Oral Diseases (2009) 15, 8–17. doi:10.1111/j.1601-0825.2008.01471.x

http://www.blackwellmunksgaard.com

INVITED REVIEW
MEDICAL REVIEW

The tumour microenvironment: a novel target for cancer therapy

E Hanna[1], J Quick[1,2], SK Libutti[1]

[1]*Tumour Angiogenesis Section, Surgery Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA;* [2]*Howard Hughes Medical Institute, National Institutes of Health Research Scholars Program, Bethesda, MD, USA*

Cancer therapy is in the midst of a major paradigm shift. Traditionally, cancer treatments have focused on tumour cells. However, studies over the past few decades have demonstrated that cancer is a vastly complex entity with multiple components affecting a tumour's growth, invasion and metastasis. These components, collectively termed the 'tumour microenvironment', include endothelial cells, pericytes, fibroblasts, inflammatory cells, leucocytes and elements of the extracellular matrix (ECM). Biological agents that target components of the tumour microenvironment may provide an interesting alternative to traditional tumour cell-directed therapy. Because of the complexity of the tumour milieu, the most beneficial therapy will likely involve the combination of one or more agents directed at this new target. This review highlights recent preclinical and clinical studies involving agents that target tumour vasculature, leucocytes, pericytes, cancer-associated fibroblasts and ECM components. We pay particular attention to combination therapies targeting multiple components of the tumour microenvironment, and aim to demonstrate that this strategy holds promise for the future of cancer treatment.
Oral Diseases (2009) **15**, 8–17

Keywords: tumour microenvironment; combination therapy; endothelial cells; leucocytes; fibroblasts; extracellular matrix

Introduction

For decades cancer therapy options have been limited to surgery, radiation or chemotherapy. While these options have been effective for the treatment of some patients, there remains a great need to reduce toxicity while simultaneously improving treatment efficacy. As our understanding of cancer biology continues to expand, there is a movement to treat tumours as a functional organ, composed of various cell types and molecules that interact in a dynamic and interdependent manner. The recognition of the tumour as a complex organ may lead to more specific and elegant methods of treating cancer that may minimize toxicity and improve patient survival. The tumour stroma, or supporting tissue, is composed of endothelial cells (ECs), pericytes adjacent to the ECs, invading inflammatory cells and leucocytes, fibroblasts and extensive extracellular matrix (ECM) components (Figure 1). The importance of the tumour stroma in cancer growth, invasion and metastasis is a fascinating and fundamental area of investigation. Recent studies have made it increasingly apparent that the tumour stroma does not exist simply as a passive support structure, but rather plays an active role in a tumour's progression (Derynck *et al*, 2001; Shekhar *et al*, 2001; Blavier *et al*, 2006).

For instance, irradiation of mammary gland stromal tissue dramatically increased the tumourigenic potential of a mutated mammary epithelial cell line (Barcellos-Hoff and Ravani, 2000). These epithelial cells were non-tumourigenic when injected orthotopically into non-irradiated syngeneic hosts. However, radiation-induced mutation of the stromal microenvironment promoted the neoplastic progression of the epithelial cells, illustrating the importance of the tumour stroma in cancer development. Furthermore, modulation of the tumour stroma using a biodegradable scaffold in squamous cell carcinoma xenografts led to decreased tumour vascularization and invasion (Willhauck *et al*, 2007). The reversion from an invasive phenotype to a minimally invasive phenotype occurred in both low- and high-grade malignant lines. Interestingly, this result was not correlated with decreased tumour cell proliferation, but rather with alterations in the tumour stroma. In particular, the authors noted normalization of epidermal differentiation, condensation of the ECM, reduction

Correspondence: SK Libutti, Tumour Angiogenesis Section, Surgery Branch, National Cancer Institute, Room 4W-5940, Building 10, 10 Center Drive, Bethesda, MD 20892, USA. Tel: 301 496 5049, Fax: 301 402 1788, E-mail: libuttis@mail.nih.gov
Received 17 June 2008; revised 14 July 2008; accepted 18 June 2008

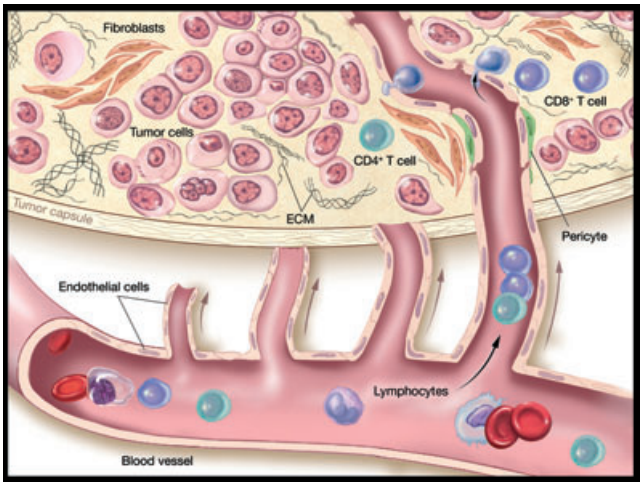

Figure 1 Schematic of the tumour microenvironment, including tumour cells, endothelial cells, pericytes, fibroblasts, $CD4^+$ and $CD8^+$ lymphocytes and extracellular matrix components

of peritumoural proteases, suppressed tumour vascularization, and an increase in endostatin and thrombospondin-1 (TSP-1), two anti-angiogenic components of the ECM. Finally, studies of inherited syndromes that predispose to cancer have also demonstrated an active role of stromal components in cancer progression (Jacoby *et al*, 1997; Howe *et al*, 1998). Specifically, these investigators showed that mutations and alterations of the stoma contribute to the development of carcinoma in patients with juvenile polyposis.

Elements of the tumour microenvironment thus present exciting and innovative targets for novel therapies (Table 1) that may overcome many of the limitations of current treatment modalities. Anti-cancer agents directed at the tumour stroma may exhibit less associated toxicity, because of unique targets in the tumour microenvironment. Unlike tumour cells, ECs are genetically stable and thus less likely to acquire mutations conferring resistance (Kerbel, 1991; Boehm *et al*, 1997). In addition, combination therapies that target multiple

Table 1 Novel agents targeting the tumour microenvironment

Target	*Class of agents*	*Examples*	*References and reviews*
Endothelial cells/ tumour-associated vasculature	Endogenous angiogenesis inhibitors	Endostatin Interferons (IFN-α,β) Interleukins (IL-4, IL-12 and IL-18) Thrombospondin-1 and -2 Angiopoietin-1 and -2	Nyberg *et al* (2005); Lawler (2000); Ahmad *et al* (2001)
	Synthetic angiogenesis inhibitors	RGD analogs Anginex and 0118	Buerkle *et al* (2002) Brandwijk *et al* (2006)
	Chemotherapeutics	5-FU-based drugs (e.g. S-1, capecitabine) Irofulven Melphalan Doxorubicin Metronomic cyclophosphamide Taxol	Miller *et al* (2001)
	Anti-vascular cytokines	TNF alpha	van Horssen *et al* (2006)
Endothelial cells and dendritic cells	Inhibitors of VEGF signalling	Bevacizumab (mAb against VEGF) DC101 (mAb against VEGF-R2) msFLK1 (soluble VEGF-R2)	McMahon (2000) Shojaei and Ferrara (2007)
Endothelial cells, pericytes, stromal cells	Small-molecule tyrosine kinase inhibitors	Sunitinib, soraenib	Steeghs *et al* (2007)
Pericytes, Fibroblasts	Inhibition of PDGF signalling	PDGFR inhibitors (e.g. Gleevec)	Bergers and Song (2005)
ECM and angiogenesis	Extracellular matrix modulators	Urokinase plasminogen activator/ receptor Matrix metalloproteinases	Dunbar *et al* (2000) Yu *et al* (1997)
ECM	Cytokines	IL-12 and IL-18	Abraham *et al* (2002)
Immune cells (lymphocytes, natural killer cells)	Cell-based immunotherapy	Adoptive cell transfer of tumour α infiltrating lymphocytes Dendritic cell therapy Depletion of regulatory T-lymphocytes	Rosenberg *et al* (2008) Jefford *et al* (2001) Elia *et al* (2007)
	Vaccines	Cytokine-gene-modified cancer cell vaccines	Bubenik (1996)
	Immunomodulators	Lenalidomide	Tohnya *et al* (2004)
Immune cells and endothelial cells	Cytokines/chemokines	IL-15/IL-15R IL-2 IL-12 Fractalkine (FKN)	Klebanoff *et al* (2004) Parmiani *et al* (2000) Ohta *et al* (2005)

RGD, arginine-glycine-aspartic acid; 5-FU, 5-fluorouracil; TNF, tumour neucrosis factor; VEGF, vascular endothelial growth factor; R, receptor; mAb, monoclonal antibody; PDGF, platelet-derived growth factor; ECM, extracellular matrix.

components of the tumour microenvironment may overcome compensatory escape mechanisms that tumours usually rely upon for survival (Mizukami *et al*, 2005; Dorrell *et al*, 2007; Blansfield *et al*, 2008). Moreover, combinatorial regimens often allow for lower doses of each individual agent, which can lead to decreased associated toxicities. Finally, the ultimate goal of this approach is to develop improved therapies that translate into prolonged survival for the patients.

Anti-angiogenic and anti-vascular therapies

The initial observation of the importance of neovasculature in malignancies was made over 60 years ago by Algire *et al* (1945). 26 years later, Folkman (1971), a pioneer in the field, coined the term 'tumour angiogenesis', identified specific molecules involved in tumour neovascularization, and laid the groundwork for future studies elucidating the importance of tumour vasculature. While anti-angiogenic agents have shown much promise in preclinical studies, these results have not always been mirrored in clinical trials (Marshall, 2002). However, ongoing studies indicate that the full potential of anti-angiogenic agents in the treatment of cancer is yet untapped. Several methods of targeting tumour vasculature are currently under investigation; some agents disrupt pathways involved in tumour angiogenesis, while others act through a direct anti-vascular effect. In either case, the addition of complementary therapies may improve clinical outcome. Several studies have shown that the efficacy of anti-angiogenic and anti-vascular agents has been improved upon by combination with additional treatment modalities.

Endostatin, a fragment of collagen XVIII, is a potent angiogenesis inhibitor in animal studies. While it appears to be moderately effective alone in preclinical studies, endostatin has been shown to enhance anti-tumour effects when combined with many conventional chemotherapeutic compounds, including paclitaxel (Li *et al*, 2008), doxorubicin (Plum *et al*, 2003; Liu *et al*, 2007) and low dose carboplatin (Abraham *et al*, 2003). The synergy noted when endostatin was administered with these chemotherapeutics support the premise that combination therapy is more potent than either agent alone.

Another approach illustrating the use of anti-angiogenic agents in combination regimens involves the RGD (arginine–glycine–aspartic acid) peptide motif and oncolytic viruses; attenuated viruses that are cytotoxic against tumour cells. Early clinical trials with oncolytic viruses have demonstrated that they may be less efficacious than expected. This prompted researchers to assess the role of the microenvironment in improving the efficacy of oncolytic viruses. The RGD motif has anti-angiogenic activity via binding to receptors expressed on ECs of tumour-associated vasculature (Arap *et al*, 1998; Hood *et al*, 2002). Delivery of an angiostatic cRGD peptide to rat gliomas prior to treatment with an oncolytic virus augmented the anti-tumour effects of the virus (Kurozumi *et al*, 2007). This again supports the hypothesis that anti-angiogenic agents may prove useful as adjuvant therapies.

Some traditional chemotherapeutic compounds, typically used for direct cytotoxic effects against the tumour cells, exhibit anti-angiogenic effects when delivered in low doses. Low doses of chemotherapeutics can be delivered at close regular intervals, rather than in large boluses. This scheme, termed metronomic chemotherapy (Hanahan *et al*, 2000; Sarmiento and Gasparini, 2008), targets the endothelium of neovasculature, inhibits angiogenesis, and may also decrease the mobilization of circulating endothelial progenitor cells. The lower doses used in this approach may lead to less associated toxicity as compared with traditional chemotherapy. In addition, chemotherapeutics targeting genetically stable ECs may overcome the challenge of treating tumours that have developed resistance to traditional chemotherapy (Browder *et al*, 2000).

One such example is 5-fluorouracil (5-FU)-based drugs. Low doses of S-1 (a modulated formulation of 5-FU) and capecitabine (a prodrug of 5-FU) administered to mice bearing human colorectal cancer xenografts resulted in inhibition of tumour growth, decreased microvessel density and induction of TSP-1 (Ooyama *et al*, 2008). Similarly, a low, non-toxic dose of the chemotherapeutic irofulven, delivered in combination with angiogenesis inhibitors (anginex and 0118), was shown to effectively inhibit growth of ovarian tumour xenografts in mice (Dings *et al*, 2008). Irofulven/anginex and irofulven/0118 combinations were more efficacious than any of the single agents.

Our laboratory has recently shown that combination therapy utilizing lenalidomide, an immunomodulatory drug, and sorafenib, a tyrosine kinase inhibitor with activity on tumour cells, ECs, pericytes and stromal cells, was effective in treating ocular melanoma in a murine model (Mangiameli *et al*, 2007). We then continued to demonstrate that the addition of metronomic cyclophosphamide to lenalidomide and sunitinib, a tyrosine kinase inhibitor, was successful in halting the growth of several types of xenografts, including melanoma, colon and pancreatic cancer (Figure 2). Treatment with each single agent leads to compensatory responses involving upregulation of pro-angiogenic molecules. This compensation was diminished following treatment with triple agent combination therapy (Blansfield *et al*, 2008). In a rat gliosarcoma model, combination therapy also abrogated pro-angiogenic compensatory pathways, underscoring the importance of targeting multiple pathways in the treatment of cancer (Dorrell *et al*, 2007).

Combination of taxol, a chemotherapeutic which is also a potent anti-angiogenic agent, and stathmin, a regulator of the microtubule cytoskeleton and mitotic spindle, has also been shown to have a synergistic anti-cancer effect (Mistry and Atweh, 2006). More recently, this group elucidated that the combination of stathmin and taxol also inhibits EC proliferation, migration and differentiation into capillary-like structures (Mistry *et al*, 2007).

Interferon-α (IFN-α) is an immunomodulatory and regulatory cytokine that downregulates pro-angiogenic molecules (von Marschall *et al*, 2003) and is thus being explored for use in anti-angiogenic cancer therapy. Combination of IFN-α with chemotherapeutics has

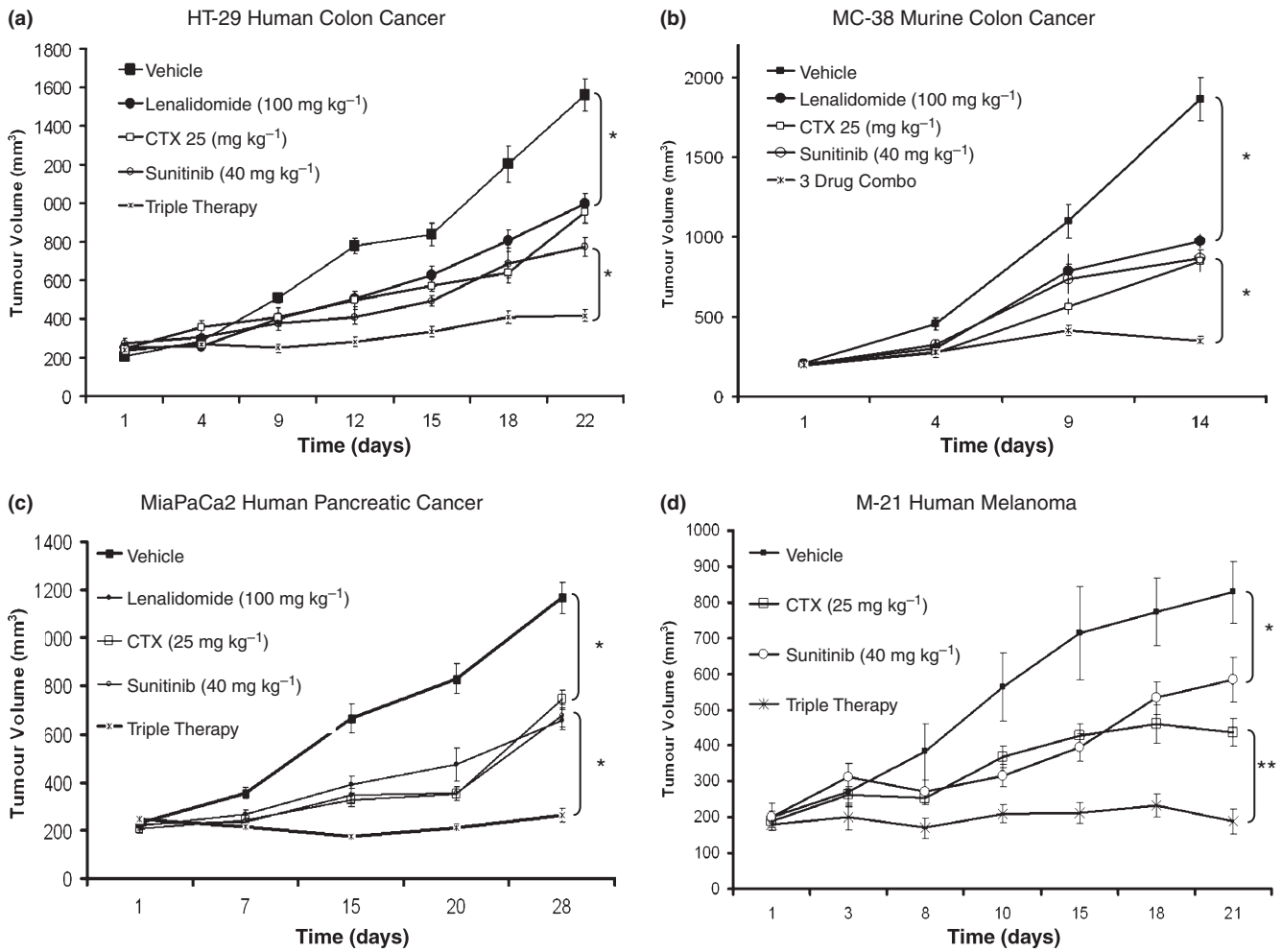

Figure 2 Effects of sunitinib, lenalidomide, metronomic cyclophosphamide alone, and triple combination therapy on xenograft models of colon cancer, pancreatic cancer and cutaneous melanoma. (**a**) In a human colon cancer xenograft model, single agent therapy can significantly slow the growth of a primary tumour compared with vehicle-treated tumours ($P < 0.001$), triple combination therapy can significantly slow the progression of the primary tumour compared with single agents alone ($P < 0.001$). (**b**) In a murine colon cancer xenograft model, single agent therapy can significantly slow the growth of a primary tumour compared with vehicle-treated tumours ($P < 0.001$), triple combination therapy can significantly slow the progression of the primary tumour compared with single agents alone ($P < 0.001$). (**c**) Single agent therapy can inhibit the growth of a pancreatic primary tumour in mice when compared with tumours in mice treated with vehicle alone. Triple therapy further inhibits the tumour growth over single agent therapy in an additive fashion (both $P < 0.001$). (**d**) Triple agent therapy can statistically significantly inhibit the growth of a primary human cutaneous melanoma tumour when compared with tumours grown in mice treated with single agents alone ($P < 0.05$, $^{*}P < 0.001$ and $^{**}P < 0.05$) (Blansfield *et al*, 2008)

shown success in murine models of ocular melanoma (Yang and Grossniklaus, 2006) and pancreatic adenocarcinoma (Zhu *et al*, 2008). IFN-α and 5-FU combination therapy has also been shown to be effective in treating advanced hepatocellular carcinoma in several clinical trials (Miyamoto *et al*, 2000; Sakon *et al*, 2002; Ota *et al*, 2005; Nagano *et al*, 2007). The efficacy of this combination therapy may be due, in part, to modulation of important angiogenesis mediators such as vascular endothelial growth factor (VEGF), angiopoietin-1 and angiopoietin-2 (Wada *et al*, 2007).

Tumour necrosis factor α (TNF-α), while once touted for its potent anti-tumour effect, has limited clinical utility because of extreme toxicity when administered systemically. Novel methods of targeting TNF-α to the tumour vasculature have opened-up the possibilities for renewed use of this agent. For example, in a phase I clinical trial, we recently demonstrated that systemic delivery of TNF-α linked to pegylated colloidal gold distributes primarily to solid tumours, and did not lead to any dose-limiting toxicities (Libutti *et al*, 2007). Of particular interest, is the combination of TNF-α with other agents, either as a way to increase the tumour sensitivity to TNF-α or to enhance the activity of traditional chemotherapeutics. Endothelial-monocyte activating polypeptide II (EMAP-II) is a tumour derived cytokine that, in low doses, can sensitize tumours to the effects of TNF-α (Kao *et al*, 1994; Marvin *et al*, 1996; Gnant *et al*, 1999; Wu *et al*, 1999). The combination of low-dose EMAP-II with tumour vasculature targeted TNF-α inhibited growth of murine lymphoma and murine melanoma tumours (Crippa *et al*, 2008). EMAP-II has also shown promise in improving anti-tumour responses in sarcoma patients receiving TNF-α

via isolated limb perfusion (Lans *et al*, 2002). Targeted delivery of TNF-α to tumour vasculature, in combination with chemotherapeutics, such as melphalan, doxorubicin, cisplatin, paclitaxel and gemcitabine, has also shown synergistic anti-tumour effects (Curnis *et al*, 2002; Sacchi *et al*, 2006). In the case of doxorubicin, this synergy appears to be related to interferon-γ (IFN-γ), as the effect was abrogated in IFN-γ knockout mice or when mice were given an anti-IFN-γ neutralizing antibody (Sacchi *et al*, 2004). The combination of melphalan and tumour vasculature targeted TNF-α resulted in tumour regression in a majority of tumour-bearing mice, as well as, induced a T-cell-specific immune response that effectively vaccinated the mice against future tumour challenges (Balza *et al*, 2006; Mortara *et al*, 2007). In addition, targeted delivery of TNF-α and IL-12 had a synergistic effect in treating teratocarcinomas in mice when administered at doses significantly less than the maximum tolerated dose, indicating that this combination may be effective with minimal toxicity (Halin *et al*, 2003).

Bevacizumab is a monoclonal antibody against VEGF, a principal mediator of tumour angiogenesis (Hicklin and Ellis, 2005). In 2004, a phase III clinical trial for patients with metastatic colorectal cancer demonstrated improved response rates, increased progression-free survival and increased overall survival when bevacizumab was added to irinotecan and 5-FU (Hurwitz *et al*, 2004). In 2004, bevacizumab became the first anti-angiogenic drug to be approved by the FDA for the treatment of cancer. A survival benefit from bevacizumab in combination with chemotherapy has also been observed in patients with advanced non-small cell lung cancer (Sandler *et al*, 2006; Manegold, 2008), prompting the FDA to approve bevacizumab for this use as well. Additionally, single agent bevacizumab led to an increase of time to tumour progression in advanced renal cell cancer (Yang, 2004). Combination therapies utilizing bevacizumab have also shown efficacy in phase III trials for metastatic breast cancer (Miller *et al*, 2005) and metastatic renal cell carcinoma (Escudier *et al*, 2007). Several other clinical trials are currently underway to investigate the activity of this agent in various other cancer histologies, including a recent phase I study in which bevacizumab, in combination with a fluorouracil/hydroxyurea/radiation therapy platform, showed anti-tumour activity in head and neck cancer patients (Seiwert *et al*, 2008).

Therapies targeting pericytes/fibroblasts/extracellular matrix

While substantial work has been performed to understand the role of ECs, much less is known about the role of pericytes, the smooth muscle cells adjacent to ECs, in tumour-associated vasculature. Colorectal and pancreatic xenografts that overexpress platelet-derived growth factor (PDGF-BB) had an increase in pericyte coverage of ECs, decreased tumour microvessel density and inhibition of tumour growth (McCarty *et al*, 2007). In a B16 mouse melanoma tumour model, combination targeting of the VEGF and PDGF receptors led to significant inhibition of tumour growth, which was associated with a decrease in both α-smooth muscle actin (α-SMA) and a subpopulation of PDGFRβ-positive pericytes. Pericytes partly detached from the endothelium were primarily affected, while those closely attached to the endothelium were not affected by this treatment, demonstrating that there was a specific subpopulation of pericytes altered by this anti-cancer regimen (Hasumi *et al*, 2007). Thus, combination therapy targeting both tumour-associated endothelium as well as adjacent pericytes may enhance the efficacy of cancer treatment.

Cancer-associated fibroblasts are also currently being investigated as a potential target for novel cancer therapies. Cancer-associated fibroblasts have been shown to alter the phenotype of malignant epithelial cells *in vitro* (Atula *et al*, 1997) and to direct tumour progression of prostate epithelium (Olumi *et al*, 1999). Ongoing studies elucidating the functional role of fibroblasts within the tumour lend credence to the hypothesis that treatments targeting fibroblasts may have an anti-cancer effect. Using a mouse model of cervical carcinogenesis, investigators have demonstrated that blockade of PDGF receptor signalling in cancer-associated fibroblasts and pericytes inhibited progression of premalignant cervical lesions as well as growth of invasive carcinomas (Pietras *et al*, 2008). Similarly, overexpression of TSP-1, an angiogenesis inhibitor, has been shown to inhibit cervical tumour growth in mice (Wu *et al*, 2008). While this reduced tumour growth was associated with a decrease in tumour vascularization, a decrease in α-SMA and desmin, two markers of activated fibroblasts, was also observed. The inhibition of tumour growth was due, in part, to decreased migration and invasion of activated fibroblasts, suggesting a novel role for TSP-1 and demonstrating the potential for therapeutics targeting cancer-associated fibroblasts.

Increased interstitial pressure in the tumour interstitium, as a result of leaky blood vessels and a lack of functional lymphatics, presents a potential obstacle to therapy. Many current anti-cancer agents are not able to disseminate through solid tumours because of this increased pressure. ECM components of the tumour microenvironment, such as proteoglycans, glycosaminoglycans and type I collagen, play a major role in the diffusion of drugs through the tumour (Netti *et al*, 2000). Enzymatic digestion of collagen and decorin, an associated proteoglycan, improved macromolecule diffusion in tumours (Magzoub *et al*, 2008), indicating that this may be a promising addition to anti-cancer pharmacological agents.

An alternative approach involves targeting enzymes that appear to be involved in cancer progression and metastasis. Urokinase plasminogen activator (uPA), a serine proteinase, is part of an endogenous proteolytic cascade, and appears to play an active role in tumour progression and metastasis (Killeen *et al*, 2008). Increased levels of uPA or its receptor correlate with aggressiveness of tumours and poor prognosis (Blasi, 1999), suggesting that uPA may be a valid target for

cancer therapy. Similarly, matrix metalloproteinases (MMPs) are a family of endopeptidases involved in the degradation of ECM components, a process essential for tumour angiogenesis and growth (Shapiro, 1998; Nagase and Woessner, 1999; Westermarck and Kahari, 1999). Proteinases of the stroma, namely MMP-3 and MMP-9, have been shown to promote tumour formation (Sternlicht *et al*, 1999; Coussens *et al*, 2000), making them attractive targets for cancer therapy as well. Capitalizing on this information, one group has shown that downregulation of both uPA and MMP-9 inhibited glioma invasion and angiogenesis, and had a synergistic effect resulting in tumour regression (Lakka *et al*, 2003).

Immunotherapy

Cells of the immune system are of particular interest for novel therapies targeting the tumour microenvironment. The inability of immune surveillance to control malignant processes (Khong and Restifo, 2002; Ferris *et al*, 2006; Zitvogel *et al*, 2006), has lead researchers to discover ways to either augment the body's natural immune system or to deliver various cells of the immune system to attack the tumour. One approach has been to deliver cytokines that are known to enhance the effects of tumour-reactive lymphocytes. *In vivo* delivery of interleukin-15 (IL-15) complexed to its soluble receptor, IL-15R has been shown to prevent tumour formation (Stoklasek *et al*, 2006) as well as, cause regression of solid tumours in two different murine tumour models (Epardaud *et al*, 2008). Similarly, enrichment of the tumour microenvironment with fractalkine (FKN), a CX3C chemokine, and IL-2 inhibited growth of neuroblastoma tumours in a syngeneic mouse model (Zeng *et al*, 2007). The anti-tumour effect was abrogated by depletion of both T cells and natural killer cells, and was primarily attributed to recruitment of $CD8^+$ T cells to the tumour. Finally, adoptive cell transfer of autologous tumour-infiltrating lymphocytes, in combination with prior immuno-depletion, produced a 50% response rate in patients with metastatic melanoma refractory to conventional treatments (Dudley *et al*, 2002, 2005).

One of the challenges that still remains in tumour immunotherapy is that tumours have many ways in which to evade or suppress the host's immune system (Ferrone and Whiteside, 2007). Recently, studies have been undertaken to investigate the importance of regulatory T lymphocytes (Treg, $CD4^+CD25^+$) in suppressing anti-tumour immune responses. Depletion of Tregs using an anti-CD25 antibody in combination with adoptive transfer of tumour-specific cytotoxic T lymphocytes (CTL) was more effective than each single therapy in a syngeneic murine renal carcinoma model. The anti-CD25 antibody and CTL combination resulted in disappearance of tumours as well as increased survival in these mice (Ohmura *et al*, 2008). Others have shown that while the use of a chemokine fusion protein (LEC/chTNT-3) caused a 40–50% reduction in tumour growth (Li *et al*, 2003a), addition of Treg depletion to LEC/chTNT-3 treatment led to complete regression of colon and renal cell carcinomas in mice (Li *et al*, 2003b). These studies suggest that modulation of the tumour's immunosuppressive environment can improve the efficacy of cancer immunotherapy.

Interleukin-15 was shown to universally improve the efficacy of various cytokine-gene-modified melanoma cell vaccines. While IL-15 and each of the cytokine producing tumour cell vaccines [TNF-α, granulocyte–macrophage colony stimulating factor (GM-CSF), IL-12 or IL-6/sIL-6R] exhibited minimal to moderate anti-tumour effects alone, the combination of IL-15 with each of the vaccines was effective in eradicating tumours in almost all animals (Basak *et al*, 2008). Likewise, in an ovarian cancer model, a synergistic anti-tumour effect was seen with the co-delivery of adenoviral vectors expressing FKN and IL-12. While there was an infiltration of immune cells into the tumour in mice receiving FKN alone, there was a deficit in the number of activated cells in these tumours and minimal inhibition of tumour growth, emphasizing the importance of IL-12 in this regimen (Gao *et al*, 2008). Furthermore, preclinical work has demonstrated that tumour targeted IL-2, when administered along with T-cell-based immunotherapy, enhanced the persistence of T cells in the tumour microenvironment and improved the anti-tumour effect (Singh *et al*, 2007). Collectively, these studies indicate that the combination of immunocytokines and cell-based immuno-therapies may provide improved results in the clinic. Augmentation of $CD8^+$ T-cell lytic activity in cancer can also be achieved by combining CTL adoptive immunotherapy with radiation therapy. Irradiation of MC38 (murine colon adenocarcinoma) subcutaneous tumours led to upregulation of Fas, sensitization of tumour cells to the lytic activity of antigen specific CTL, and improved efficacy of tumour rejection by CTLs (Chakraborty *et al*, 2003).

Anti-angiogenic compounds have also shown promising results when combined with immune-based therapy. These treatment modalities complement each other in a way that may prove to be more efficacious in the clinic. Anti-angiogenic therapy may reduce the tumour burden by depriving it of a blood supply, while immunotherapy may provide long-lasting immune responses, which can eliminate any residual cancer cells. Combination of DC101 (an anti-angiogenic monoclonal antibody against VEGF receptor 2), with tumour-targeted vaccination resulted in regression of a breast cancer mouse model (Manning *et al*, 2007). While the VEGF-R2 antibody induced an anti-tumour response, the authors' observed that addition of the tumour-specific vaccine produced greater immune responses than either single therapy alone. Similarly, the combination of anti-angiogenic agents with a tumour vaccine expressing macrophage colony stimulating factor (M-CSF) had a synergistic effect in treating intracranial gliomas in rats, while additionally providing a long-lasting anti-tumour immune response (Jeffes *et al*, 2005). In a renal cell carcinoma model, simultaneous delivery of IL-2 and soluble VEGF receptor 2 (msFlk1) led to significant inhibition of subcutaneous tumour growth. Lung metastases that resulted from intravenous injection of renal cancer cells were also reduced after treatment with the IL-2/msFlk1 combination. Further-

more, these mice exhibited increased survival as compared with controls (Yockman *et al*, 2007).

In addition to VEGF's role in tumour angiogenesis, recent work has demonstrated that VEGF may inhibit dendritic cell activity, thereby contributing to the evasion of tumours from immune surveillance (Gabrilovich *et al*, 1996; Dhodapkar *et al*, 2001). Therefore, it has been postulated that blockade of VEGF may improve dendritic cell function, leading to enhanced anti-tumour immunity (Osada *et al*, 2008). Combination of anti-VEGF antibodies with dendritic cell immunotherapy led to prolonged and more pronounced anti-tumour effects in preclinical murine studies (Gabrilovich *et al*, 1999). These dual functions of VEGF make it a promising adjuvant to immunotherapy.

Conclusions and future directions

Although the above studies establish a hopeful platform for the future of cancer therapy, there is still a vast amount of information about the tumour microenvironment that remains to be discovered. For instance, tumour-associated macrophages (TAMs) have recently been identified as playing a role in tumour progression (Bingle *et al*, 2002; Sica and Bronte, 2007). However, the precise function of TAMs and other cells of the tumour microenvironment in cancer progression have yet to be fully elucidated. The development of therapies targeting TAMs, in addition to the aforementioned targets, will be an exciting course to follow. Studies comparing cells of the tumour milieu with their normal counterparts may lead to discovery of unique microenvironment signatures (Grover *et al*, 2006; Hanson *et al*, 2006; Rodriguez-Canales *et al*, 2007). Such information could be utilized for the development of additional targeted therapies. Novel means of dissecting tumour components from their native surroundings will yield insight into the signalling and cross-talk that occurs between the individual cell types (Tangrea *et al*, 2004).

By understanding the interactions between cells of the tumour microenvironment, and how these interactions affect tumour progression, we can develop better therapies involving complementary and synergistic combinations. Although Paget (1889) first defined the seed and soil hypothesis over a century ago, the biological complexity of tumours is still far from being completely understood. However, enormous strides have been made in understanding and targeting the tumour microenvironment over the past few decades. Combination therapies that target multiple elements of the tumour microenvironment are beginning to address the complex nature of cancer progression and provide promising new strategies for the treatment of patients with cancer.

Author contributions

E Hanna participated in the conception and design of the paper, drafted and revised the article critically for important intellectual content, and was involved with the final approval of the version to be published. J Quick participated in the conception and design of the paper, revised it critically for important intellectual content, and was involved with the final approval of the version to be published. SK Libutti made substantial contributions to the conception and design of the paper, drafted and revised the article critically for important intellectual content, and was involved with the final approval of the version to be published. All authors read and approved the final manuscript.

References

Abraham M, Shapiro S, Lahat N, Miller A (2002). The role of IL-18 and IL-12 in the modulation of matrix metalloproteinases and their tissue inhibitors in monocytic cells. *Int Immunol* **14:** 1449–1457.

Abraham D, Abri S, Hofmann M, Holtl W, Aharinejad S (2003). Low dose carboplatin combined with angiostatic agents prevents metastasis in human testicular germ cell tumor xenografts. *J Urol* **170:** 1388–1393.

Ahmad SA, Liu W, Jung YD *et al* (2001). The effects of angiopoietin-1 and -2 on tumor growth and angiogenesis in human colon cancer. *Cancer Res* **61:** 1255–1259.

Algire GH, Chalkley HW, Legallais FY, Park HD (1945). Vascular reactions of normal and malignant tissues in vivo. I. Vascular reactions of mice to wounds and to normal and neoplastic transplants. *J Natl Cancer Inst* **6:** 73–85.

Arap W, Pasqualini R, Ruoslahti E (1998). Cancer treatment by targeted drug delivery to tumor vasculature in a mouse model. *Science* **279:** 377–380.

Atula S, Grenman R, Syrjanen S (1997). Fibroblasts can modulate the phenotype of malignant epithelial cells in vitro. *Exp Cell Res* **235:** 180–187.

Balza E, Mortara L, Sassi F *et al* (2006). Targeted delivery of tumor necrosis factor-alpha to tumor vessels induces a therapeutic T cell-mediated immune response that protects the host against syngeneic tumors of different histologic origin. *Clin Cancer Res* **12:** 2575–2582.

Barcellos-Hoff MH, Ravani SA (2000). Irradiated mammary gland stroma promotes the expression of tumorigenic potential by unirradiated epithelial cells. *Cancer Res* **60:** 1254–1260.

Basak GW, Zapala L, Wysocki PJ, Mackiewicz A, Jakobisiak M, Lasek W (2008). Interleukin 15 augments antitumor activity of cytokine gene-modified melanoma cell vaccines in a murine model. *Oncol Rep* **19:** 1173–1179.

Bergers G, Song S (2005). The role of pericytes in blood-vessel formation and maintenance. *Neuro Oncol* **7:** 452–464.

Bingle L, Brown NJ, Lewis CE (2002). The role of tumour-associated macrophages in tumour progression: implications for new anticancer therapies. *J Pathol* **196:** 254–265.

Blansfield JA, Caragacianu D, Alexander HR III *et al* (2008). Combining agents that target the tumor microenvironment improves the efficacy of anticancer therapy. *Clin Cancer Res* **14:** 270–280.

Blasi F (1999). The urokinase receptor. A cell surface, regulated chemokine. *APMIS* **107:** 96–101.

Blavier L, Lazaryev A, Dorey F, Shackleford GM, DeClerck YA (2006). Matrix metalloproteinases play an active role in Wnt1-induced mammary tumorigenesis. *Cancer Res* **66:** 2691–2699.

Boehm T, Folkman J, Browder T, O'Reilly MS (1997). Antiangiogenic therapy of experimental cancer does not induce acquired drug resistance. *Nature* **390:** 404–407.

Brandwijk RJ, Dings RP, van der Linden E, Mayo KH, Thijssen VL, Griffioen AW (2006). Anti-angiogenesis and anti-tumor activity of recombinant anginex. *Biochem Biophys Res Commun* **349:** 1073–1078.

Browder T, Butterfield CE, Kraling BM *et al* (2000). Antiangiogenic scheduling of chemotherapy improves efficacy against experimental drug-resistant cancer. *Cancer Res* **60:** 1878–1886.

Bubenik J (1996). Cytokine gene-modified vaccines in the therapy of cancer. *Pharmacol Ther* **69:** 1–14.

Buerkle MA, Pahernik SA, Sutter A, Jonczyk A, Messmer K, Dellian M (2002). Inhibition of the alpha-nu integrins with a cyclic RGD peptide impairs angiogenesis, growth and metastasis of solid tumours in vivo. *Br J Cancer* **86:** 788–795.

Chakraborty M, Abrams SI, Camphausen K *et al* (2003). Irradiation of tumor cells up-regulates Fas and enhances CTL lytic activity and CTL adoptive immunotherapy. *J Immunol* **170:** 6338–6347.

Coussens LM, Tinkle CL, Hanahan D, Werb Z (2000). MMP-9 supplied by bone marrow-derived cells contributes to skin carcinogenesis. *Cell* **103:** 481–490.

Crippa L, Gasparri A, Sacchi A, Ferrero E, Curnis F, Corti A (2008). Synergistic damage of tumor vessels with ultra low-dose endothelial-monocyte activating polypeptide-II and neovasculature-targeted tumor necrosis factor-alpha. *Cancer Res* **68:** 1154–1161.

Curnis F, Sacchi A, Corti A (2002). Improving chemotherapeutic drug penetration in tumors by vascular targeting and barrier alteration. *J Clin Invest* **110:** 475–482.

Derynck R, Akhurst RJ, Balmain A (2001). TGF-beta signaling in tumor suppression and cancer progression. *Nat Genet* **29:** 117–129.

Dhodapkar MV, Steinman RM, Krasovsky J, Munz C, Bhardwaj N (2001). Antigen-specific inhibition of effector T cell function in humans after injection of immature dendritic cells. *J Exp Med* **193:** 233–238.

Dings RP, Laar ES, Webber J *et al* (2008). Ovarian tumor growth regression using a combination of vascular targeting agents anginex or topomimetic 0118 and the chemotherapeutic irofulven. *Cancer Lett* **265:** 270–280.

Dorrell MI, Aguilar E, Scheppke L, Barnett FH, Friedlander M (2007). Combination angiostatic therapy completely inhibits ocular and tumor angiogenesis. *Proc Natl Acad Sci USA* **104:** 967–972.

Dudley ME, Wunderlich JR, Yang JC *et al* (2002). A phase I study of nonmyeloablative chemotherapy and adoptive transfer of autologous tumor antigen-specific T lymphocytes in patients with metastatic melanoma. *J Immunother* **25:** 243–251.

Dudley ME, Wunderlich JR, Yang JC *et al* (2005). Adoptive cell transfer therapy following non-myeloablative but lymphodepleting chemotherapy for the treatment of patients with refractory metastatic melanoma. *J Clin Oncol* **23:** 2346–2357.

Dunbar SD, Ornstein DL, Zacharski LR (2000). Cancer treatment with inhibitors of urokinase-type plasminogen activator and plasmin. *Expert Opin Investig Drugs* **9:** 2085–2092.

Elia L, Aurisicchio L, Facciabene A *et al* (2007). CD4 + CD25 + regulatory T-cell-inactivation in combination with adenovirus vaccines enhances T-cell responses and protects mice from tumor challenge. *Cancer Gene Ther* **14:** 201–210.

Epardaud M, Elpek KG, Rubinstein MP *et al* (2008). Interleukin-15/interleukin-15R alpha complexes promote destruction of established tumors by reviving tumor-resident CD8 + T cells. *Cancer Res* **68:** 2972–2983.

Escudier B, Pluzanska A, Koralewski P *et al* (2007). Bevacizumab plus interferon alfa-2a for treatment of metastatic renal cell carcinoma: a randomised, double-blind phase III trial. *Lancet* **370:** 2103–2111.

Ferris RL, Whiteside TL, Ferrone S (2006). Immune escape associated with functional defects in antigen-processing machinery in head and neck cancer. *Clin Cancer Res* **12:** 3890–3895.

Ferrone S, Whiteside TL (2007). Tumor microenvironment and immune escape. *Surg Oncol Clin N Am* **16:** 755–774.

Folkman J (1971). Tumor angiogenesis: therapeutic implications. *N Engl J Med* **285:** 1182–1186.

Gabrilovich DI, Chen HL, Girgis KR *et al* (1996). Production of vascular endothelial growth factor by human tumors inhibits the functional maturation of dendritic cells. *Nat Med* **2:** 1096–1103.

Gabrilovich DI, Ishida T, Nadaf S, Ohm JE, Carbone DP (1999). Antibodies to vascular endothelial growth factor enhance the efficacy of cancer immunotherapy by improving endogenous dendritic cell function. *Clin Cancer Res* **5:** 2963–2970.

Gao JQ, Kanagawa N, Xu DH *et al* (2008). Combination of two fiber-mutant adenovirus vectors, one encoding the chemokine FKN and another encoding cytokine interleukin 12, elicits notably enhanced anti-tumor responses. *Cancer Immunol Immunother* Epub ahead of print.

Gnant MF, Berger AC, Huang J *et al* (1999). Sensitization of tumor necrosis factor alpha-resistant human melanoma by tumor-specific in vivo transfer of the gene encoding endothelial monocyte-activating polypeptide II using recombinant vaccinia virus. *Cancer Res* **59:** 4668–4674.

Grover AC, Tangrea MA, Woodson KG *et al* (2006). Tumor-associated endothelial cells display GSTP1 and RARbeta2 promoter methylation in human prostate cancer. *J Transl Med* **4:** 13.

Halin C, Gafner V, Villani ME *et al* (2003). Synergistic therapeutic effects of a tumor targeting antibody fragment, fused to interleukin 12 and to tumor necrosis factor alpha. *Cancer Res* **63:** 3202–3210.

Hanahan D, Bergers G, Bergsland E (2000). Less is more, regularly: metronomic dosing of cytotoxic drugs can target tumor angiogenesis in mice. *J Clin Invest* **105:** 1045–1047.

Hanson JA, Gillespie JW, Grover A *et al* (2006). Gene promoter methylation in prostate tumor-associated stromal cells. *J Natl Cancer Inst* **98:** 255–261.

Hasumi Y, Klosowska-Wardega A, Furuhashi M, Ostman A, Heldin CH, Hellberg C (2007). Identification of a subset of pericytes that respond to combination therapy targeting PDGF and VEGF signaling. *Int J Cancer* **121:** 2606–2614.

Hicklin DJ, Ellis LM (2005). Role of the vascular endothelial growth factor pathway in tumor growth and angiogenesis. *J Clin Oncol* **23:** 1011–1027.

Hood JD, Bednarski M, Frausto R *et al* (2002). Tumor regression by targeted gene delivery to the neovasculature. *Science* **296:** 2404–2407.

van Horssen R, Ten Hagen TL, Eggermont AM (2006). TNF-alpha in cancer treatment: molecular insights, antitumor effects, and clinical utility. *Oncologist* **11:** 397–408.

Howe JR, Roth S, Ringold JC *et al* (1998). Mutations in the SMAD4/DPC4 gene in juvenile polyposis. *Science* **280:** 1086–1088.

Hurwitz H, Fehrenbacher L, Novotny W *et al* (2004). Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. *N Engl J Med* **350:** 2335–2342.

Jacoby RF, Schlack S, Sekhon G, Laxova R (1997). Del(10)(q22.3q24.1) associated with juvenile polyposis. *Am J Med Genet* **70:** 361–364.

Jeffes EW, Zhang JG, Hoa N *et al* (2005). Antiangiogenic drugs synergize with a membrane macrophage colony-stimulating factor-based tumor vaccine to therapeutically treat rats with an established malignant intracranial glioma. *J Immunol* **174:** 2533–2543.

Jefford M, Maraskovsky E, Cebon J, Davis ID (2001). The use of dendritic cells in cancer therapy. *Lancet Oncol* **2:** 343–353.

Kao J, Houck K, Fan Y *et al* (1994). Characterization of a novel tumor-derived cytokine. Endothelial-monocyte activating polypeptide II. *J Biol Chem* **269:** 25106–25119.

Kerbel RS (1991). Inhibition of tumor angiogenesis as a strategy to circumvent acquired resistance to anti-cancer therapeutic agents. *Bioessays* **13:** 31–36.

Khong HT, Restifo NP (2002). Natural selection of tumor variants in the generation of ''tumor escape'' phenotypes. *Nat Immunol* **3:** 999–1005.

Killeen S, Hennessey A, El Hassan Y, Waldron B (2008). The urokinase plasminogen activator system in cancer: a putative therapeutic target? *Drug News Perspect* **21:** 107–116.

Klebanoff CA, Finkelstein SE, Surman DR *et al* (2004). IL-15 enhances the in vivo antitumor activity of tumor-reactive CD8+ T cells. *Proc Natl Acad Sci USA* **101:** 1969–1974.

Kurozumi K, Hardcastle J, Thakur R *et al* (2007). Effect of tumor microenvironment modulation on the efficacy of oncolytic virus therapy. *J Natl Cancer Inst* **99:** 1768–1781.

Lakka SS, Gondi CS, Yanamandra N *et al* (2003). Synergistic down-regulation of urokinase plasminogen activator receptor and matrix metalloproteinase-9 in SNB19 glioblastoma cells efficiently inhibits glioma cell invasion, angiogenesis, and tumor growth. *Cancer Res* **63:** 2454–2461.

Lans TE, de Wilt JH, van Geel AN, Eggermont AM (2002). Isolated limb perfusion with tumor necrosis factor and melphalan for nonresectable sSewart-Treves lymphangiosarcoma. *Ann Surg Oncol* **9:** 1004–1009.

Lawler J (2000). The functions of thrombospondin-1 and-2. *Curr Opin Cell Biol* **12:** 634–640.

Li J, Hu P, Khawli LA, Epstein AL (2003a). Complete regression of experimental solid tumors by combination LEC/chTNT-3 immunotherapy and CD25(+) T-cell depletion. *Cancer Res* **63:** 8384–8392.

Li J, Hu P, Khawli LA, Epstein AL (2003b). LEC/chTNT-3 fusion protein for the immunotherapy of experimental solid tumors. *J Immunother* **26:** 320–331.

Li J, Dong X, Xu Z *et al* (2008). Endostatin gene therapy enhances the efficacy of paclitaxel to suppress breast cancers and metastases in mice. *J Biomed Sci* **15:** 99–109.

Libutti SK, Paciotti GF, Myer L *et al* (2007). Preliminary results of a phase I clinical trial of CYT-6091: a pegylated colloidal gold-TNF nanomedicine. *J Clin Oncol*, 2007 ASCO Meeting Proceedings Part I. **25:** 3603.

Liu F, Tan G, Li J, Dong X, Krissansen GW, Sun X (2007). Gene transfer of endostatin enhances the efficacy of doxorubicin to suppress human hepatocellular carcinomas in mice. *Cancer Sci* **98:** 1381–1387.

Magzoub M, Jin S, Verkman AS (2008). Enhanced macromolecule diffusion deep in tumors after enzymatic digestion of extracellular matrix collagen and its associated proteoglycan decorin. *FASEB J* **22:** 276–284.

Manegold C (2008). Bevacizumab for the treatment of advanced non-small-cell lung cancer. *Expert Rev Anticancer Ther* **8:** 689–699.

Mangiameli DP, Blansfield JA, Kachala S *et al* (2007). Combination therapy targeting the tumor microenvironment is effective in a model of human ocular melanoma. *J Transl Med* **5:** 38.

Manning EA, Ullman JG, Leatherman JM *et al* (2007). A vascular endothelial growth factor receptor-2 inhibitor enhances antitumor immunity through an immune-based mechanism. *Clin Cancer Res* **13:** 3951–3959.

von Marschall Z, Scholz A, Cramer T *et al* (2003). Effects of interferon alpha on vascular endothelial growth factor gene transcription and tumor angiogenesis. *J Natl Cancer Inst* **95:** 437–448.

Marshall E (2002). Cancer therapy. Setbacks for endostatin. *Science* **295:** 2198–2199.

Marvin MR, Libutti SK, Kayton M *et al* (1996). A novel tumor-derived mediator that sensitizes cytokine-resistant tumors to tumor necrosis factor. *J Surg Res* **63:** 248–255.

McCarty MF, Somcio RJ, Stoeltzing O *et al* (2007). Overexpression of PDGF-BB decreases colorectal and pancreatic cancer growth by increasing tumor pericyte content. *J Clin Invest* **117:** 2114–2122.

McMahon G (2000). VEGF receptor signaling in tumor angiogenesis. *Oncologist* **5**(Suppl. 1)**:** 3–10.

Miller KD, Sweeney CJ, Sledge GW Jr (2001). Redefining the target: chemotherapeutics as antiangiogenics. *J Clin Oncol* **19:** 1195–1206.

Miller KD, Chap LI, Holmes FA *et al* (2005). Randomized phase III trial of capecitabine compared with bevacizumab plus capecitabine in patients with previously treated metastatic breast cancer. *J Clin Oncol* **23:** 792–799.

Mistry SJ, Atweh GF (2006). Therapeutic interactions between stathmin inhibition and chemotherapeutic agents in prostate cancer. *Mol Cancer Ther* **5:** 3248–3257.

Mistry SJ, Bank A, Atweh GF (2007). Synergistic antiangiogenic effects of stathmin inhibition and taxol exposure. *Mol Cancer Res* **5:** 773–782.

Miyamoto A, Umeshita K, Sakon M *et al* (2000). Advanced hepatocellular carcinoma with distant metastases, successfully treated by a combination therapy of alpha-interferon and oral tegafur/uracil. *J Gastroenterol Hepatol* **15:** 1447–1451.

Mizukami Y, Jo WS, Duerr EM *et al* (2005). Induction of interleukin-8 preserves the angiogenic response in HIF-1alpha-deficient colon cancer cells. *Nat Med* **11:** 992–997.

Mortara L, Balza E, Sassi F *et al* (2007). Therapy-induced antitumor vaccination by targeting tumor necrosis factor alpha to tumor vessels in combination with melphalan. *Eur J Immunol* **37:** 3381–3392.

Nagano H, Miyamoto A, Wada H *et al* (2007). Interferon-alpha and 5-fluorouracil combination therapy after palliative hepatic resection in patients with advanced hepatocellular carcinoma, portal venous tumor thrombus in the major trunk, and multiple nodules. *Cancer* **110:** 2493–2501.

Nagase H, Woessner JF Jr (1999). Matrix metalloproteinases. *J Biol Chem* **274:** 21491–21494.

Netti PA, Berk DA, Swartz MA, Grodzinsky AJ, Jain RK (2000). Role of extracellular matrix assembly in interstitial transport in solid tumors. *Cancer Res* **60:** 2497–2503.

Nyberg P, Xie L, Kalluri R (2005). Endogenous inhibitors of angiogenesis. *Cancer Res* **65:** 3967–3979.

Ohmura Y, Yoshikawa K, Saga S, Ueda R, Kazaoka Y, Yamada S (2008). Combinations of tumor-specific CD8+ CTLs and anti-CD25 mAb provide improved immunotherapy. *Oncol Rep* **19:** 1265–1270.

Ohta M, Tanaka F, Yamaguchi H, Sadanaga N, Inoue H, Mori M (2005). The high expression of Fractalkine results in a better prognosis for colorectal cancer patients. *Int J Oncol* **26:** 41–47.

Olumi AF, Grossfeld GD, Hayward SW, Carroll PR, Tlsty TD, Cunha GR (1999). Carcinoma-associated fibroblasts direct tumor progression of initiated human prostatic epithelium. *Cancer Res* **59:** 5002–5011.

Ooyama A, Oka T, Zhao HY, Yamamoto M, Akiyama SI, Fukushima M (2008). Anti-angiogenic effect of 5-Fluorouracil-based drugs against human colon cancer xenografts. *Cancer Lett* **267:** 26–36.

Osada T, Chong G, Tansik R *et al* (2008). The effect of anti-VEGF therapy on immature myeloid cell and dendritic cells in cancer patients. *Cancer Immunol Immunother* **57:** 1115–1124.

Ota H, Nagano H, Sakon M *et al* (2005). Treatment of hepatocellular carcinoma with major portal vein thrombosis by combined therapy with subcutaneous interferon-alpha and intra-arterial 5-fluorouracil; role of type 1 interferon receptor expression. *Br J Cancer* **93:** 557–564.

Paget S (1889). he distribution of secondary growths in cancer of the breast. *Lancet* **1:** 571–573.

Parmiani G, Rivoltini L, Andreola G, Carrabba M (2000). Cytokines in cancer therapy. *Immunol Lett* **74:** 41–44.

Pietras K, Pahler J, Bergers G, Hanahan D (2008). Functions of paracrine PDGF signaling in the proangiogenic tumor stroma revealed by pharmacological targeting. *PLoS Med* **5:** e19.

Plum SM, Hanson AD, Volker KM *et al* (2003). Synergistic activity of recombinant human endostatin in combination with adriamycin: analysis of in vitro activity on endothelial cells and in vivo tumor progression in an orthotopic murine mammary carcinoma model. *Clin Cancer Res* **9:** 4619–4626.

Rodriguez-Canales J, Hanson JC, Tangrea MA *et al* (2007). Identification of a unique epigenetic sub-microenvironment in prostate cancer. *J Pathol* **211:** 410–419.

Rosenberg SA, Restifo NP, Yang JC, Morgan RA, Dudley ME (2008). Adoptive cell transfer: a clinical path to effective cancer immunotherapy. *Nat Rev Cancer* **8:** 299–308.

Sacchi A, Gasparri A, Curnis F, Bellone M, Corti A (2004). Crucial role for interferon gamma in the synergism between tumor vasculature-targeted tumor necrosis factor alpha (NGR-TNF) and doxorubicin. *Cancer Res* **64:** 7150–7155.

Sacchi A, Gasparri A, Gallo-Stampino C, Toma S, Curnis F, Corti A (2006). Synergistic antitumor activity of cisplatin, paclitaxel, and gemcitabine with tumor vasculature-targeted tumor necrosis factor-alpha. *Clin Cancer Res* **12:** 175–182.

Sakon M, Nagano H, Dono K *et al* (2002). Combined intraarterial 5-fluorouracil and subcutaneous interferon-alpha therapy for advanced hepatocellular carcinoma with tumor thrombi in the major portal branches. *Cancer* **94:** 435–442.

Sandler A, Gray R, Perry MC *et al* (2006). Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer. *N Engl J Med* **355:** 2542–2550.

Sarmiento R, Gasparini G (2008). Antiangiogenic metronomic chemotherapy. *Onkologie* **31:** 161–162.

Seiwert TY, Haraf DJ, Cohen EE *et al* (2008). Phase I study of bevacizumab added to fluorouracil- and hydroxyurea-based concomitant chemoradiotherapy for poor-prognosis head and neck cancer. *J Clin Oncol* **26:** 1732–1741.

Shapiro SD (1998). Matrix metalloproteinase degradation of extracellular matrix: biological consequences. *Curr Opin Cell Biol* **10:** 602–608.

Shekhar MP, Werdell J, Santner SJ, Pauley RJ, Tait L (2001). Breast stroma plays a dominant regulatory role in breast epithelial growth and differentiation: implications for tumor development and progression. *Cancer Res* **61:** 1320–1326.

Shojaei F, Ferrara N (2007). Antiangiogenic therapy for cancer: an update. *Cancer J* **13:** 345–348.

Sica A, Bronte V (2007). Altered macrophage differentiation and immune dysfunction in tumor development. *J Clin Invest* **117:** 1155–1166.

Singh H, Serrano LM, Pfeiffer T *et al* (2007). Combining adoptive cellular and immunocytokine therapies to improve treatment of B-lineage malignancy. *Cancer Res* **67:** 2872–2880.

Steeghs N, Nortier JW, Gelderblom H (2007). Small molecule tyrosine kinase inhibitors in the treatment of solid tumors: an update of recent developments. *Ann Surg Oncol* **14:** 942–953.

Sternlicht MD, Lochter A, Sympson CJ *et al* (1999). The stromal proteinase MMP3/stromelysin-1 promotes mammary carcinogenesis. *Cell* **98:** 137–146.

Stoklasek TA, Schluns KS, Lefrancois L (2006). Combined IL-15/IL-15Ralpha immunotherapy maximizes IL-15 activity in vivo. *J Immunol* **177:** 6072–6080.

Tangrea MA, Chuaqui RF, Gillespie JW *et al* (2004). Expression microdissection: operator-independent retrieval of cells for molecular profiling. *Diagn Mol Pathol* **13:** 207–212.

Tohnya TM, Ng SS, Dahut WL *et al* (2004). A phase I study of oral CC-5013 (lenalidomide, Revlimid), a thalidomide derivative, in patients with refractory metastatic cancer. *Clin Prostate Cancer* **2:** 241–243.

Wada H, Nagano H, Yamamoto H *et al* (2007). Combination therapy of interferon-alpha and 5-fluorouracil inhibits tumor angiogenesis in human hepatocellular carcinoma cells by regulating vascular endothelial growth factor and angiopoietins. *Oncol Rep* **18:** 801–809.

Westermarck J, Kahari VM (1999). Regulation of matrix metalloproteinase expression in tumor invasion. *FASEB J* **13:** 781–792.

Willhauck MJ, Mirancea N, Vosseler S *et al* (2007). Reversion of tumor phenotype in surface transplants of skin SCC cells by scaffold-induced stroma modulation. *Carcinogenesis* **28:** 595–610.

Wu PC, Alexander HR, Huang J *et al* (1999). In vivo sensitivity of human melanoma to tumor necrosis factor (TNF)-alpha is determined by tumor production of the novel cytokine endothelial-monocyte activating polypeptide II (EMAPII). *Cancer Res* **59:** 205–212.

Wu MP, Young MJ, Tzeng CC *et al* (2008). A novel role of thrombospondin-1 in cervical carcinogenesis: Inhibit stroma reaction by inhibiting activated fibroblasts from invading cancer. *Carcinogenesis* **29:** 1115–1123.

Yang JC (2004). Bevacizumab for patients with metastatic renal cancer: an update. *Clin Cancer Res* **10:** 6367S–6370S.

Yang H, Grossniklaus HE (2006). Combined immunologic and anti-angiogenic therapy reduces hepatic micrometastases in a murine ocular melanoma model. *Curr Eye Res* **31:** 557–562.

Yockman JW, Kim WJ, Chang CW, Kim SW (2007). Non-viral delivery of interleukin-2 and soluble Flk-1 inhibits metastatic and primary tumor growth in renal cell carcinoma. *Gene Ther* **14:** 1399–1405.

Yu AE, Hewitt RE, Connor EW, Stetler-Stevenson WG (1997). Matrix metalloproteinases. Novel targets for directed cancer therapy. *Drugs Aging* **11:** 229–244.

Zeng Y, Huebener N, Fest S *et al* (2007). Fractalkine (CX3CL1)- and interleukin-2-enriched neuroblastoma microenvironment induces eradication of metastases mediated by T cells and natural killer cells. *Cancer Res* **67:** 2331–2338.

Zhu Y, Tibensky I, Schmidt J *et al* (2008). Interferon-alpha in combination with chemotherapy has potent antiangiogenic properties in an orthotopic mouse model for pancreatic adenocarcinoma. *J Immunother* **31:** 28–33.

Zitvogel L, Tesniere A, Kroemer G (2006). Cancer despite immunosurveillance: immunoselection and immunosubversion. *Nat Rev Immunol* **6:** 715–727.