NEOPLASIA
www.neoplasia.com

Volume 11 Number 9 September 2009 pp. 874–881 874

Tumor Interstitial Fluid Pressure as an Early-Response Marker for Anticancer Therapeutics

Stephane Ferretti, Peter R. Allegrini, Mike M. Becquet and Paul M.J. McSheehy

Oncology Research, Novartis Institute of Biomedical Research, 4002 Basel, Switzerland

Abstract

Solid tumors have a raised interstitial fluid pressure (IFP) due to high vessel permeability, low lymphatic drainage, poor perfusion, and high cell density around the blood vessels. To investigate tumor IFP as an early-response biomarker, we have tested the effect of seven anticancer chemotherapeutics including cytotoxics and targeted cytostatics in 13 experimental tumor models. IFP was recorded with the wick-in-needle method. Models were either ectopic or orthotopic and included mouse and rat syngeneic as well as human xenografts in nude mice. The mean basal IFP was between 4.4 and 15.2 mm Hg; IFP was lowest in human tumor xenografts and highest in rat syngeneic models. Where measured, basal IFP correlated positively with relative tumor blood volume (rTBV) determined by dynamic contrast-enhanced magnetic resonance imaging. Most chemotherapeutics sooner (2 or 3 days) or later (6 or 7 days) lowered tumor IFP significantly, and the cytotoxic patupilone caused the greatest decrease in IFP. In rat mammary orthotopic BN472 tumors, significant drug-induced decreases in IFP and rTBV correlated positively with each other for both patupilone and the cytostatic vatalanib. In the two orthotopic models studied, early decreases in IFP were significantly ($P \leq .005$) correlated with late changes in tumor volume. Thus, drug-induced decreases in tumor IFP are an early marker of response to therapy, which could aid clinical development.

Neoplasia (2009) 11, 874–881

Introduction

The initial growth of a solid tumor makes use of existing vasculature, but further growth requires formation of new blood vessels, that is, angiogenesis, and these blood vessels tend to be leaky, highly irregular, and tortuous and exhibit a low blood flow [1,2]. Compression of the blood vessels by proliferating cells also increases the microvascular pressure, and an inadequate lymphatic system limits drainage of excess fluid and thus reduces fluid movement through the interstitium. Together, these factors cause an elevated interstitial fluid pressure (IFP) in solid tumors compared with normal tissues [1]. In experimental tumor models, IFP is uniform across the whole tumor except at the periphery where it drops precipitously [1]. In the clinic, a raised IFP has been demonstrated in several tumor types [1], although data from cervical tumors indicated much greater heterogeneity than in experimental models [3].

IFP has been used as a prognostic factor where it was shown to be the single best indicator of survival for patients with cancer of the cervix [3]. Furthermore, chemotherapy has been shown to reduce IFP. Preclinically, this has been shown for dexamethasone [4], hydralazine [5], imatinib [6], PGE1 [7], and patupilone [8]. In the latter study, decreases in IFP were negatively correlated with apoptosis ($P < .001$) and positively correlated with tumor blood volume ($P = .0005$). In the clinic, bevacizumab [9] and paclitaxel [10] have been shown to reduce the IFP of rectal and breast tumors, respectively. However, whether the drug-induced decrease in IFP is, in general, predictive of subsequent response has not been investigated. This could be very useful because generic markers of tumor response can allow optimization of 1) current therapies, for example, discontinuation in the event of a lack of response and 2) new drugs in development, for example, attainment of an optimal biologic dose that provides an alternative to the maximum tolerated dose. Although measurement of IFP is invasive, it has been used in the clinic for breast, cervix, colorectal, head and neck, lung, melanoma, and renal tumors [1,3,9,10].

Abbreviations: rTBV, relative tumor blood volume; DCE-MRI, dynamic contrast-enhanced magnetic resonance imaging; IFP, interstitial fluid pressure; TVol, tumor volume

Address all correspondence to: Stephane Ferretti, Novartis Pharma AG, WKL-125.2.05B, CH-4002 Basel, Switzerland. E-mail: stephane.ferretti@novartis.com

Received 1 April 2009; Revised 2 June 2009; Accepted 3 June 2009

DOI 10.1593/neo.09554

The aim of this study was to investigate 1) the relationship between IFP and the tumor vasculature and 2) the effects of several different chemotherapeutics, including cytotoxics and cytostatic targeted agents on IFP to better understand the nature of IFP and whether it could be a marker of tumor response to chemotherapy.

Materials and Methods

Animals

All animal experiments were done in strict adherence to the Swiss law for animal protection. Female C3H/He, black C57BL/6, HsdEZ *nu/nu* (Harlan nude), and BALB/c nude mice were obtained from Iffa Credo (Charles River, St Germain Sur L Arbresle, France) or Novartis (Basel, Switzerland). Female BDIX and Brown-Norwegian (BN) rats were obtained from Charles Rivers or Harlan Netherlands BV (Horst, Netherlands). Nude mice were kept in a pathogen-controlled environment.

Tumor Models

Cell culture materials were obtained from Life Technologies (Paisley, United Kingdom) or BioConcept (Allschwil, Switzerland).

Orthotopic tumor models. BN472 rat mammary carcinomas were established in female BN rats by orthotopic (mammary fat pad) transplantation of BN472 tumor fragments obtained from donor rats [8]. B16/BL6 mice melanoma cells were injected intradermally (ID) in the ear from where they rapidly metastasize to the lymph nodes, primarily at the neck, and these LN-mets were studied in this report [11]. RIF-1 mice fibrosarcoma tumors were induced by concentrating 2 to 5×10^6 cells in 100 µl of Hank's balanced salt solution and injecting subcutaneously (SC) in the top of the right thigh of C3H/He mice [12].

Ectopic tumor models. PROb rat colon adenocarcinoma cells (a.k.a. DHD/K12/TrB) were a gift from the laboratory of Chandra Rubin, Uppsala, Sweden. They were cultured in Dulbecco's modified Eagle medium (DMEM) + F10 medium with the addition of 2 mM glutamine and 10% fetal calf serum (FCS). U87MG human glioma cells were cultured in DMEM high glucose supplemented with 10% FCS, 1% glutamine. 1A9 [13] and 1A9ptx10 paclitaxel-resistant variant [14] human ovarian carcinoma cells were cultured in RPMI-1640 medium supplemented with 20% FCS and 1% glutamine. For the culture of 1A9ptx10 cells, this medium was supplemented with 17.5 nM paclitaxel and 5 µg/ml verapamil. C6 rat glioma cells were cultured in RPMI-1640 supplemented with 10% FCS and 1% glutamine. HeLa human cervical adenocarcinoma cells were cultured in DMEM low glucose supplemented with 10% FCS and 1% glutamine. All the tumors were induced by concentrating 5×10^6 to 10^7 cells in 100 µl of phosphate-buffered saline and injecting SC in the right flank of Harlan nude mice or, in some cases, of BALB/c (C6 cells) or BDIX rats (PROb cells).

Drug Preparation and Treatment

Animals were randomized into different treatment groups based on tumor size (200-400 mm^3 in mice; 500-1000 mm^3 in rats). IFP measurements were made in each individual animal immediately before treatment and at days 2 to 3 and days 6 to 7 after initiation of treatment (when applicable, treatment occurred 2 hours before the second or third IFP measurement).

Patupilone (EPO906), imatinib (STI571), vatalanib (PTK/ZK), everolimus (RAD001), and AEE788 were obtained from Chemical Development, Novartis, and the powders were stored at +4°C. Cyclophosphamide (CP) and gemcitabine were obtained from Sigma-Aldrich Chemie, GmbH, (Buchs SG, Switzerland) and Lilly SA (Fegersheim, France), respectively. All the drugs were freshly prepared on each treatment day by using the appropriate formulation. The doses and schedules used in the experiments described in the next paragraphs were selected based on previous experience with the compounds that gave significant inhibition of tumor growth but were tolerated by the animals [8,11,12,15–19]. In general, cytostatics were very well tolerated and caused little or no change in body weight (BW), although the cytotoxics induced BW loss, but at the doses and schedules used, this was tolerated and normally transient (2-3 days). Animals showing an individual BW loss more than 15% or with ulcerating tumors or altered behavior (self-mutilation, immobile) were culled.

Cytostatics. All cytostatics were administered by oral gavage using 5 ml/kg for rats and 10 ml/kg for mice. Imatinib is an inhibitor of Bcr-Abl, c-kit, and the platelet-derived growth factor receptor; it was administered at 50 to 200 mg/kg daily or twice daily for 3 or 7 days. Vatalanib is a pan–vascular endothelial growth factor receptor (VEGFR) and platelet-derived growth factor receptor inhibitor; it was administered at 30 to 200 mg/kg daily. Everolimus is a mammalian target of rapamycin inhibitor; it was administered at 5 mg/kg thrice weekly or daily. NVP-AEE788 is an inhibitor of VEGFR-2 (kdr) and epidermal growth factor receptor; it was administered at 30 or 60 mg/kg thrice weekly.

Cytotoxics. Patupilone is a microtubule stabilizer; it was administered intravenously (IV) once at 0.15 to 1.5 and 1 to 4 mg/kg for rats and mice, respectively, using 2 ml/kg for rats and 5 ml/kg for mice. Gemcitabine is an antimetabolite; it was administered intraperitoneally (10 ml/kg) at 75 mg/kg twice weekly or 150 mg/kg once weekly. CP is an alkylating agent; it was administered at 50 and 150 mg/kg (10 ml/kg) for rats and mice, respectively.

Efficacy and IFP

Tumor volume (TVol) was determined from the formula: $l \times w \times h \times \pi / 6$. Efficacy was quantified as the T/C_{TVol} determined from the mean difference in TVol (ΔTVol) of drug-treated animals divided by the mean difference in volume of vehicle-treated animals.

Tumor IFP was measured using the wick-in-needle technique [15] in anesthetized animals (1.5%-2.5% isoflurane delivered at 1-2 L/min) lying on an electrically warmed pad. A standard 30-gauge needle connected to a pressure transducer was inserted into the central part of the tumors, and the pressure was monitored for a period of 10 minutes. This setup enabled continuous and stable recordings of fluid pressure at one site in the tumor. Previous measurements have shown that, in experimental tumors, the IFP is uniform except at the edge [1]. We were able to confirm this in our BN472 tumor model where two widely separated sites gave IFP values that differed by approximately 5%. In all models, the response to drug treatment was expressed as a T/C_{IFP} determined from the mean fractional change in IFP (IFP-F) of drug-treated animals divided by the IFP-F of vehicle-treated animals.

Dynamic Contrast-Enhanced Magnetic Resonance Imaging

Animals were anesthetized using 1.5% isoflurane (Abbott, Cham, Switzerland) in a 1:1 mixture of O_2/N_2 and placed on an electrically

warmed pad for cannulation of one lateral tail vein as previously described [11]. Magnetic resonance imaging (MRI) experiments were performed on a Bruker DBX 47/30 or Avance 2 spectrometer (Bruker Medical, Fällanden, Switzerland) at 4.7 T equipped with a self-shielded 12-cm bore gradient system. The iron oxide particle intravascular contrast agent Endorem was injected (6 mmol of iron per kilogram) for determining the relative tumor blood volume (rTBV) and an index of blood flow (BFI). The Endorem uptake curve was fitted with a sigmoid curve (software: BioMap based in IDL, Research Systems, Inc, Boulder, CO) to provide a value for the rTBV (plateau) and the BFI, which was the rTBV divided by the steepest part of the slope. Note that the line slope for Endorem uptake was very rapid, meaning the measurement of BFI was less precise than rTBV. Values shown in the Results section are in arbitrary units, and the principles behind measurement of these parameters have been fully described [11]. At the end of all experiments, animals were killed by CO_2 inhalation.

Histology

Blood vessels. Perfused blood vessels were visualized in frozen sections of tumor tissue using the nuclear staining dye H33342, which was injected IV (20 mg/kg, 10 ml/kg) in the lateral tail vein 45 seconds before killing the rodent [11]. Tumors were ablated, embedded in Tissue-Tek (Sakura, the Netherlands), and frozen slowly on dry ice. Cryosections of embedded tissue with a thickness of 10 μm were taken from the central tumor region. Blood vessel outlines were identified by the surrounding halo of fluorescent H33342-labeled cells. For measuring vessel density, all vessels, independent of size, were counted over the whole tumor section, and this was expressed as vessels per squared millimeters. Vessel sizes reported are external diameters.

Hypoxia

Tumor hypoxia was assessed quantitatively by use of the hydroxyprobe-1 assay kit, HP-100 (Chemicon International, Billerica, MA). Before culling (90 minutes), pimonidazole hydrochloride was injected IV (20 mg/kg, 2 ml/kg) in the lateral tail vein of rodents. The tumor was removed and embedded in Tissue-Tek and prepared for histology as previously described. Hypoxia was visualized by detection of bound pimonidazole using the antipimonidazole monoclonal antibody at a dilution of 1:50 according to the kit instructions. Microscopic analysis was performed at a magnification of ×100. The whole area of each tumor slice was photographed, and both hypoxic and nonhypoxic areas were quantified pixel by pixel and expressed as the percentage hypoxic area of the tumor total area.

Statistical Analysis

The data are expressed as means ± SEM. The statistical significance of differences between changes in drug-treated groups and vehicle-treated groups at a specific time point was determined by using a 2-tailed *t*-test or one-way analysis of variance (ANOVA) with Tukey test *post hoc* for multiple comparisons (using the software from SigmaStat version 3.1, Systat Software, Chicago, IL). To quantify the relationship between various parameters, the Pearson linear correlation coefficient was used to provide the coefficient of determination, r^2, and the associated P value. In the data sets of BN472 tumors treated with 1) vehicle or vatalanib and 2) vehicle or patupilone, one rat was excluded from all analyses in each set because it was an outlier by Grubb's test for the BFI-F and IFP-F data, respectively, on day 2 (see http://www.graphpad.com). For all statistical evaluations, a probability value of $P < .05$ was considered to be significant.

Results

Basal IFP of Ectopic and Orthotopic Tumor Models

Tumor IFP (mm Hg) was evaluated in a wide range of experimental tumor models ($n = 13$) including human tumors in nude mice as well as syngeneic models in mice and rats (Table 1). The highest mean IFPs were all associated with rat tumors whether grown in mice or rats SC or orthotopically. Rat colon PROb tumors implanted SC (ectopically) in rats had an IFP of 15.2 ± 0.7 mm Hg, ($n = 45$), whereas the same tumor also implanted SC in the flank of nude mice had a significantly ($P = .01$) lower IFP (10.5 ± 2.3 mm Hg, $n = 14$). In contrast, the IFP of C6 tumors was not significantly altered when grown SC in two different types of nude mouse (Harlan or BALB/c). Similarly, when BN472 (in rats) and B16/BL6 (in mice) tumors were grown both SC and orthotopically in the same animal, there was no significant difference in IFP (Table 1, $P > .7$). The human ovarian xenograft 1A9 (wild type) and its paclitaxel-resistant counterpart (1A9ptx10), which differed only by one amino acid in β-tubulin, also had no significant difference in IFP. In general, most human tumor SC xenografts had rather low IFPs and, in some cases, for example, U87MG, there were several tumors that were almost unmeasurable, with IFPs less than 2.0 mm Hg. Collectively, these observations emphasize the important role of both the tumor type and the host species in contributing to the basal IFP.

Effect of Cytostatics on IFP and Tumor Growth

Imatinib. Daily treatment (100 mg/kg) for 1 week of BDIX rats bearing rat PROb tumors significantly inhibited growth ($T/C_{TVol} = 0.32$ on day 7) and showed a trend to reduce IFP by approximately 20% with no evidence that longer treatment had greater effects on IFP (Table 2). A 3-day experiment incorporated dynamic contrast-enhanced MRI (DCE-MRI) for the measurement of rTBV and BFI and histology *ex vivo* for the measurements of hypoxia (Figure 1). Basal IFP was positively correlated with basal rTBV ($r^2 = 0.34$, $P = .06$) but not at all with BFI ($r^2 = 0.03$, $P = .7$), see Figure 1*A*. This experiment confirmed a small but significant decrease (–22%) in IFP (Figure 1*B*) and trends for decreases in rTBV and BFI (Figure 1*C*). Histologic diagnosis showed that imatinib also had no significant effect on hypoxia: $T/C_{pimo} = 0.8$, $P = .2$ (Figure 1*D*). The same tumors grown in Harlan nude mice showed much greater decreases in IFP (–85%), although

Table 1. Basal IFP in Function of the Type of Tumor and the Host.

Tumor Model	Species	Injection Site	Host and Strain	*n*	IFP
PROb, colon	Rat	SC	BDIX rat	45	15.2 ± 0.7
C6, glioma	Rat	SC	Harlan nude mice	36	14.6 ± 1.5
C6, glioma	Rat	SC	BALB/c nude mice	35	13.7 ± 1.6
BN472, breast	Rat	SC	BN rat	12	13.1 ± 5.4
BN472, breast	Rat	Mammary gland	BN rat	218	12.3 ± 0.3
HeLa, cervical	Human	SC	Harlan nude mice	15	11.2 ± 1.5
PROb, colon	Rat	SC	Harlan nude mice	14	10.5 ± 2.3*
1A9, ovarian	Human	SC	Harlan nude mice	62	7.2 ± 0.6
B16/BL6, melanoma	Mouse	ID	C57BL/6 mice	35	6.7 ± 0.5
B16/BL6 melanoma	Mouse	SC	C57BL/6 mice	14	6.3 ± 3.3
RIF-1, fibrosarcoma	Mouse	SC	C3H/He mice	29	6.3 ± 0.6
1A9ptx10, ovarian	Human	SC	Harlan nude mice	65	6.2 ± 0.5
U87, glioma	Human	SC	Harlan nude mice	24	4.4 ± 0.7

Tumor initiation is described in the Materials and Methods section; they were studied 2 to 7 weeks after cell injection/transplantation, and IFP (mm Hg) was measured using the wick-in-needle method. Results show the mean ± SEM for basal IFP of all the tumor models used for experiments and the number of animals (*n*).

*$P = .01$, compared with the same tumor grown in BDIX rats.

Table 2. Effect of Cytostatics on the Growth and IFP of Several Tumor Models.

Treatment	Dose (mg/kg) and Schedule	Tumor	Host	*n*	Day 2/3	Day 6/7	
					T/C_{IFP}	T/C_{TVol}	T/C_{IFP}
Imatinib	100, daily		BDIX rat	6	0.78	0.32*	0.80
				12	0.72*	—	—
		PROb		7	0.50†	1.14	0.15‡
			Harlan nude mouse	12	0.56‡	—	—
		C6	BALB/c nude mouse	11	0.85	—	—
Vatalanib	100, daily	PROb	BDIX rat	6	0.91	0.26§	0.84
	200, daily	BN472	BN rat	5	0.66†	0.3†	0.5‡
AEE788	60, thrice weekly			12	0.48§	0.48‡	0.59‡
Everolimus	5, thrice weekly			10	0.88	0.56	0.75‡

Tumor initiation is described in the Materials and Methods section; after 2 to 7 weeks of growth, IFP was determined before (day 0), during (day 2 or 3), and after treatment (day 6 or 7) with imatinib, vatalanib, AEE788, or everolimus at the doses and schedules shown. Results show the effect of one dose for each drug in a specific tumor model as the T/C for TVol and IFP.

*$P < .01$, significantly different from the change in the vehicle.

†The T/C was low but not significantly different to the change in the vehicle ($P < .3$; 2-tailed *t*-test or one-way ANOVA with Tukey test *post hoc*).

‡$P < .05$, significantly different from the change in the vehicle.

§$P < .001$, significantly different from the change in the vehicle.

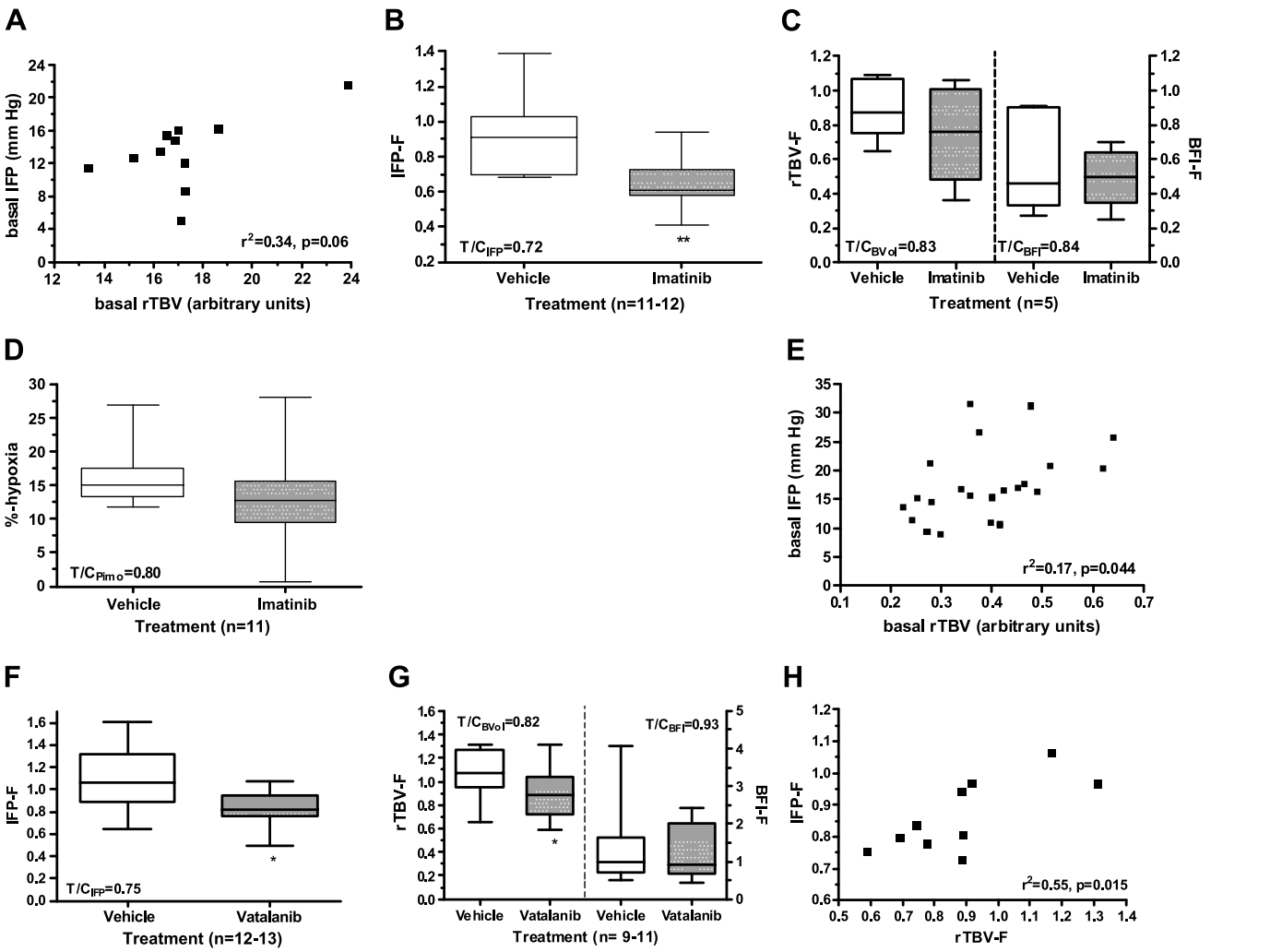

Figure 1. Effects of imatinib and vatalanib on the IFP and vasculature of rat syngeneic tumors. PROb tumors in BDIX rats (A–D) and BN472 rat mammary carcinomas in BN rats (E–H) were created as described in the Materials and Methods section. After 3 to 7 weeks of growth, IFP was determined, followed by DCE-MRI measurement of rTBV and BFI using the intravascular contrast agent Endorem before (day 0) and 3 days after initiation of treatment with vehicle or imatinib (100 mg/kg daily; A–D) or vatalanib (100 mg/kg daily; E–H). After measurements *in situ* on day 3, the rats treated with imatinib were injected with pimonidazole, killed 90 minutes later, and the tumors were ablated for histologic diagnosis. Results show the Pearson correlation for individual tumors between basal rTBV and IFP in PROb (A) and BN472 tumors (E) and the IFP-F and rTBV-F on day 3 for treatment with vatalanib in BN472 tumors (H) and also the median and 25th and 75th quartiles for the effect of treatment on the different parameters of IFP-F, rTBV-F, BFI-F, and percentage hypoxia, where *$P < .05$ and **$P < .01$.

there was no effect on tumor growth. Thus, the effects of imatinib on rat PROb tumors seemed to be host-dependent. To a certain extent, similar data were obtained with C6 tumors, where a significant decrease (−44%) was detectable when Harlan nude mice were the host but not with BALB/c nude mice (Table 2). These experiments with imatinib further emphasize the role of the host vasculature in contributing to tumor IFP and the effect of treatment on IFP.

Vatalanib. Vatalanib was also tested in the PROb model using BDIX rats and, despite strongly inhibiting growth, did not significantly affect IFP (Table 2). Vatalanib was tested at several doses in the BN472 model and showed a dose-dependent reduction of IFP and tumor growth, and these two effects were significantly correlated (Figure 2). One experiment is summarized in Table 2. In another experiment, tumor IFP, rTBV, and BFI were measured in the same mice treated with vehicle or vatalanib. These data showed that basal IFP was positively correlated with basal rTBV (Figure 1*E*) and, to a similar extent, with BFI (r^2 = 0.19, P = .04). Treatment with vatalanib decreased IFP (Figure 1*F*) and also rTBV but did not affect BFI, which, overall, was unchanged in both treatment groups (Figure 1*G*). The fractional changes in rTBV induced by vatalanib correlated significantly with IFP (Figure 1*H*) but not with BFI (r^2 = 0.19, P = .21). These changes in noninvasive measurements of vasculature were not associated with histologic changes in blood vessel density (14.2 ± 1.6 to 16.1 ± 1.9 vessels per mm^2, P = .46) but were associated with narrower vessels (20.8 ± 1.3 μm reduced to 17.7 ± 1.0 μm, P = .07). Finally, overall, in BN472 tumors, there was a significant positive correlation between early IFP changes and the eventual change in TVol in response to vatalanib (r^2 = 0.37, P = .0006, n = 28; Figure 3*A*). These experiments with vatalanib further demonstrate the relationship between IFP and the host vasculature, in particular, rTBV, which consistently correlated with basal IFP and changes in IFP in response to treatment.

AEE788 and everolimus on BN472 tumors. AEE788 (60 mg/kg thrice weekly) significantly inhibited tumor growth (T/C_{TVol} = 0.48 on day 7) and reduced IFP to a similar degree during the same period (T/C_{IFP} = 0.48 and 0.59 on days 3 and 7, respectively; Table 2). Similar data were obtained using a lower dose of 30 mg/kg: $T/C_{TVol\ day\ 7}$ = 0.60 and $T/C_{IFP\ day\ 7}$ = 0.47.

As previously reported [20], everolimus (5 mg/kg thrice weekly) also decreased BN472 tumor growth and IFP, but in both cases, this was only after a week of treatment probably because this model is not very sensitive to everolimus (Table 2).

Thus, these further experiments with the BN472 model confirmed a consistent relationship between drug-induced changes in IFP and inhibition of tumor growth.

Effect of Cytotoxic Drugs on IFP and Tumor Growth

Patupilone. Patupilone was tested in several different ectopic and orthotopic tumor models (Table 3). The drug potently inhibited growth and decreased IFP of all tumors with significant effects normally detectable after 3 to 7 days (max T/C_{TVol} = −0.2 to 0.3 and max T/C_{IFP} = 0.2-0.5). The exception was the U87 tumor model where the greatest antitumor effect was only attained on day 7 (T/C_{TVol} = 0.37), and this did not reach significance (P = .14). Consistent with this, there was also no significant change in the IFP of U87 tumors at either time point. In BN472 tumors, a dose-dependent effect on IFP was apparent, and there was a highly significant positive correlation between early IFP changes and the eventual change in $TVol_{day\ 7}$ (r^2 = 0.33, P < .0001; Figure 3*B*). In RIF-1 tumors, there was also a significant positive correlation between early IFP changes and the eventual change in $TVol_{day\ 7}$ (r^2 = 0.46, P = .005; Figure 3*C*).

Thus, in general, for patupilone, across all models, a drug-induced decrease in IFP was paralleled by decreases in TVol.

CP and gemcitabine. CP was tested in the two orthotopic tumor models RIF-1 and BN472. In both models, CP had a rapid inhibitory effect on tumor growth inducing stasis or even minor regression (Table 3). In contrast, rapid effects on IFP were not significant ($T/C_{IFP\ day\ 3}$ = 0.78-0.80) and only in BN472 tumors was there a significant effect after 7 days of treatment (T/C_{IFP} = 0.65, P < .05). Consequently, there was a significant correlation between IFP-$F_{day\ 7}$ with $TVol_{day\ 7}$ (r^2 = 0.41, P = .008) in the BN472 model but not in the RIF-1 model (Table 4). In a separate study, DCE-MRI studies showed that in RIF-1 tumors, CP actually increased tumor rTBV: T/C_{rTBV} on days 3 and 7 was 1.56 and 1.43, respectively, which may explain the minimal effects of CP on the IFP of RIF-1 tumors.

The antimetabolite gemcitabine was also tested in the BN472 model. A schedule of 150 mg/kg once weekly showed similar potency to CP causing TVol stasis after 2 to 7 days; IFP was significantly lowered after 2 days (T/C_{IFP} = 0.51, P = .03) and returned to baseline by day 7

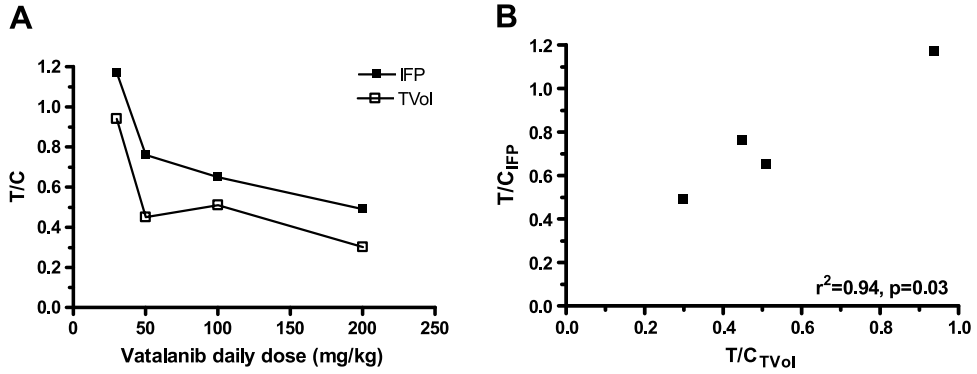

Figure 2. Dose-dependent effects of vatalanib on BN472 IFP and TVol. BN472 rat mammary carcinomas were established in female BN rats as described in the Materials and Methods section. After 2 to 3 weeks of growth, rats were treated with vatalanib at the doses shown (per os, daily), and IFP and TVol were determined on days 2 and 7. Results show the T/C on day 7 after treatment for IFP and TVol (A) and the correlation between the two on day 7 (B).

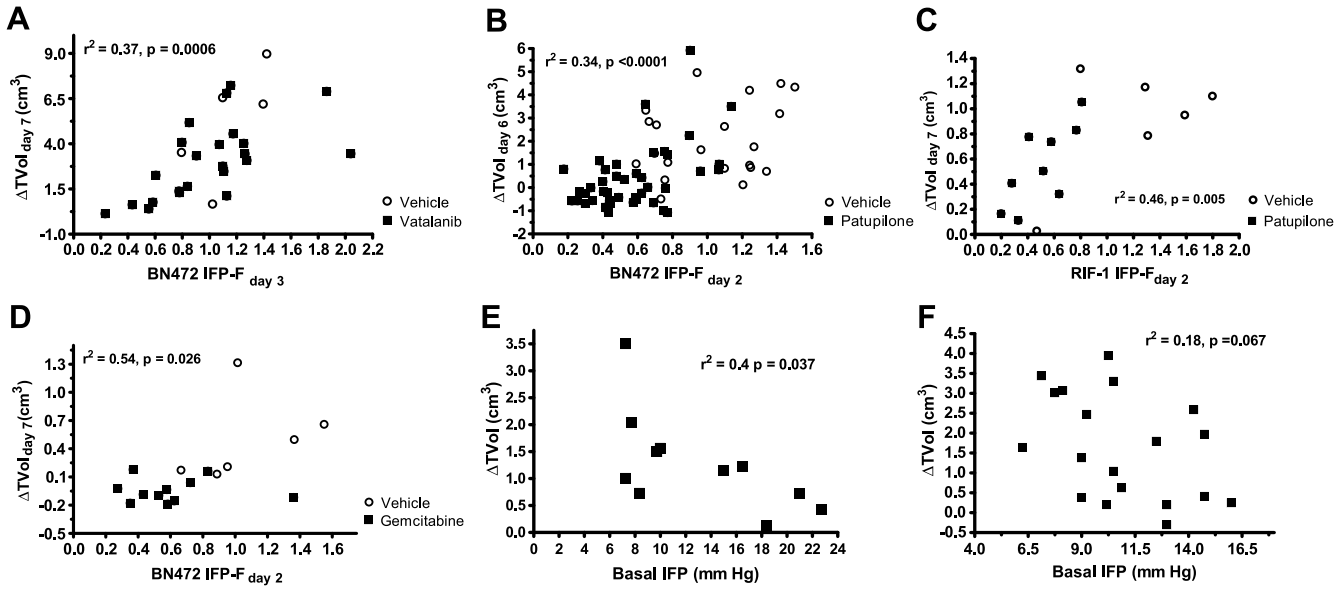

Figure 3. Correlations between IFP and tumor response to treatment. BN472 rat mammary carcinomas were established in female BN rats (A, B, D–F) and RIF-1 tumors in C3H/He mice (C) were created as described in the Materials and Methods section. After 2 to 3 weeks of growth, IFP was determined before (day 0), during (day 2 or 3), and after treatment (day 6 or 7) with patupilone (0.15-4 mg/kg once), vatalanib (30-200 mg/kg daily) or gemcitabine (75 mg/kg twice weekly and 150 mg/kg once). Results show Pearson correlations for individual tumors between IFP-F on days 2 to 3 and ΔTVol on days 6 to 7 for treatment with vatalanib (A) patupilone (B, C) or gemcitabine (D) and between the response of individual tumors (ΔTVol) and the pretreatment basal IFP for rats treated with 0.3 mg/kg patupilone once (E) or 100 mg/kg vatalanib daily (F).

(Table 3). An alternative schedule of 75 mg/kg twice a week improved the efficacy ($T/C_{TVol\ day\ 7} = -0.16$) and gave a similar effect on IFP after 2 to 3 days ($T/C_{IFP} = 0.59$, $P = .07$). There was also a significant positive correlation between early IFP changes and the eventual change in $TVol_{day\ 7}$ (Figure 3*D*).

Thus, in general, for all three cytotoxics, a drug-induced decrease in IFP was paralleled by decreases in TVol.

Drug-Induced Changes in IFP as an Early Marker of Response

As already described, significant linear correlations were seen for patupilone, vatalanib, and gemcitabine in BN472 tumors between early changes in IFP, that is, day 2 or 3 and the eventual change in TVol on day 7 (Figure 3, *A*, *B*, and *D*). A similar observation was also made for patupilone in the RIF-1 model (Figure 3*C*), suggesting some relationship between IFP and rTBV. Because the IFP changes tended to occur before those in TVol, the correlations imply that IFP could be used as an early marker of response. Interestingly, this effect seemed stronger in the orthotopic tumor models. For example, for patupilone, which was tested in two orthotopic and three ectopic models, significant correlations between early IFP changes and late TVol changes were only seen in orthotopic tumors but not in the xenografts.

Finally, in the BN472 tumor, there was a trend for higher basal levels of IFP to be associated with response to both low-dose patupilone ($P = .04$) and vatalanib ($P = .07$), suggesting that pretreatment levels of IFP might also have the potential be used to predict response (Figure 3, *E* and *F*).

Table 3. Effect of Cytotoxics on the Growth and IFP of Several Tumor Models.

Drugs	Dose (mg/kg), Once	Tumor	Host	*n*	Day 2/3	Day 6/7	
					T/C_{IFP}	T/C_{TVol}	T/C_{IFP}
Patupilone	4	Human 1A9	Harlan nude mouse	24	0.47*	0.14†	0.18†
	4	Human 1A9ptx10		24	0.55*	0.50‡	0.37†
	4	Human U87		8	1.2	0.37‡	0.89
	4	Murine RIF-1	C3H/He mouse	5	0.40*	0.24*	0.82
	1.5	Rat BN472	BN rat	5	0.52†	0.06§	0.32†
Gemcitabine	150			6	0.51§	0.07*	1.18
CP	50			8	0.82	–0.2†	0.65§
	150	Murine RIF-1	C3H/He mouse	6	0.78	0.06*	0.84

Tumor initiation is described in the Materials and Methods section; after 2 to 3 weeks of growth, IFP was determined before (day 0), during (day 2 or 3), and after treatment (day 6 or 7) with patupilone, gemcitabine, or CP at the doses and schedules shown. Results show the effect of one dose for each drug in a specific tumor model as the T/C for TVol and IFP.

*$P < .01$, significantly different from the change in the vehicle.

†$P < .001$, significantly different from the change in the vehicle.

‡The T/C is low but not significantly different from the change in the vehicle ($P < .3$; 2-tailed *t*-test or one-way ANOVA with Tukey test *post hoc*).

§$P < .05$, significantly different from the change in the vehicle.

Discussion

The basal IFP has previously been shown to be dependent on the type of tumor and the host [21]. We have also investigated this phenomenon by comparing four different tumors in different backgrounds and sites within the host. Our data suggest that the implantation site has no effect on the basal IFP of rat BN472 or murine B16/BL6 tumors (ID or intramammary *vs* SC) or on rat C6 tumors grown in different nude mice (Harlan *vs* BALB/c). However, when the rat PROb tumor was grown in Harlan nude mice (both SC), there was a significantly lower IFP compared with the same tumor in BDIX rats (10.5 *vs* 15.2 mm Hg). Indeed, in general, rat tumors tended to have a higher IFP. Tumor IFP is dependent on many factors, including vessel permeability and flow [1,2], and perhaps also rTBV because we have recently found that rat tumors have a greater rTBV and permeability compared with the same tumors grown in mice [22]. These observations support the concept that IFP is strongly affected by the host vasculature and, in particular, the rTBV. Furthermore, we found in both untreated rat BN472 and PROb tumors that basal IFP was significantly positively correlated with basal rTBV. This correlation was weaker for BFI because it was only observed in one of the two models, which may reflect the fact that this parameter could not be so accurately measured by the DCE-MRI method that we have used (see Materials and Methods section).

To further investigate the nature of tumor IFP, we studied the effect of seven different chemotherapeutics (four cytostatics and three cytotoxics) on IFP. All seven agents were able to reduce IFP sooner (days 2 to 3) or later (days 6 to 7), but the magnitude of these effects was also dependent on the type of tumor and, to a certain extent, the host. Thus, imatinib reduced the IFP of PROb tumors in rats by 20% to 30% and significantly inhibited growth but caused a much greater decrease (−85%) when the same tumor was grown in nude mice, despite a lack of effect on tumor growth in the latter model. In the rat model, there was no evidence that an IFP decrease was associated with changes in vasculature in volume, flow, or hypoxia. Vatalanib also strongly inhibited PROb growth in rats but had minimal effect on IFP. In contrast, vatalanib dose-dependently inhibited growth and IFP of rat BN472 tumors. In this model, there was clear evidence of a relationship between changes in IFP and tumor vasculature, with decreases in IFP correlating with decreases in rTBV and a trend for a decrease in blood vessel width. The hypothesis of Jain [23] predicted, and provided evidence, that pruning of the vasculature by antiangiogenics would lead to narrower vessels, with decreased density and permeability and thus a lower IFP with, in some cases, increased blood flow and oxygenation. This seemed to be the case for vatalanib in BN472 tumors: partial growth inhibition was associated with reduced IFP and rTBV, and as we have previously reported, permeability was strongly decreased in this model [17]. Thus, considering two rat models, in one (vatalanib on BN472), an IFP decrease reflected tumor response and vascular normalization, whereas in the other (imatinib on PROb), an IFP decrease was apparently independent of vascular changes despite a strong effect on tumor growth.

Two other cytostatics, everolimus and AEE788, also reduced BN472 IFP by 25% to 50%, but further comparisons with the vasculature were not made. The data with these two drugs, however, confirmed that decreases in IFP paralleled inhibition of tumor growth.

The cytotoxic patupilone dose-dependently decreased the IFP of BN472 tumors, and in comparison to the cytostatics and two other cytotoxics (gemcitabine and CP) caused much greater decreases in IFP of up to 70%. As previously reported [8], IFP decreases in this model were strongly correlated to decreases in rTBV and increases in apoptosis. Because IFP, in theory, reflects not only the vasculature but also high tumor cell density around the blood vessels, the strong antivascular effect and direct antitumor effect may account for the very large decreases in IFP caused by patupilone in this well-vascularized model.

Others have reported that chemotherapeutics can reduce the IFP of experimental tumors, for example, imatinib on PROb tumors [6], dexamethasone [4], and an anti-VEGF monoclonal antibody [24] on human tumor xenografts. IFP decreases have also been reported in the clinic for bevacizumab [9] and paclitaxel [10] in rectal and breast tumors, respectively. However, these decreases in IFP have not been correlated with eventual response in the individual tumors. Using this approach, we have shown that decreases in IFP that were detectable after 2 to 3 days, in most cases before a change in TVol was detectable, can show a significant positive linear correlation (r^2 = 0.23-0.56, P < .05) with the eventual response as measured after 6 to 7 days. These data suggest an important relationship between IFP and tumor response, although it may not always be a linear relationship, and of course, other factors dictate tumor response. Nevertheless, these data imply that a decrease in IFP could be used as an early marker of response in the clinic. Interestingly, this was only apparent for the orthotopic tumors and, except in one case (vatalanib on BN472), was limited to cytotoxics. Given the evidence described previously for a relationship between IFP and rTBV, this may be because of orthotopic tumors being better

Table 4. Summary of Correlations between IFP-F and ΔTVol.

Tumor	Host	Compound	IFP-$F_{day\ 2/3}$ *vs* $\Delta TVol_{day\ 6/7}$			IFP-$F_{day\ 6/7}$ *vs* $\Delta TVol_{day\ 6/7}$		
			n	r^2	P	n	r^2	P
Rat BN472	BN rat	Patupilone	**63**	**0.33**	**.0000008**	**64**	**0.18**	**.0004**
		Vatalanib	**28**	**0.37**	**.0006**	**27**	**0.38**	**.0007**
		Gemcitabine	**17**	**0.54**	**.026**	17	0.009	.92
		CP	16	0.13	.17	**16**	**0.41**	**.008**
		AEE788	26	0.01	.6	26	0.005	.72
		Everolimus	14	0.008	.77	14	0.09	.30
Rat PROb	BDIX rat	Imatinib	12	0.20	.14	12	0.07	.40
	Harlan nude mouse		14	0.09	.30	14	0.10	.26
Murine RIF-1	C3H mouse	CP	11	0.11	.32	11	0.04	.58
		Patupilone	**14**	**0.46**	**.005**	14	0.09	.28
Human 1A9	Harlan nude mouse		50	0.04	.19	**50**	**0.15**	**.005**
Human 1A9ptx10			58	0.05	.08	58	0.01	.40
Human U87MG			22	0.002	.85	22	0.0004	.95

Data limited to models where the mean IFP-F was ≥ 0.70 and/or significantly affected with TVol data available at 6 to 7 days after initiation of treatment. Data in boldface emphasis show a significant relationship (P < .05).

vascularized than xenografts [12] and, because cytotoxics can kill both tumor and endothelial cells, would therefore cause much larger decreases in IFP. The exception to this hypothesis was CP, which, although showing strong a efficacy in two models, had weaker effects on IFP and no significant correlations between early IFP-F and TVol. However, in the RIF-1 model, CP increased rTBV, which should increase IFP and thus counteract decreases in IFP resulting from tumor cell kill. This observation underlines the relationship between tumor IFP and rTBV. Furthermore, where IFP is not readily measurable in patients, alternative, entirely noninvasive measurements could be used instead to measure rTBV or perhaps BFI, for example, DCE-MRI, ultrasound, or Doppler.

Finally, there was evidence that the basal IFP in BN472 tumors, and thus by implication rTBV, was related to response for both patupilone and vatalanib, that is, more vascularized tumors were more likely to respond to treatment. Interestingly, this is consistent with recent clinical data, which showed that renal cell carcinomas with a higher permeability were more sensitive to the multitargeted cytostatic sorafenib [25].

In conclusion, our data add to the accumulating literature that tumor IFP is dependent on the host vasculature and, in particular, the blood volume (rTBV) of the tumor. Treatment with cytotoxic or cytostatic agents significantly decreased the IFP of ectopic and orthotopic tumor models, and this paralleled the decreases in rTBV. Rapid decreases in IFP preceded significant changes in tumor size, and this seemed more prevalent in orthotopic models perhaps because these models are more vascularized than ectopic models. Our data suggest an important potential clinical application of measuring IFP, that is, that decreases in tumor IFP could be a generic marker of response to therapy.

References

[1] Jain RK (1994). Barrier to drug delivery in solid tumours. *Sci Am* **271** (1), 58–65.

[2] Brown JM and Giaccia AJ (1998). The unique physiology of solid tumours: opportunities (and problems) for cancer therapy. *Cancer Res* **58**, 1408–1416.

[3] Milosevic M, Fyles A, Hedley D, Pintilie M, Levin W, Manchul L, and Hill R (2001). Interstitial fluid pressure predicts survival in patients with cervix cancer independent of clinical prognostic factors and tumour oxygen measurements. *Cancer Res* **61** (17), 6400–6405.

[4] Kristjansen PE, Boucher Y, and Jain RK (1993). Dexamethasone reduces the interstitial fluid pressure in a human colon adenocarcinoma xenograft. *Cancer Res* **53** (20), 4764–4766.

[5] Podobnik B, Sersa G, and Miklavcic D (2001). Effect of hydralazine on interstitial fluid pressure in experimental tumours and in normal tissue. *In Vivo* **15** (5), 417–424.

[6] Pietras K, Ostman A, Sjöquist M, Buchdunger E, Reed RK, Heldin CH, and Rubin K (2001). Inhibition of platelet-derived growth factor receptors reduces interstitial hypertension and increases transcapillary transport in tumours. *Cancer Res* **61**, 2929–2934.

[7] Salnikov AV, Iversen VV, Koisti M, Sundberg C, Johansson L, Stuhr LB, Sjöquist M, Ahlström H, Reed RK, and Rubin K (2003). Lowering of tumour interstitial fluid pressure specifically augments efficacy of chemotherapy. *FASEB J* **17** (12), 1756–1758.

[8] Ferretti S, Allegrini PR, O'Reilly T, Schnell C, Stumm M, Wartmann M, Wood J, and McSheehy PM (2005). Patupilone induced vascular disruption in orthotopic rodent tumour models detected by magnetic resonance imaging and interstitial fluid pressure. *Clin Cancer Res* **11** (21), 7773–7784.

[9] Willett CG, Boucher Y, di Tomaso E, Duda DG, Munn LL, Tong RT, Chung DC, Sahani DV, Kalva SP, Kozin SV, et al. (2004). Direct evidence that the VEGF-specific antibody bevacizumab has antivascular effects in human rectal cancer. *Nat Med* **10**, 145–147.

[10] Taghian AG, Abi-Raad R, Assaad SI, Casty A, Ancukiewicz M, Yeh E, Molokhia P, Attia K, Sullivan T, Kuter I, et al. (2005). Paclitaxel decreases the interstitial fluid pressure and improves oxygenation in breast cancer in patients treated with neoadjuvant chemotherapy: clinical implications. *J Clin Oncol* **23**, 1951–1961.

[11] Rudin M, McSheehy PM, Allegrini PR, Rausch M, Baumann D, Becquet M, Brecht K, Brueggen J, Ferretti S, Schaeffer F, et al. (2005). PTK787/ZK222584, a tyrosine kinase inhibitor of vascular endothelial growth factor receptor, reduces uptake of the contrast agent GdDOTA by murine orthotopic B16/BL6 melanoma tumours and inhibits their growth *in vivo*. *NMR Biomed* **18**, 308–321.

[12] Ebenhan T, Honer M, Ametamey SM, Schubiger PA, Becquet M, Ferretti S, Cannet C, Rausch M, and McSheehy PM (2009). Comparison of [^{18}F]-tracers in various experimental tumour models by PET imaging and identification of an early response biomarker for the novel microtubule stabiliser patupilone. *Mol Imaging Biol* [May 22, Epub ahead of print].

[13] Eva A, Robbins KC, Andersen PR, Srinivasan A, Tronick SR, Reddy EP, Ellmore NW, Galen AT, Lautenberger JA, Papas TS, et al. (1982). Cellular genes analogous to retroviral *onc* genes are transcribed in human tumour cells. *Nature* **295**, 116–119.

[14] Giannakakou P, Sackett DL, Kang YK, Zhan Z, Buters JT, Fojo T, and Poruchynsky MS (1997). Paclitaxel-resistant human ovarian cancer cells have mutant β-tubulins that exhibit impaired paclitaxel-driven polymerization. *J Biol Chem* **272** (27), 17118–17125.

[15] Pietras K, Stumm M, Hubert M, Buchdunger E, Rubin K, Heldin CH, McSheehy P, Wartmann M, and Ostman A (2003). STI571 enhances the therapeutic index of Epothilone B by a tumour-selective increase of drug uptake. *Clin Cancer Res* **9**, 3779–3787.

[16] Traxler P, Allegrini PR, Brandt R, Brueggen J, Cozens R, Fabbro D, Grosios K, Lane HA, McSheehy P, Mestan J, et al. (2004). AEE788: a dual family epidermal growth factor receptor/ErbB2 and vascular endothelial growth factor receptor tyrosine kinase inhibitor with antitumour and antiangiogenic activity. *Cancer Res* **64** (14), 4931–4941.

[17] Schnell CR, Stauffer F, Allegrini PR, O'Reilly T, McSheehy PM, Dartois C, Stumm M, Cozens R, Littlewood-Evans A, García-Echeverría C, et al. (2008). Effects of the dual phosphatidylinositol 3-kinase/mammalian target of rapamycin inhibitor NVP-BEZ235 on the tumor vasculature: implications for clinical imaging. *Cancer Res* **68** (16), 6598–6607.

[18] Carpinelli G, Bucci B, D'Agnano I, Canese R, Caroli F, Raus L, Brunetti E, Giannarelli D, Podo F, and Carapella CM (2006). Gemcitabine treatment of experimental C6 glioma: the effects on cell cycle and apoptotic rate. *Anticancer Res* **26** (4B), 3017–3024.

[19] Kolfschoten GM, Pinedo HM, Scheffer PG, Schlüper HM, Erkelens CA, and Boven E (2000). Development of a panel of 15 human ovarian cancer xenografts for drug screening and determination of the role of the glutathione detoxification system. *Gynecol Oncol* **76** (3), 362–368.

[20] Lane HA, Wood JM, McSheehy PM, Allegrini PR, Boulay A, Brueggen J, Littlewood-Evans A, Maira SM, Martiny-Baron G, Schnell CR, et al. (2009). mTOR inhibitor RAD001 (everolimus) has antiangiogenic/vascular properties distinct from a VEGFR tyrosine kinase inhibitor. *Clin Cancer Res* **15** (5), 1612–1622.

[21] Lunt SJ, Kalliomaki TM, Brown A, Yang VX, Milosevic M, and Hill RP (2008). Interstitial fluid pressure, vascularity and metastasis in ectopic, orthotopic and spontaneous tumours. *BMC Cancer* **8**, 2.

[22] O'Reilly T, McSheehy PMJ, Kawai R, Kretz O, McMahon L, Brueggen J, Bruelisauer A, Gschwind HP, Allegrini PR, and Lane HA (2009). Comparative pharmacokinetics of RAD001 (everolimus) in normal and tumor-bearing rodents. *Cancer Chemother Pharm* [Epub ahead of print].

[23] Jain RK (2001). Normalizing tumour vasculature with anti-angiogenic therapy: a new paradigm for combination therapy. *Nat Med* **7**, 987–989.

[24] Lee CG, Heijn M, di Tomaso E, Griffon-Etienne G, Ancukiewicz M, Koike C, Park KR, Ferrara N, Jain RK, Suit HD, et al. (2000). Anti–vascular endothelial growth factor treatment augments tumour radiation response under normoxic or hypoxic conditions. *Cancer Res* **60**, 5565–5570.

[25] Flaherty KT, Rosen MA, Heitjan DF, Gallagher ML, Schwartz B, Schnall MD, and O'Dwyer PJ (2008). Pilot study of DCE-MRI to predict progression-free survival with sorafenib therapy in renal cell carcinoma. *Cancer Biol Ther* **7** (4), 496–501.