macrophages was 91% +/- 5.6 and of sorted neutrophils was 94% +/- 4.4. Values represent mean +/- SD.

For the tdTomato+ adoptive transfer, monocytes with a purity higher than 98% were used.

Gating strategy

The morphologic gate (FSC/SSC) included all cells, but debris. Consequently doublets (FSC-H/FSC-A and SSC-H/SSC-A) and dead cells were excluded. Immune cells were then gated based on their specific markers. See Supplementary Figure 3 and Supplementary Fig. 5. For markers like CD80, CD86, PD1L, CCR2 and CX3CR1, the boundaries between “positive” and “negative” were defined with a FMO (fluorescence minus one) sample. For Supplementary Fig.6d, morphologic gate (FSC/SSC) included only lymphocytes.

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