Supplementary Box 1 | Emerging trend where patient response rate and PFS does not translate into significantly increased overall survival in phase III trials

Patient response rate and progression-free survival (PFS) has not always translated into significantly increased overall survival in phase III trials (Tables 1 and 2). This has been observed in trials assessing bevacizumab (Tables 1 and 2) in patients with RCC patients treated in combination with interferon-α (AVOREN[1] and *CALGB 90206*[2] studies); in patients with MBC treated in combination with chemotherapy (E2100,[3] AVADO,[4] Ribbon-1,[5] and Ribbon-2[6]); in patients with NSCLC treated in combination with gemcitabine and cisplatin (AVAiL[7] study); and in patients with prostate or pancreatic cancer treated in combination with chemotherapy (CALGB80303,[8] CALGB90401,[9] and AviTA[10] (Table 1).

1. Escudier, B. *et al.* Phase III trial of bevacizumab plus interferon alfa-2a in patients with metastatic renal cell carcinoma (AVOREN): final analysis of overall survival. *J. Clin. Oncol.* **28**, 2144–2150 (2010).
2. Rini, B. I. *et al.* Phase III trial of bevacizumab plus interferon alfa versus interferon alfa monotherapy in patients with metastatic renal cell carcinoma: final results of CALGB 90206. *J. Clin. Oncol.* **28**, 2137–2143 (2010).
3. Miller, K. *et al.* Paclitaxel plus bevacizumab versus paclitaxel alone for metastatic breast cancer. *N. Engl. J. Med.* **357**, 2666–2676 (2007).
4. Miles, D. W. *et al.* Phase III study of bevacizumab plus docetaxel compared with placebo plus docetaxel for the first-line treatment of human epidermal growth factor receptor 2-negative metastatic breast cancer. *J. Clin. Oncol.* **28**, 3239–3247 (2010).
5. Robert, N. J. *et al.* RIBBON-1: Randomized, double-blind, placebo-controlled, phase III trial of chemotherapy with or without bevacizumab (B) for first-line treatment of HER2-negative locally recurrent or metastatic breast cancer (MBC). *J. Clin. Oncol.* **27** (Suppl.), abstr. 1005 (2009).
6. Brufsky, A. *et al.* Progression-free survival (PFS) in patient subgroups in RIBBON-2, a phase III trial of chemotherapy (chemo) plus or minus bevacizumab (BV) for second-line treatment of HER2-negative, locally recurrent or metastatic breast cancer (MBC). *J. Clin. Oncol.* **28** (Suppl.), abstr. 1021 (2010).
7. Reck, M. *et al.* Overall survival with cisplatin-gemcitabine and bevacizumab or placebo as first-line therapy for nonsquamous non-small-cell lung cancer: results from a randomised phase III trial (AVAiL). *Ann. Oncol.* **21**, 1804–1809 (2010).
8. Kindler, H. L. *et al.* Gemcitabine plus bevacizumab compared with gemcitabine plus placebo in patients with advanced pancreatic cancer: phase III trial of the Cancer and Leukemia Group B (CALGB 80303). *J. Clin. Oncol.* **28**, 3617–3622 (2010).
9. Kelly, W. K. *et al.* A randomized, double-blind, placebo-controlled phase III trial comparing docetaxol, prednisone, and placebo with docetaxel, prednisone, and bevacizumab in men with metastatic castration-resistant prostate cancer (mCRPC): Survival results of CALB 90401. *J. Clin. Oncol.* **28** (Suppl.), LBA4511 (2010).
10. Van Cutsem, E. *et al.* Phase III trial of bevacizumab in combination with gemcitabine and erlotinib in patients with metastatic pancreatic cancer. *J. Clin. Oncol.* **27**, 2231–2237 (2009).

Supplementary Box 2

The first exception includes the aforementioned use of bevacizumab as a second-line monotherapy in patients with glioblastoma multiforme, where approval was based on improvements in quality of life and objective response rates in a phase II trial, which has yet to translate into improvements in overall survival. Whether this improvement arises as a result of tumor shrinkage or a noted (beneficial) consequence of VEGF blockade, namely, reduction in edema, remains unknown.[1] The second example comes from a recent announcement of improved progression-free survival when vandetanib—which has additional activity against EGFR—was given in combination with docetaxol in non-small-cell lung cancer.[2]

1. Gerstner, E. R. *et al*. VEGF inhibitors in the treatment of cerebral edema in patients with brain cancer. *Nat. Rev. Clin. Oncol.* **6**, 229–236 (2009).
2. Herbst,R.S. et al. Vandetanib plus docetaxel versus docetaxel as second-line treatment for patients with advanced non-small-cell lung cancer (ZODIAC): a double-blind, randomised, phase 3 trial. Lancet Oncol. (2010).

Supplementary Box 3

In general, two methods have been employed to disrupt VEGF pathway signaling, both of which have been reviewed recently.[1,2] In one group are extracellular, large-molecule based inhibitors such as antibodies to VEGF (bevacizumab), VEGFR2 (ramucirumab), chimeric soluble receptors (aflibercept), or fusion proteins (angiocept) and others in clinical development that specifically disrupt ligand–receptor binding and prevent receptor dimerization. In the other group are small-molecule-based inhibitors that (much less specifically) block intracellular phosphorylation and downstream signaling pathways, including the aforementioned inhibitory effects against PDGFR and other receptors.

1. Ivy, S. P., Wick, J. Y. & Kaufman, B. M. An overview of small-molecule inhibitors of VEGFR signaling. *Nat. Rev. Clin. Oncol.* **6**, 569–579 (2010).
2. Grothey, A. & Galanis, E. Targeting angiogenesis: progress with anti-VEGF treatment with large molecules. *Nat. Rev. Clin. Oncol.* **6**, 507–518 (2009).

Supplementary Box 4

Such results were observed with multiple forms of the aforementioned large-molecule inhibitors of VEGF or VEGFR binding, such as bevacizumab, VEGF-trap, RNA-based VEGF-targeted aptamers (pegaptanib), and the mouse VEGFR2 neutralizing antibody, DC101. More recently, Paez-Ribes *et al*.[1] used DC101 or various VEGF receptor tyrosine kinase inhibitors as monotherapies (both short-term and long-term treatments) in transgenic mice with genetically engineered *RIP1*-Tag2 pancreatic islet cell tumors and in mice bearing orthotopically transplanted GBMs. In drug treated mice, or in mice with *VEGF* selectively knocked out, tumors acquired an adaptive or evasive phenotype capable of increased infiltrative or aggressive patterns with wide fronts of invasion, whereas the majority of control tumors were predominantly encapsulated or microinvasive.

1. Paez-Ribes, M. *et al.* Antiangiogenic therapy elicits malignant progression of tumors to increased local invasion and distant metastasis. *Cancer Cell* **15**, 220–231 (2009).

Supplementary Box 5

The tumor microenvironment consists of multiple interacting components, such as the extracellular matrix (ECM); several cell types including endothelial cells, fibroblasts, numerous bone-marrow-derived dendritic cells (BMDCs), and infiltrating inflammatory cells; a myriad of different growth factors, hormones, chemokines, cytokines, and proteases; and processes which drive (often via tumor hypoxia) invasion and distant metastasis, such as low pH, low glucose concentrations, altered adhesion, ECM alterations, among other factors.[1] Disruption of the VEGF pathway could potentially affect all or some of these functions. For example, stromal cells (including tumor-associated fibroblasts [TAFs]) can upregulate compensatory growth factors (such as PDGF-C) in response to VEGF inhibition.[2] Also, pericytes could alter vascular

function[3-5] and, thus, have an important role in observed rapid rebounds in revascularization after cessation of VEGFR tyrosine kinase inhibitor therapy by providing a scaffold for regrowth.[6,7] Also, various proangiogenic BMDCs, such as $Gr1^+CD11b^+$ myeloid suppressor-type cells, TIE2 expressing monocytes, and tumor-associated macrophages might home to the tumor microenvironment and mediate resistance to VEGF pathway blockade via the production of the aforementioned compensatory proangiogenic factors, including Bv8 (prokineticin), G-CSF, angiopoietin-2, among others.[8–12] Of course, the tumor likely has a critical role in adaption as well, directly and indirectly, and likely includes mechanisms involving initial (intrinsic) or adaptive (acquired) changes in response to therapy. Intrinsic resistance likely depends on disease history, stage, genetic factors, and acquired resistance to antiangiogenic therapy can include vascular co-option[13] and/or numerous complimentary/supplemental proangiogenic pathways, which could compensate for VEGF inhibition,[14] among others.[5,15]

1. Steeg, P. S. & Theodorescu, D. Metastasis: a therapeutic target for cancer. *Nat. Clin. Pract. Oncol*. **5**, 206–219 (2008).
2. Crawford, Y. *et al.* PDGF-C mediates the angiogenic and tumorigenic properties of fibroblasts associated with tumors refractory to anti-VEGF treatment. *Cancer Cell* **15**, 21–34 (2009).
3. Bergers, G., Song, S., Meyer-Morse, N., Bergsland, E. & Hanahan, D. Benefits of targeting both pericytes and endothelial cells in the tumor vasculature with kinase inhibitors. *J. Clin. Invest*. **111**, 1287–1295 (2003).
4. Hirschi, K. K. & D'Amore, P. A. Control of angiogenesis by the pericyte: molecular mechanisms and significance. *EXS* **79**, 419 –428 (1997).
5. Bergers, G. & Hanahan, D. Modes of resistance to anti-angiogenic therapy. *Nat. Rev. Cancer* **8**, 592–603 (2008).
6. Mancuso, M. R. *et al.* Rapid vascular regrowth in tumors after reversal of VEGF inhibition. *J. Clin. Invest*. **116**, 2610–2621 (2006).
7. Levashova, Z. *et al.* Molecular imaging of changes in the prevalence of vascular endothelial growth factor receptor in sunitinib-treated murine mammary tumors. *J. Nucl. Med*. **51**, 959–966 (2010).
8. Lewis, C. E., De Palma, M. & Naldini, L. Tie2-expressing monocytes and tumor angiogenesis: regulation by hypoxia and angiopoietin-2. *Cancer Res*. **67**, 8429–8432 (2007).
9. Shojaei, F. *et al.* Tumor refractoriness to anti-VEGF treatment is mediated by CD11b+Gr1+ myeloid cells. *Nat. Biotechnol*. **25**, 911–920 (2007).
10. Joyce, J. A. & Pollard, J. W. Microenvironmental regulation of metastasis. *Nat. Rev. Cancer* **9**, 239–252 (2009).
11. Shojaei, F. *et al.* G-CSF-initiated myeloid cell mobilization and angiogenesis mediate tumor refractoriness to anti-VEGF therapy in mouse models. *Proc. Natl Acad. Sci. USA* **106**, 6742–6747 (2009).
12. Kerbel, R. S. Tumor angiogenesis. *N. Engl. J. Med*. **358**, 2039–2049 (2008).
13. Rubenstein, J. L. *et al.* Anti-VEGF antibody treatment of glioblastoma prolongs survival but results in increased vascular cooption. *Neoplasia* **2**, 306–314 (2000).
14. Relf, M. *et al.* Expression of the angiogenic factors vascular endothelial cell growth factor, acidic and basic fibroblast growth factor, tumor growth factor beta-1, platelet-derived endothelial cell growth factor, placenta growth factor, and pleiotrophin in human primary breast cancer and its relation to angiogenesis. *Cancer Res*. **57**, 963–969 (1997).
15. Ellis, L. M. & Hicklin, D. J. Pathways mediating resistance to vascular endothelial growth factor-targeted therapy. *Clin. Cancer Res*. **14**, 6371–6375 (2008).

Supplementary Box 6

Sometimes the term neoadjuvant therapy is used to describe any treatment of disease prior to surgery, which can include several different scenarios of patients with both primary and secondary metastatic lesions present (referred to as 'synchronous' or 'concurrent' therapy) at different stages of progression.[1] End points of objective response in the primary tumor remain the focus—such as downstaging of tumors to

decrease surgical margins (for example nephron-sparing in RCC[2])—but it is also used as a determinant of treatment efficacy, that is, primary tumor response used as surrogate determinant of whether a particular patient's metastatic disease is likely to respond to the same therapy.[3,4]

1. Cowey, C. L. *et al.* Neoadjuvant clinical trial with sorafenib for patients with stage II or higher renal cell carcinoma. *J. Clin. Oncol.* **28**, 1502–1507 (2010).
2. Silberstein, J. L. *et al.* Feasibility and efficacy of neoadjuvant sunitinib before nephron-sparing surgery. *BJU Int.* **106**, 1270–1276 (2010).
3.. Bex, A. *et al.* Neoadjuvant sunitinib for surgically complex advanced renal cell cancer of doubtful resectability: initial experience with downsizing to reconsider cytoreductive surgery. *World J. Urol.* **27**, 533–539 (2009).
4.. van der Veldt, A. A. *et al.* Sunitinib for treatment of advanced renal cell cancer: primary tumor response. *Clin. Cancer Res.* **14**, 2431–2436 (2008).

Supplementary Box 7

The dearth in neoadjuvant-model therapy testing could be owing to the extreme difficulty of such assays, as it is cumbersome to balance several variables, including establishing tumor cells that readily metastasize; determining how to assess a threshold for micrometastatic or macrometastatic disease; duration of therapy; monitoring of disease progression; among many other challenges. Theoretical limitations also include the purpose of such studies. For example, is it better to study anti-primary tumor effects (that is, tumor shrinkage) or antimetastatic effects (that is, increased survival), or both; which drugs and treatment regimens (including combinations) can be compared; can an antibody with a long half life be compared to a tyrosine kinase inhibitor that has a short-half life? These questions increase the complexity of undertaking such studies in mice, but such efforts are urgently needed, particularly given the potential that antiangiogenic effects observed in localized tumors may be quite different when treating micrometastatic and macrometastatic disease. At present, only a handful of preclinicial studies have attempted to use VEGF pathway inhibitors to test this and, thus, there is no compelling body of evidence to support clinical testing in this setting.

Supplementary Table 1 | Examples of therapy-induced metastasis in preclinical models

Treatment	**Treatment period (in relation to tumor cell inoculation)***
Chemotherapy (low-dose metronomic cyclophosphamide, maximum tolerated dose cyclophosphamide, bleomycin dacarbazine, actinomycin D, mithramycin, doxorubicin, methotrexate, cytosine arabinoside, 5-azacytidine and aphidicolin, bleomycin)	Pretreatment[1–12‡]
	Post-treatment[13,14§]
Irradiation (whole body and/or localized [usually to thorax])[¶]	Pretreatment[1,3,8–10,12,15–31‡]
	Post-treatment[32–39§]
	Human case reports[40–43]
Acute and/or chronic hypoxic stress	Post-treatment[44–46]
Inflammation (LPS, calcium pyrophosphate microcrystal injection)	Pretreatment[47,48]
	Post-treatment[49,50]
Antidepressants (desipramine and fluoxetine)	Pretreatment[51]
Surgery	Pretreatment[52–54]
Steroid hormone (cortisone)	Pretreatment[31]
Analgesic (morphine)	Post-Treatment[55,56§]
Monoclonal antibody (targeting capillary endothelial cells)	Pretreatment[57]
Hyperoxia	Pretreatment[58]
	Post-treatment[59§]
Hyperthermia	Post-treatment[60–63‖]
VEGF Receptor tyrosine kinase inhibitors (sunitinib, sorafenib, SU10944)	Pretreatment[64]

*Pretreatment studies involve experimental metastasis with treatment occurring only prior to tumor inoculation. Post-treatment studies involve treatment after tumor inoculation, including treatment of primary localized tumors or intravenous injection of tumor cells. [‡]References 12 and 19 report enhanced experimental metastasis but no enhancement or reduction of spontaneous metastasis. [§]Spontaneous metastasis increased. [‖]These references include examples of primary tumor treatment examining later metastasis development (without tumor resection). [¶]The irradiation pretreatment studies were largely conducted in mice, but some studies were carried out in rabbits, rats and dogs. Abbreviation: LPS, lipopolysaccharide.

1. van Putten, L. M., Kram, L. K., van Dierendonck, H. H., Smink, T. & Fuzy, M. Enhancement by drugs of metastatic lung nodule formation after intravenous tumour cell injection. *Int. J. Cancer.* **15**, 588–595 (1975).
2. Carmel, R. J. & Brown, J. M. The effect of cyclophosphamide and other drugs on the incidence of pulmonary metastases in mice. *Cancer Res.* **37**, 145–151 (1977).
3. Steel, G. G. & Adams, K. Enhancement by cytotoxic agents of artificial pulmonary metastasis. *Br. J. Cancer.* **36**, 653–658 (1977).
4. Vollmer, T. L. & Conley, F. K. Effect of cyclophosphamide on survival of mice and incidence of metastatic tumor following intravenous and intracardial inoculation of tumor cells. *Cancer Res.* **44**, 3902–3906 (1984).
5. Lev, D. C. *et al.* Exposure of melanoma cells to dacarbazine results in enhanced tumor growth and metastasis in vivo. *J. Clin. Oncol.* **22**, 2092–2100 (2004).
6. Wu, Y. J. *et al.* Cyclophosphamide enhances human tumor growth in nude rat xenografted tumor models. *Neoplasia.* **11**, 187–195 (2009).
7. Yamauchi, K. *et al.* Induction of cancer metastasis by cyclophosphamide pretreatment of host mice: an opposite effect of chemotherapy. *Cancer Res.* **68**, 516–520 (2008).
8. Brown, J. M. & Parker, E. T. Host treatments affecting artificial pulmonary metastases: interpretation of loss of radioactively labelled cells from lungs. *Br. J. Cancer.* **40**, 677–688 (1979).
9. Biswas, S. *et al.* Inhibition of TGF-beta with neutralizing antibodies prevents radiation-induced acceleration of metastatic cancer progression. *J. Clin. Invest.* **117**, 1305–1313 (2007).
10. Brown, J. M. & Marsa, G. W. Effect of dose fractionation on the enhancement by radiation or cyclophosphamide of artificial pulmonary metastases. *Br. J. Cancer.* **37**, 1020–1025 (1978).
11. Orr, F. W., Adamson, I. Y. R. & Young, L. Promotion of pulmonary metastasis in mice by bleomycin-induced endothelial injury. *Cancer Res* **46**, 891–897 (1986).
12. Dubravsky, N. B. *et al.* Long-term effects of pulmonary damage in mice on lung weight, compliance, hydroxyproline content and formation of metastases. *Br. J. Radiol.* **54**, 1075–1080 (1981).
13. Man, S., Zhang, Y., Gao, W., Yan, L. & Ma, C. Cyclophosphamide promotes pulmonary metastasis on mouse lung adenocarcinoma. *Clin. Exp. Metastasis.* **25**, 855–864 (2008).
14. Moore, J. V. & Dixon, B. Metastasis of a transplantable mammary tumour in rats treated with

cyclophosphamide and/or irradiation. *Br. J. Cancer.* **36**, 221–226 (1977).
15. Withers, H. R. & Milas, L. Influence of preirradiation of lung on development of artificial pulmonary metastases of fibrosarcoma in mice. *Cancer Res.* **33**, 1931–1936 (1973).
16. Brown, J. M. The effect of lung irradiation on the incidence of pulmonary metastases in mice. *Br. J. Radiol.* **46**, 613–618 (1973).
17. Dao, T. L. & Yogo, H. Enhancement of pulmonary metastases by x-irradiation in rats bearing mammary cancer. *Cancer.* **20**, 2020–2025 (1967).
18. Peters, L. J. Letter: The potentiating effect of prior local irradiation of the lungs on the development of pulmonary metastases. *Br. J. Radiol.* **47**, 827–829 (1974).
19. Peters, L. J., Mason, K., McBride, W. H. & Patt, Y. Z. Enhancement of lung colony-forming efficiency by local thoracic irradiation: interpretation of labeled cell studies. *Radiology.* **126**, 499–505 (1978).
20. Hirata, H. & Tanaka, K. Artificial metastases and decrease of fibrinolysis in the nude mouse lung after hemithoracic irradiation. *Clin. Exp. Metastasis.* **2**, 311–319 (1984).
21. Suzuki, N. Spontaneous versus artificial lung metastasis: discrepant effect of whole-body irradiation in NFSA2ALM and NFSA1SLM tumor systems. *J. Natl. Cancer Inst.* **71**, 835–839 (1983).
22. Tanaka, Y. Effect of lung irradiation on the incidence of pulmonary metastases and its mechanism. *Acta Radiol. Ther. Phys. Biol.* **15**, 142–148 (1976).
23. Thompson, S. C. Tumour colony growth in the irradiated mouse lung. *Br. J. Cancer.* **30**, 337–341 (1974).
24. Van den Brenk, H. A. & Sharpington, C. Effect of local x-irradiation of a primary sarcoma in the rat on dissemination and growth of metastases: dose-response characteristics. *Br. J. Cancer.* **25**, 812–830 (1971).
25. Milas, L., Hunter, N., Basic, I. & Withers, H. R. Protection by corynebacterium granulosum against radiation-induced enhancement of artificial pulmonary metastases of a murine fibrosarcoma. *J. Natl. Cancer Inst.* **52**, 1875–1880 (1974).
26. Van den Brenk, H. A. & Kelly, H. Stimulation of growth of metastases by local x-irradiation in kidney and liver. *Br. J. Cancer.* **28**, 349–353 (1973).
27. Van den Brenk, H. A., Burch, W. M., Orton, C. & Sharpington, C. Stimulation of clonogenic growth of tumour cells and metastases in the lungs by local x-radiation. *Br. J. Cancer.* **27**, 291–306 (1973).
28. Fidler, I. J. & Zeidman, I. Enhancement of experimental metastasis by x-ray: a possible mechanism. *J. Med.* **3**, 172–177 (1972).
29. Zeidman, I. & Fidler, I. J. Effect of irradiation on experimental metastases via lymph and blood streams. *J. Med.* **1**, 9–14 (1970).
30. Fisher, E. R. & Fisher, B. Effects of x-irradiation on parameters of tumor growth, histology, and ultrastructure. *Cancer.* **24**, 39–55 (1969).
31. KOIKE, A., NAKAZATO, H. & MOORE, G. E. The fate of Ehrlich cells injected into the portal system. *Cancer.* **16**, 716**–20**, 716–720 (1963).
32. KAAE, S. Metastatic frequency of spontaneous mammary carcinoma in mice following biopsy and following local roentgen irradiation. *Cancer Res.* **13**, 744–747 (1953).
33. Rappaport, D. S. & Brown, J. M. Influence of localized preinoculation irradiation of the foot on lymphatic metastasis from a primary tumor growing in the foot. *Radiat. Res.* **78**, 108–121 (1979).
34. Camphausen, K. *et al.* Radiation therapy to a primary tumor accelerates metastatic growth in mice. *Cancer Res.* **61**, 2207–2211 (2001).
35. de Ruiter, J., Cramer, S. J., Lelieveld, P. & van Putten, L. M. Comparison of metastatic disease after local tumour treatment with radiotherapy or surgery in various tumour models. *Eur. J. Cancer Clin Oncol.* **18**, 281–289 (1982).
36. Milas, L., Hirata, H., Hunter, N. & Peters, L. J. Effect of radiation-induced injury of tumor bed stroma on metastatic spread of murine sarcomas and carcinomas. *Cancer Res.* **48**, 2116–2120 (1988).
37. Monnier, Y. *et al.* CYR61 and alphaVbeta5 integrin cooperate to promote invasion and metastasis of tumors growing in preirradiated stroma. *Cancer Res.* **68**, 7323–7331 (2008).
38. Rofstad, E. K., Mathiesen, B. & Galappathi, K. Increased metastatic dissemination in human melanoma xenografts after subcurative radiation treatment: radiation-induced increase in fraction of hypoxic cells and hypoxia-induced up-regulation of urokinase-type plasminogen activator receptor. *Cancer Res.* **64**, 13–18 (2004).
39. Rofstad, E. K., Mathiesen, B., Henriksen, K., Kindem, K. & Galappathi, K. The tumor bed effect: increased metastatic dissemination from hypoxia-induced up-regulation of metastasis-promoting gene products. *Cancer Res.* **65**, 2387–2396 (2005).
40. Diehl, L. F., Hurwitz, M. A., Johnson, S. A., Butler, W. M. & Taylor, H. G. Skin metastases confined to a field of previous irradiation. Report of two cases and review of the literature. *Cancer.* **53**, 1864–1868 (1984).
41. Ito, H., Kubo, A., Shigematsu, N. & Hashimoto, S. Skin metastases within the previous radiation field after prophylactic postoperative radiotherapy for breast cancer. *Clin. Exp. Metastasis.* **2**, 235–239 (1984).
42. Marley, N. F. & Marley, W. M. Skin metastases in an area of radiation dermatitis. *Arch. Dermatol.* **118**, 129–131 (1982).
43. Meltzer, J., Ahmed, S. A. & Archambeau, J. O. The development of metastases within a field of previous irradiation: a case report. *Cancer.* **48**, 717–720 (1981).
44. Cairns, R. A. & Hill, R. P. Acute hypoxia enhances spontaneous lymph node metastasis in an orthotopic murine model of human cervical carcinoma. *Cancer Res.* **64**, 2054–2061 (2004).

45. Cairns, R. A., Kalliomaki, T. & Hill, R. P. Acute (cyclic) hypoxia enhances spontaneous metastasis of KHT murine tumors. *Cancer Res.* **61**, 8903–8908 (2001).
46. Rofstad, E. K., Gaustad, J. V., Egeland, T. A., Mathiesen, B. & Galappathi, K. Tumors exposed to acute cyclic hypoxic stress show enhanced angiogenesis, perfusion and metastatic dissemination. *Int. J. Cancer.* **%20.**, (2010).
47. Orr, F. W., Adamson, I. Y. & Young, L. Pulmonary inflammation generates chemotactic activity for tumor cells and promotes lung metastasis. *Am. Rev. Respir. Dis.* **131**, 607–611 (1985).
48. Florentin, I., Nolibe, D. & Giroud, J. P. Acute non-immunological inflammation inhibits natural killer (NK) activity in the lungs and enhances metastatic development. *Int. J. Exp. Pathol.* **72**, 83–95 (1991).
49. Pidgeon, G. P. *et al.* The role of endotoxin/lipopolysaccharide in surgically induced tumour growth in a murine model of metastatic disease. *Br. J. Cancer.* **81**, 1311–1317 (1999).
50. Harmey, J. H. *et al.* Lipopolysaccharide-induced metastatic growth is associated with increased angiogenesis, vascular permeability and tumor cell invasion. *Int. J. Cancer.* **101**, 415–422 (2002).
51. Kubera, M. *et al.* Age-dependent stimulatory effect of desipramine and fluoxetine pretreatment on metastasis formation by B16F10 melanoma in male C57BL/6 mice. *Pharmacol. Rep.* **61**, 1113–1126 (2009).
52. Al Sahaf, O., Wang, J. H., Browne, T. J., Cotter, T. G. & Redmond, H. P. Surgical injury enhances the expression of genes that mediate breast cancer metastasis to the lung. *Ann. Surg.* **252**, 1037–1043 (2010).
53. Baker, D. G., Masterson, T. M., Pace, R., Constable, W. C. & Wanebo, H. The influence of the surgical wound on local tumor recurrence. *Surgery.* **106**, 525–532 (1989).
54. Hofer, S. O., Shrayer, D., Reichner, J. S., Hoekstra, H. J. & Wanebo, H. J. Wound-induced tumor progression: a probable role in recurrence after tumor resection. *Arch. Surg.* **133**, 383–389 (1998).
55. Simon, R. H. & Arbo, T. E. Morphine increases metastatic tumor growth. *Brain Res. Bull.* **16**, 363–367 (1986).
56. Farooqui, M. *et al.* COX-2 inhibitor celecoxib prevents chronic morphine-induced promotion of angiogenesis, tumour growth, metastasis and mortality, without compromising analgesia. *Br. J. Cancer.* **97**, 1523–1531 (2007).
57. Kennel, S. J., Lankford, T. K., Ullrich, R. L. & Jamasbi, R. J. Enhancement of lung colony formation by treatment of mice with monoclonal antibodies to pulmonary capillary endothelial cells. *Cancer Res* **48**, 4964–4968 (1988).
58. Adamson, I. Y., Young, L. & Orr, F. W. Tumor metastasis after hyperoxic injury and repair of the pulmonary endothelium. *Lab Invest.* **57**, 71–77 (1987).
59. Shewell, J. & Thompson, S. C. The effect of hyperbaric oxygen treatment on pulmonary metastasis in the C3H mouse. *Eur. J. Cancer.* **16**, 253–259 (1980).
60. Nathanson, S. D. *et al.* Regional lymph node and pulmonary metastases after local hyperthermia of melanomas in C57BL/6 mice. *Int. J. Radiat. Oncol. Biol. Phys.* **13**, 243–249 (1987).
61. Baker, D., Sager, H. & Constable, W. The influence of levamisole and hyperthermia on the incidence of metastases from an X-irradiated tumor. *Cancer Invest.* **4**, 287–292 (1986).
62. Walker, A., McCallum, H. M., Wheldon, T. E., Nias, A. H. & Abdelaal, A. S. Promotion of metastasis of C3H mouse mammary carcinoma by local hyperthermia. *Br. J. Cancer.* **38**, 561–563 (1978).
63. Yerushalmi, A. Influence on metastatic spread of whole-body or local tumor hyperthermia. *Eur. J. Cancer.* **12**, 455–463 (1976).
64. Ebos, J. M. *et al.* Accelerated metastasis after short-term treatment with a potent inhibitor of tumor angiogenesis. *Cancer Cell.* **15**, 232–239 (2009).

Supplementary Table 2 | Preclinical testing of VEGF pathway targeted agents in mouse models of metastasis*

Drug	Cancer (species)	Metastasis model	Methodology	Effect of therapy
Studies include survival studies where metastatic disease mimics late-stage clinical metastasis and mice are given treatment until end point				
Sunitinib	Breast, melanoma (human)	Experimental	Intravenous injection	Biphasic survival effects: either unchanged, better, or worse[1]
Axitinib and bevacizumab	Melanoma (human)	Spontaneous	Resection of ectopic primary tumor	Moderate survival benefit (non significant) [2]
Sorafenib	Renal (mouse)	Experimental	Intravenous injection	Survival Benefit[3]
		Spontaneous	Resection of orthotopic primary tumor	No effect on survival[3]
VEGF-trap	Renal (mouse)	Spontaneous	Orthotopic implantation, treatment, monitor for survival.	Primary tumor and visual lung metastasis reduced[4]
pcDNA3.1-FLK1ECD (oral DNA vaccine immunotargeting VEGFR2)	Melanoma (mouse)	Experimental	Intravenous injection	Survival benefit[5]
AAV (adeno-associated virus for human VEGF)	Prostate (human)	Experimental	Intravenous injection	Survival benefit[6] Watanabe et al., 2010)
Cediranib	Prostate (human)	Experimental	Intracardiac injection	Survival benefit[7]
PTK/787	Breast (human)	Experimental	Internal carotid injection	No survival benefit[8]
Studies include those where metastasis is monitored as a secondary measure, such as in primary tumors injected orthotopically or ectopically, as xenograft or synegenic, or in spontaneous tumors generated in genetically engineered mouse models where the end point is either arbitrarily assigned or based on institutional or ethical limitations for localized disease				
Sunitinib	Lung (mouse)	Spontaneous	Transgenic (lung)	Survival benefit[9]
PTK787	Pancreatic (mouse)	Spontaneous	Transgenic (Rip-Tag)	Primary tumor reduced, no effect on metastasis[10]
PTK787	Melanoma (mouse)	Spontaneous	Orthotopic primary tumor growth; metastasis monitored at defined end point	PTK787: Primary tumor and metastasis reduced[10,11]
DC101				DC101: Primary tumor reduced, no effect on metastasis[10,11]
Sunitinib, DC101, SU10944	Pancreatic (mouse)	Spontaneous	Transgenic (Rip-Tag)	Primary tumor reduced, survival benefit, but metastasis increased[12]
Cediranib	Fibrosarcoma (mouse)	Spontaneous	Resection of ectopic (ear) tumors; treatments given in both neoadjuvant and adjuvant setting	Cediranib: reduced metastasis in neoadjuvant setting, no effect in adjuvant[13]
Vandetanib				Vandetanib: no effect in either setting[13]
SU5416, SU6668	Colorectal (human)	Spontaneous	Ectopic (intra-splenic) tumor growth; metastasis monitored at primary end point	Liver metastasis reduced[14]
NVP-AAL881	Pancreatic (human)	Spontaneous	Orthotopic primary tumor growth; metastasis monitored at defined end point	Lymph metastasis reduced, liver metastasis not reduced[15]
E7080	Breast (human)	Spontaneous	Orthotopic primary tumor growth; metastasis monitored with primary tumors present during treatment	Reduced metastasis[16]
			Orthotopic primary tumor growth; primary tumor resected, treatment initiated and metastasis monitored	No change in metastasis[16]
ZD6474	NSCLC (human), ADC (human), SSC (human)	Experimental	Intravenous injection	Reduced size of metastases (but not number) in lung[17]
Bevacizumab	Colon (human)	Spontaneous	Orthotopic primary tumor (intra-cecal) resected (along with meso-appendix lymph node); metastasis	Lung metastases reduced[18]

			monitored at defined end point (includes adjuvant treatment)	
Bevacizumab	NSCLC (human), melanoma (human)	Experimental	Intra-carotid injection; metastasis monitored at defined end point	Micrometastatic disease progression slowed (but not halted) in two out of three cell lines[19]
Bevacizumab	Uveal melanoma (mouse and human)	Spontaneous	Orthotopic (intra-ocular) primary tumor; metastasis monitored at defined end point	Decreased liver micrometastasis[20]
A4.6.1 (anti-human monoclonal antibody against VEGF)	Prostate (human)	Spontaneous	Ectopic (subcutaneous) primary tumor; metastasis monitored at defined end point (luminescence)	Lung metastasis reduced[21]
A4.6.1	Wilhms (human)	Spontaneous	Orthotopic (renal) primary tumor; metastasis monitored at defined end point (IHC)	Primary tumor and lung metastases reduced[22]
A4.6.1	Neuroblastoma (human)	Spontaneous	Orthotopic (renal) primary tumor; metastasis monitored at defined end point (IHC)	Primary tumor reduced, no effect on lung or liver metastasis, slight increase in lung metastasis[23]
A4.6.1	Colon (human)	Experimental	Intrasplenic portal-vein injection followed immediately by splenectomy; metastasis monitored at defined end point (visually or by weight in liver)	Liver metastasis reduced[24]
R&D (anti-human monoclonal antibody against VEGF)	Melanoma (human)	Spontaneous	Orthotopic primary tumor; tumor VEGF expression and metastasis driven by acute hypoxia; metastasis monitored at defined end point (visual in lung)	Reduced visible lung nodules[25]
R&D	Melanoma (human)	Spontaneous	Orthotopic primary tumor; tumor VEGF expression and metastasis driven by acute hypoxia; metastasis monitored at defined end point (visual in lung, IHC)	Reduced visible lung nodules[26]
2C3 (anti-human monoclonal antibody against VEGF)	Breast (human)	Spontaneous	Orthotopic primary tumor; metastasis monitored at defined end point (luciferase)	Reduced lung and lymph node metastasis[27]
VEGF-trap	Renal (human)	Spontaneous	Orthotopic (renal) primary tumor; metastasis monitored at defined end point (IHC)	Reduced lung metastasis[28]
VEGF-trap	HCC (human)	Spontaneous	Ectopic (subcutaneous) primary tumor; metastasis monitored at defined end point (IHC)	VEGF-trap; reduced primary tumor and lung metastasis; no change in lymph metastasis[29]
VEGFR31-Ig (VEGF A and C trap)				VEGFR31-Ig;Reduced primary, lung, and lymph metastasis[29]
DC101	Prostate (human)	Spontaneous	Orthotopic (prostate) primary tumor; metastasis monitored at defined end point (IHC)	Lymph metastasis reduced in short-term/early setting, no effect for longer/later treatments[30]
B20.4.1 (mouse monoclonal anti-VEGF antibody)	Melanoma (mouse)	Experimental	Intravenous injection.	Reduced visible lung nodules[31]
Sorafenib	HCC (human)	Spontaneous	Orthotopic (hepatic) tumor; metastasis monitored at defined end point (IHC)	Reduced lung metastasis[32]
CT322	Breast (human)	Spontaneous	Resection of orthotopic (mammary fat pad) tumor; metastasis monitored at defined end point (visual)	Reduced visible lung nodules[33]
DC101	Breast (genetically engineered mouse model)	Spontaneous	Mouse mammary tumor virus (MMTV)-driven Polyoma Middle T Antigen (PyMT) model	No reduction in visible metastasis[34]
DC101	Renal, colon	Experimental	Intravenous injection, intrasplenic	Reduced size of lung

	(mouse)			nodules, no reduction in number[35]
*All studies must include a measure of metastasis after anti-VEGF pathway therapy and a single drug treatment arm. Studies were excluded if they included any orthotopic or ectopic localized tumor implantation models, including glioma, ovarian, HCC and prostate that exclude tumor resection, caused death because of primary tumor, and/or did not measure distant metastasis. Please note, there is large variability in assessment and quantification in preclinical studies, often with large variations in the quality of metastatic assessment. Abbreviations: ADC, adenocarcinoma; IHC, immunohistochemistry; NSCLC, non-small-cell lung cancer; SCC, squamous cell carcinoma.				

1. Ebos, J. M. *et al.* Accelerated metastasis after short-term treatment with a potent inhibitor of tumor angiogenesis. *Cancer Cell* **15**, 232–239 (2009).
2. Hu-Lowe, D. D. *et al.* Nonclinical antiangiogenesis and antitumor activities of axitinib (AG-013736), an oral, potent, and selective inhibitor of vascular endothelial growth factor receptor tyrosine kinases 1, 2, 3. *Clin. Cancer Res.* **14**, 7272–7283 (2008).
3. Amagai, Y. *et al.* Combination therapy of interleukin-2 and sorafenib improves survival benefits and prevents spontaneous pulmonary metastasis in murine renal cell carcinoma models. *Jpn. J. Clin. Oncol.* **40**, 503–507 (2010).
4. Verheul, H. M. *et al.* Vascular endothelial growth factor trap blocks tumor growth, metastasis formation, and vascular leakage in an orthotopic murine renal cell cancer model. *Clin. Cancer Res.* **13**, 4201–4208 (2007).
5. Zuo, S. G. *et al.* Orally administered DNA vaccine delivery by attenuated *Salmonella typhimurium* targeting fetal liver kinase 1 inhibits murine Lewis lung carcinoma growth and metastasis. *Biol. Pharm. Bull.* **33**, 174–182 (2010).
6. Watanabe, M., Boyer, J. L. & Crystal, R. G.AAVrh.10-mediated genetic delivery of bevacizumab to the pleura to provide local anti-VEGF to suppress growth of metastatic lung tumors. *Gene Ther*. **17**, 1042–1051 (2010).
7. Yin, J. J., Zhang, L., Munasinghe, J., Linnoila, R. I. & Kelly, K. Cediranib/AZD2171 inhibits bone and brain metastasis in a preclinical model of advanced prostate cancer. *Cancer Res.* **70**, 8662–8673 (2010).
8. Kim, L. S., Huang, S., Lu, W., Lev, D. C. & Price, J. E. Vascular endothelial growth factor expression promotes the growth of breast cancer brain metastases in nude mice. *Clin. Exp. Metastasis* **21**, 107–118 (2004).
9. Gandhi, L. *et al.* Sunitinib prolongs survival in genetically engineered mouse models of multistep lung carcinogenesis. *Cancer Prev. Res. (Phila Pa).* **2**, 330–337 (2009).
10. Schomber, T. *et al.* Differential effects of the vascular endothelial growth factor receptor inhibitor PTK787/ZK222584 on tumor angiogenesis and tumor lymphangiogenesis. *Mol. Cancer Ther.* **8**, 55–63 (2009).
11. Sini, P. *et al.* Inhibition of multiple vascular endothelial growth factor receptors (VEGFR) blocks lymph node metastases but inhibition of VEGFR-2 is sufficient to sensitize tumor cells to platinum-based chemotherapeutics. *Cancer Res.* **68**, 1581–1592 (2008).
12. Paez-Ribes, M. *et al.* Antiangiogenic therapy elicits malignant progression of tumors to increased local invasion and distant metastasis. *Cancer Cell* **15**, 220–231 (2009).
13. Padera, T. P. *et al.* Differential response of primary tumor versus lymphatic metastasis to VEGFR-2 and VEGFR-3 kinase inhibitors cediranib and vandetanib. *Mol. Cancer Ther.* **7**, 2272–2279 (2008).
14. Shaheen, R. M. *et al.* Antiangiogenic therapy targeting the tyrosine kinase receptor for vascular endothelial growth factor receptor inhibits the growth of colon cancer liver metastasis and induces tumor and endothelial cell apoptosis. *Cancer Res.* **59**, 5412–5416 (1999).
15. Lang SA. *et al.* Dual targeting of Raf and VEGF receptor 2 reduces growth and metastasis of pancreatic cancer through direct effects on tumor cells, endothelial cells, and pericytes. *Mol. Cancer Ther*. **7**, 3509–3518 (2008).
16. Matsui J. *et al.* Multi-kinase inhibitor E7080 suppresses lymph node and lung metastases of human mammary breast tumor MDA-MB-231 via inhibition of vascular endothelial growth factor-receptor (VEGF-R) 2 and VEGF-R3 kinase. *Clin. Cancer Res*. **14**, 5459–5465 (2008).
17. Matsumori, Y. *et al.* ZD6474, an inhibitor of vascular endothelial growth factor receptor tyrosine kinase, inhibits growth of experimental lung metastasis and production of malignant pleural effusions in a non-small cell lung cancer model. *Oncol. Res.* **16**, 15–26 (2006).
18. Mizobe, T. *et al.* Efficacy of the combined use of bevacizumab and irinotecan as a postoperative adjuvant chemotherapy in colon carcinoma. *Oncol. Rep.* **20**, 517–523 (2008).
19. Kienast, Y. *et al.* Real-time imaging reveals the single steps of brain metastasis formation. *Nat. Med.* **16**, 116–122 (2010).
20. Yang, H., Jager, M. J. & Grossniklaus, H. E. Bevacizumab suppresses establishment of micrometastases in experimental ocular melanoma. *Invest. Ophthalmol. Vis. Sci*. doi:10.1167/iovs.09-4755
21. Melnyk, O., Zimmerman, M., Kim, K. J. & Shuman, M. Neutralizing anti-vascular endothelial growth factor antibody inhibits further growth of established prostate cancer and metastases in a pre-clinical model. *J. Urol*. **161**, 960–963 (1999).
22. Rowe, D. H. *et al.* Anti-VEGF antibody suppresses primary tumor growth and metastasis in an experimental model of Wilms' tumor. *J. Pediatr. Surg*. **35**, 30–32 (2000).
23. Rowe, D. H. *et al.* Suppression of primary tumor growth in a mouse model of human neuroblastoma. *J. Pediatr. Surg.* **35**, 977–981 (2000).
24. Warren, R. S., Yuan, H., Mati, M. R., Gillett, N. A. & Ferrara, N. Regulation by vascular endothelial growth

factor of human colon cancer tumorigenesis in a mouse model of experimental liver metastasis. *J. Clin. Invest.* **95**, 1789–1797 (1995).
25. Rofstad, E. K. & Halsor, E. F. Hypoxia-associated spontaneous pulmonary metastasis in human melanoma xenografts: involvement of microvascular hot spots induced in hypoxic foci by interleukin 8. *Br. J. Cancer* **86**, 301–308 (2002).
26. Rofstad, E. K., Gaustad, J. V., Egeland, T. A., Mathiesen, B. & Galappathi, K. Tumors exposed to acute cyclic hypoxic stress show enhanced angiogenesis, perfusion and metastatic dissemination. *Int. J. Cancer* **127**, 1535–1546 (2010).
27. Whitehurst, B. *et al.* Anti-VEGF-A therapy reduces lymphatic vessel density and expression of VEGFR-3 in an orthotopic breast tumor model. *Int. J. Cancer* **121**, 2181–2191 (2007).
28. Huang, J. *et al.* Regression of established tumors and metastases by potent vascular endothelial growth factor blockade. *Proc. Natl Acad. Sci. USA* **100**, 7785–7790 (2003).
29. Zhang, D. *et al.* Suppression of tumor growth and metastasis by simultaneously blocking vascular endothelial growth factor (VEGF)-A and VEGF-C with a receptor-immunoglobulin fusion protein. *Cancer Res.* **70**, 2495–2503 (2010).
30. Sweeney, P. *et al.* Anti-vascular endothelial growth factor receptor 2 antibody reduces tumorigenicity and metastasis in orthotopic prostate cancer xenografts via induction of endothelial cell apoptosis and reduction of endothelial cell matrix metalloproteinase type 9 production. *Clin. Cancer Res.* **8**, 2714–2724 (2002).
31. Bais, C. *et al.* PlGF blockade does not inhibit angiogenesis during primary tumor growth. *Cell* **141**, 166–177 (2010).
32. Zhang, W. *et al.* Depletion of tumor-associated macrophages enhances the effect of sorafenib in metastatic liver cancer models by antimetastatic and antiangiogenic effects. *Clin. Cancer Res.* **16**, 3420–3430 (2010).
33. Mamluk, R. *et al.* Anti-tumor effect of CT-322 as an adnectin inhibitor of vascular endothelial growth factor receptor-2. *MAbs* **2**, 199–208 (2010).
34. Hassan, S. *et al*. CXCR4 peptide antagonist inhibits primary breast tumor growth, metastasis and enhances the efficacy of anti-VEGF treatment or docetaxel in a transgenic mouse model. *Int. J. Cancer* doi:10.1002/ijc.25665
35. Lee, Y. J. *et al.* Differential effects of VEGFR-1 and VEGFR-2 inhibition on tumor metastases based on host organ environment. *Cancer Res.* **70**, 8357–8367 (2010).