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Antiangiogenics and radiotherapy

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Abstract

Antiangiogenic therapies are one of the fore-runners of the new generation of anticancer drugs aimed at tumour-specific molecular targets. Up until the beginning of this century, the general opinion was that targeted agents should show antitumour activity when used as single agents. However, it has now become clear that much greater improvements in therapeutic activity may be achieved by combining the novel agents with conventional cytotoxic therapies already in use in the clinic. Radiotherapy is currently used to treat half of all cancer patients at some stage in their therapy, although the development of radioresistance is an ongoing problem. It is therefore reasonable to expect that any novel molecularly-targeted agent which reaches the clinic will be used in combination with radiotherapy. The rationale for combining antiangiogenics in particular with radiotherapy exists, as radiotherapy has been shown to kill proliferating endothelial cells, suggesting that inhibiting angiogenesis may sensitise endothelial cells to the effects of radiation. Furthermore, targeting the vasculature may paradoxically increase oxygenation within tumours, thereby enhancing radiotherapy efficacy. In this review we present an update on the use of antiangiogenic methods in combination with radiotherapy.

Angiogenesis

Angiogenesis is a physiological process involving the growth of new blood vessels from pre-existing vessels. It is a critical step in tumour progression, as tumours cannot grow beyond approximately 2 mm^3 without a vascular supply, due to a lack of oxygen and nutrients (Folkman 1971). In response, tumour cells secrete angiogenic growth factors such as vascular endothelial growth factor A (VEGF-A). VEGF, also secreted by monocytes, binds to its receptors on the existing endothelium and stimulates endothelial cell proliferation and migration into the tumour (Shweiki et al 1992; Dvorak et al 1995). VEGF acts as a survival factor for endothelial cells by inhibiting apoptosis (Liu et al 2000). It is therefore a pivotal driver of tumour angiogenesis, leading to the formation of new blood vessels within the tumour, allowing tumour progression from in-situ lesions to widespread disease, as well as providing the tumour with a route by which cells can get into the circulation and form distant metastases (Folkman 1971; Byrne et al 2005).

Angiogenesis is regulated by activator proteins such as VEGF, basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), granulocyte colony-stimulating factor (GCSF), placental growth factor (PlGF) and tumour necrosis factor (TNF)-α, and inhibitor molecules such as angiostatin, endostatin and the interferons. Usually the inhibitor proteins dominate and block growth, however the shift in balance from the anti- to the pro-angiogenic factors in a process termed the 'angiogenic switch' causes a transition from the dormant to the angiogenic state (Hanahan & Folkman 1996). Tumour hypoxia is a significant factor in the highly complex 'switch' process, stimulating the production of pro-angiogenic factors by the tumour cells (Shweiki et al 1992). Tumour blood vessels are distinct from those of normal tissue however, as they are structurally and functionally abnormal, and unevenly distributed throughout the tumour leading to avascular areas. The dysfunctional vascular architecture often consists of elongated, dilated and twisted blood vessels with blind ends. They can be alternately functional as they close and re-open leading to sluggish and fluctuating blood-flow (Folkman 1971; Vaupel et al 1989, 2001a). These vascular abnormalities lead to hypoxia (Figure 1) and acidosis in the tumour microenvironment, and the hyperpermeable vessels combined with the lack of functional lymphatic vessels inside solid tumours lead to elevated interstitial fluid pressure (Warburg 1956; Galarraga et al 1986; Raghunand et al 2003).

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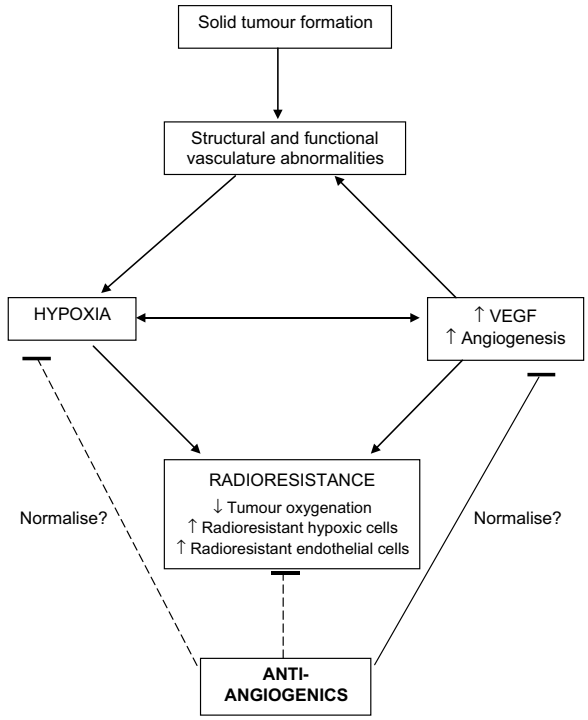

Figure 1 Hypoxia, angiogenesis and radioresistance: antiangiogenics may 'normalise' the vasculature which decreases tumour hypoxia and resistance to radiotherapy, therefore increasing the radioresponsiveness of tumours.

Hypoxia

Hypoxia occurs in tumour tissue when there is an inadequate supply of oxygen which compromises normal biological processes in the cell (Höckel & Vaupel 2001; Harris 2002). Tumour hypoxia results from inadequate perfusion in the microenvironment as a result of tumour vasculature abnormalities, and can also be caused by an increase in diffusion distances as cells distant (>70 μm) from the blood vessel receive less oxygen than required (Vaupel et al 1989, 2001b; Höckel & Vaupel 2001). Hypoxia induces a wide range of responses in cells and tissues, and the degree of intra-tumoral hypoxia is positively correlated with the expression of the transcription factor hypoxia-inducible factor (HIF)-1 (Semenza 2000). Biological pathways that are regulated by hypoxia-inducible genes, usually under the control of HIF-1, include angiogenesis, glycolysis, apoptosis, cell cycle arrest, pH regulation and erythropoiesis (Semenza et al 1991; Maxwell et al 1997; Shih & Claffey 1998; Harris 2002).

HIF-1 is composed of two subunits, HIF-1α and HIF-1β. The status of HIF-1α is the key element in allowing cells to survive and adapt in a hostile hypoxic environment; it is stabilised only under conditions of low oxygen tension and rapidly degraded under aerobic conditions (Wang et al 1995; Huang et al 1998). Under hypoxic conditions, nuclear accumulation of HIF-1α occurs and it dimerises with HIF-1β to form the HIF-1 complex. HIF-1 then transactivates target genes such as VEGF, thereby leading to increased angiogenesis and vascular permeability in tumours (Forsythe et al 1996). Deregulation of HIF-regulated pathways, e.g. loss of cell cycle arrest, leads to a more aggressive tumour phenotype (Sutherland 1998). However, the prognostic value of HIF-1α is controversial, and some clinical research data has indicated that expression correlates with longer survival of patients (Beasley et al 2002; Fillies et al 2005). Nevertheless, it is generally accepted that hypoxia is an adverse prognostic indicator and a determinant of malignant progression, metastatic development and resistance to radiotherapy and chemotherapeutic agents (Vaupel et al 2001b; Shannon et al 2003; O'Donnell et al 2006). Gray et al (1953) established that hypoxia leads to radiation resistance as oxygen is required to chemically modify free-radical damage to the target DNA; when radiation is absorbed by the tissue, it creates ionised oxygen species that react with and damage cellular DNA. In general, a two- to threefold higher radiation dose is required to kill hypoxic cells compared with well-oxygenated cells, known as the 'oxygen-enhancement effect' (Höckel et al 1993; Okunieff et al 1993). Furthermore, HIF-1 may increase radioresistance of solid tumours, independently of the tumour oxygenation status (Williams et al 2005).

Targeting the vasculature as a cancer therapy

As angiogenesis plays a major role in tumour growth and metastasis, the process is an attractive target for therapeutic intervention. Attacking the tumour vasculature deprives it of nutrients and oxygen necessary for its growth and should also inhibit metastasis, theoretically leading to tumour regression. Antiangiogenic agents are a unique therapeutic group as they have highly specific targets, while simultaneously having the potential to be effective against a broad range of tumour types (Chan & Camphausen 2003). However, current antiangiogenics have unfortunately not lived up to earlier promise. Most antiangiogenic strategies undertaken to date have focussed on directly targeting tumour-associated endothelial cells by blocking endothelial growth factors such as VEGF and its corresponding receptors, using small molecule inhibitors of VEGF or its receptors (Cediranib/AZD2171 AstraZeneca), anti-VEGF antibodies (bevacizumab/Avastin), soluble VEGF receptor (VEGFR) analogues, and ribozymes (angiozyme). Other strategies have aimed to inhibit functions of activated endothelial cells such as proliferation and migration (Byrne et al 2005). Compounds such as thalidomide and its analogues also have antiangiogenic properties (D'Amato et al 1994; Dredge et al 2002). The US National Cancer Institute Database reported that twenty antiangiogenic inhibitors were being tested in clinical trials in 1999, many of which were discontinued in phase III trials due to lack of activity or toxicities (Kerbel 2000). There were over 300 antiangiogenic drug candidates in various stages of testing by 2001, however clinical trial results failed to match results observed in preclinical studies (Matter 2001). Errors in the concept and design of trials and in the definition of clinical endpoints could account for many of these results (McCarty et al 2003).

A second generation of antiangiogenic trials is beginning to show significant potential, and recent reports of positive results in phase II and III trials have raised expectations (Quesada et al 2006). Hurwitz et al (2004) demonstrated that a humanised anti-VEGF monoclonal antibody bevacizumab (Avastin;

Roche) showed a significant and clinically meaningful improvement in survival among patients with metastatic colorectal cancer, when used in combination with fluorouracil-based combination chemotherapy. The US Food and Drug Administration approved bevacizumab in 2004 for use in combination with standard chemotherapy in the treatment of metastatic colon cancer and most forms of metastatic non-small cell lung cancer. The data implied that the future success of antiangiogenics may depend on their combination with conventional therapies such as chemotherapy or radiotherapy.

Rationale for combining radiotherapy with antiangiogenics

Approximately half of all cancer patients receive radiotherapy over the course of their treatment (Steel 1997), although tumour radioresistance is a major problem. As the patient population treated with radiotherapy is so enormous, enhancing therapeutic outcome for even a relatively small proportion of these has the potential to translate to a highly significant clinical benefit. Therefore, in recent years there has been increasing interest in combining antiangiogenic therapies with radiation (Wachsberger et al 2004). However, the mechanisms of interaction between angiogenic-targeting agents and ionising radiation are complex and involve interactions between the tumour's stroma, the vasculature and the tumour cells themselves.

Although antiangiogenics theoretically have the potential to increase tumour hypoxia by damaging blood vessels and therefore restrict delivery of oxygen and nutrients to the tumour, this does not appear to be the case. Rakesh Jain (2001, 2005) explained that rather than obliterating tumour blood vessels, antiangiogenics instead destroyed immature vessels, reduced vascular permeability and interstitial fluid pressure, and increased pericyte recruitment to stabilise intact vessels. This process was termed 'normalisation' and resulted in a more stable, organised vasculature, which could deliver oxygen and nutrients to the tumour more efficiently via well-functioning vessels, thereby decreasing hypoxia. As previously mentioned, hypoxia is an important risk factor for poor locoregional control and survival in cancer, and is associated with malignant progression and treatment resistance (Vaupel et al 2001a; Semenza 2002). Both in-vitro and in-vivo studies have demonstrated the benefits of disrupting hypoxia-inducible transcription, such as suppression of tumour growth and inhibition of angiogenesis (Maxwell et al 1997; Ryan et al 1998; Kung et al 2000). Therefore, an increase in oxygenation within the tumour environment may increase the efficacy of radiotherapy by reducing the aggressive radioresistant hypoxic cell compartment as well as increasing the oxygen-enhancement effect of radiotherapy (Figure 1). However, the benefits of such combination therapy may be dependent upon a transient normalisation window of opportunity when blood flow and tumour oxygenation are increased. Following this, the caveat is that extended treatment with vascular-targeting agents may exacerbate hypoxic conditions by reducing vessel density, with the end result being a negative impact on radiotherapy outcome (Wedge et al 2005; Franco et al 2006; Riesterer et al 2006).

Radiation may be pro- or antiangiogenic through its direct pro-apoptotic effect on endothelial cells or through pro- or antiangiogenic signals released by irradiated cancer cells (Garcia-Barros et al 2003). Radiation-induced cytokines, which positively regulate angiogenesis, include transforming growth factor (TGF)-β, bFGF, platelet-derived growth factor (PDGF), interferon (IFN)-γ. TNF-α, interleukin (IL)-4, IL-5 and VEGF (McBride et al 2004). Consequently, tumours protect their own vasculature from the damaging effects of ionising radiation. Increased VEGF production in response to radiotherapy may be via the mitogen-activated protein kinase pathway (Park et al 2001) or through HIF-1 induction (Forsythe et al 1996); following radiotherapy, tumour reoxygenation leads to nuclear accumulation of HIF-1 in response to reactive oxygen (Moeller et al 2004). VEGF may act as an endothelial cell survival factor as previously mentioned (Liu et al 2000), and also as a tumour cell survival factor (Harmey & Bouchier-Hayes 2002). Furthermore, up-regulated VEGF expression appears to enhance endothelial cell survival by protecting them from radiation-induced cell death (Teicher et al 1992; Gorski et al 1999; Grunstein et al 1999; Geng et al 2001; Hess et al 2001).

In one study, both VEGF and bFGF were found to be up-regulated in prostate cancer cells following irradiation, and VEGFR2 expression was augmented in endothelial cells (Abdollahi et al 2003). Tumour-infiltrating macrophages may be an additional source of VEGF production following chemoradiotherapy (McDonnell et al 2003). Sonveaux et al (2003) found that irradiation induced the activation of the pro-angiogenic nitric oxide pathway in endothelial cells, which led to their migration and initiation of endothelial cell sprouting, the first steps in angiogenesis. The relevance of these findings was verified by Garcia-Barros et al (2003), who demonstrated that the major determining factor controlling tumour response to radiotherapy was microvascular damage. The surviving endothelium remaining after radiotherapy is thought to play a major role in the overall radiation response, by stimulating neovascularisation to support tumour regrowth after radiation-induced damage (Moeller et al 2005; Williams et al 2007). Small degrees of vascular destruction may translate to large-scale tumour damage (Denekamp 1993). This suggests a further rationale for targeting VEGF and other angiogenic mechanisms, to potentiate radiotherapy toxicity by increasing the radiosensitivity of endothelial cells (Gupta et al 2002) (Figure 1).

Combination of antiangiogenics and radiotherapy

Efforts have been made to increase the effectiveness of radiotherapy by combining it with vasculature-targeting agents in preclinical studies for over ten years, some examples of which are detailed here. Teicher et al (1995) showed that antiangiogenic agents increased the radiation response in a gliosarcoma xenograft model by decreasing tumour hypoxia. Mauceri et al (1998) reported how angiostatin was combined with radiation to target endothelial rather than tumour cells, whilst Gorski et al (1999) reported that blocking VEGF with a neutralising antibody increased the antitumour effects of radiation in murine tumour models. The latter group also

demonstrated in in-vitro assays that VEGF inhibited the lethal effects of radiation on endothelial cells, and that VEGF blockade increased radiation-induced endothelial cell damage. Other earlier studies combining antiangiogenics with radiotherapy include the utilisation of a VEGFR-2-blocking antibody to increase the effectiveness of radiation-induced long-term control of two different human tumour xenografts (Kozin et al 2001). Subsequent studies using the same antibody (DC101) investigated the pathophysiological consequences of its combination with radiotherapy (Fenton et al 2004). These studies revealed that although the combined therapy had no immediate effect on tumour hypoxia, hypoxia was increased at protracted times post-treatment which correlated with an apparent degeneration of vessels formed post-treatment (Fenton et al 2004). Other studies used a VEGFR tyrosine kinase inhibitor (PTK787/ZK222584) to enhance tumour growth delay in radiation-resistant tumour xenografts (Hess et al 2001). Inhibition of the VEGFR receptor by the VEGFR2 kinase inhibitor SU5416 was demonstrated to revert radioresistance of refractory tumour vessels and enhance the cytotoxic effects of radiotherapy in glioblastoma and melanoma xenografts (Geng et al 2001). Lee et al (2000) demonstrated that an anti-VEGF monoclonal antibody decreased interstitial fluid pressure by 73–74% in two murine xenograft models of human colon adenocarcinoma. Radiation-induced tumour growth delay was enhanced in both models, one of which had decreased tumour hypoxia while the other did not. This suggested that VEGF targeting could overcome radioresistance without necessarily reducing overall tumour hypoxia, although it was conceivable that perfusion was improved in both models as a consequence of the changes in interstitial fluid pressure. The complex mechanisms by which tumours increase endothelial cell radioresistance may have initially been underestimated, and it now appears that numerous factors other than VEGF may be involved. Ning et al (2002) demonstrated that a small molecule inhibitor of the VEGFR combined with an inhibitor of VEGF, FGF and PDGF receptors in combination with fractionated radiotherapy was significantly more effective at radiosensitising the tumour endothelium than VEGFR blockade alone. This implied that achieving optimal tumour vasculature radiosensitisation may require inhibition of all endothelial cell radioresistance-inducing signals by disrupting a common pathway such as HIF-1, as demonstrated by Moeller et al (2004), thereby endorsing the hypothesis of blocking endothelial cell radioprotective responses before the protective cytokines are ever released. In that study, inhibiting HIF-1 activation post-irradiation significantly increased tumour radiosensitivity in a murine tumour model, as a result of enhanced vascular destruction (Moeller et al 2004).

Development of antiangiogenics and radiotherapy combination therapy in the clinic is still in its early stages. In a phase II study of the antiangiogenic agent thalidomide in combination with radiotherapy and temozolomide in patients with recurrent malignant glioma, the combination strategy was relatively well tolerated with increased survival outcomes compared with patients who received nitrosourea adjuvant chemotherapy (Chang et al 2004). As previously mentioned, promising results were achieved in patients with metastatic colorectal cancer using bevacizumab in combination with fluorouracil-based combination chemotherapy (Hurwitz et al 2004). Overall survival was increased by 4.7 months compared with chemotherapy alone, a statistically significant increase. However, a more recent report indicated that bevacizumab may have caused severe bowel complications in patients who had previously received radiotherapy (Lordick et al 2006). This demonstrates the potential for increased normal tissue toxicity when novel agents are integrated into the neoadjuvant treatment of cancers.

The importance of scheduling in combining antiangiogenics and radiotherapy

Few studies to date have considered the importance of scheduling when combining antiangiogenic treatment and radiotherapy. The timing of employing vasculature-targeting agents concomitantly with, as a neo-adjuvant or sequentially to radiotherapy may be of utmost importance for optimal radiation enhancement (Table 1). A mathematical model was used to determine the optimum schedule for combining antiangiogenic methods with radiotherapy (Ergun et al 2003). The results indicated that the best performance may be achieved by maintaining a constant 20:1 ratio of tumour cell volume to supporting vasculature volume using antiangiogenic agents, and increasing the dose only during the latter portion of the radiation fractionation schedule. However, a number of variables come into play regarding the optimal schedule in an in-vivo setting.

Antiangiogenic strategies: neo-adjuvant or concomitantly with radiotherapy

As described previously, preclinical data suggested that vasculature normalisation created a 'therapeutic window' whereby improved oxygenation for radiotherapy efficacy was achieved (Jain 2001), and that antiangiogenics may sensitise the vasculature to radiation damage (Gupta et al 2002). This implied that treating with antiangiogenic agents before or concomitantly with radiation was the most advantageous scheduling option. One study showed that in a preclinical model, a delay in radiotherapy following VEGFR2 blockade was associated with improved tumour response compared with concurrent delivery (Winkler et al 2004). The authors showed that the antiangiogenic therapy increased pericyte coverage of tumour vessels via up-regulation of angiopoietin-1 and degraded the thick basement membrane via matrix metalloproteinase activation during the normalisation window. Other studies have investigated concomitant administration, with the option of continuing antiangiogenic treatment on completion of radiotherapy. SU11248, an inhibitor of VEGFR2, PDGFR, c-kit and fetal liver tyrosine kinase 3 was administered daily during seven days of radiation. This protocol significantly reduced tumour volume in a murine tumour model compared with either modality alone, while simultaneously destroying the tumour vasculature (Schueneman et al 2003). Maintenance of the drug following completion of radiotherapy prolonged tumour growth control. Cediranib (AZD2171), a highly potent, orally active inhibitor of VEGFR signalling, has been shown to sensitise human tumour xenografts to radiation (Cao et al 2006; Williams et al

Table 1 Examples of antiangiogenic and ionising radiation (IR) combination regimens which improved therapeutic response

Antiangiogenic (combined with IR)	Schedule of antiangiogenic with IR	Author
Angiostatin	Concomitant	Mauceri et al (1998)
Anti-VEGF-antibody	Concomitant	Gorski et al (1999)
	Neo-adjuvant	Lee et al (2000)
	Concomitant	Gupta et al (2002)
Anti-VEGFR-2 antibody DC101	Concomitant and continued post-IR	Kozin et al (2001); Fenton et al (2004)
	Neo-adjuvant	Winkler et al (2004)
VEGFR (kinase) inhibitors:		
PTK787/ZK222584	Concomitant	Hess et al (2001)
	Sequential	Zips et al (2003)
SU5416	Concomitant and continued post-IR	Geng et al (2001)
		Ning et al (2002)
AZD2171	Concomitant	Cao et al (2006)
	Concomitant and continued post-IR, and sequential	Williams et al (2007)
ZD6474	Sequential	Williams et al (2004)
Inhibitor of VEGF, FGF and PDGF receptors: SU6668	Concomitant and continued post-IR	Ning et al (2002)
Inhibitor of VEGFR2, PDGFR, c-kit and foetal tyrosine kinase 3: SU11248	Concomitant and continued post-IR	Scheueneman et al (2003)
TNP-470	Sequential	Murata et al (1997)

2007). The latter study demonstrated that although cediranib treatment alone reduced total vessel density, the proportion of perfused or functional vessels was increased compared with control tumours. However, when combined concomitantly with radiotherapy the antivascular effect was augmented and tumour hypoxia was increased, although this did not impair the therapeutic benefit of the combination when cediranib was maintained post-radiotherapy.

Antiangiogenic after radiotherapy (sequential)

As a single dose as well as a fractionated dose of radiation can impair tumour vasculature (Solesvik et al 1984; Zywietz et al 1994), the rationale exists that radiation-damaged tumour vessels should be more sensitive to vasculature targeting agents than un-irradiated vessels. Furthermore, in cases where the antiangiogenic agent disrupts the vasculature to the extent at which perfusion is impaired and normalisation does not occur, oxygenation will be reduced and radiation efficacy therefore diminished. One of the earliest studies to demonstrate that post-radiation scheduling of antiangiogenics may be most beneficial came from the work of Murata et al (1997). Here, administration of the antiangiogenic agent TNP-470 during radiotherapy reduced tumour curability, whereas growth delay studies showed a beneficial interaction when TNP-470 was administered after radiation (Murata et al 1997). More recent studies have similarly demonstrated that post-radiotherapy may be optimal. Williams et al (2004) established that the therapeutic benefit of administering ZD6474 (Vandetanib), a potent VEGFR2 tyrosine kinase inhibitor, after radiotherapy was markedly greater than that achieved when ZD6474 was administered before each fraction of radiation. The authors demonstrated that this was as a result of reduced tumour vascular perfusion caused by administration of ZD6474, which impaired reoxygenation between radiation fractions, thereby decreasing radiosensitivity. Zips et al (2003) confirmed that application of a VEGFR tyrosine kinase inhibitor PTK787/ZK222584 (Valatanib) was of no benefit if given before or during fractionated irradiation, however it enhanced radiotherapy outcome when administered after radiation treatment. Two years later the same group demonstrated that tumours transplanted into normal un-irradiated tissues did not respond to the VEGFR tyrosine kinase inhibitor PTK787/ZK222584, whereas those growing in pre-irradiated tissues supplied by radiation-damaged vessels responded with longer latency and slower growth rates (Zips et al 2005). The authors attributed the results to increased sensitivity of the irradiated tumour vasculature to VEGFR targeting, which has been supported by more recent studies (Cao et al 2006; Kozin et al 2007; Williams et al 2007).

Conclusion

It is becoming increasingly apparent that anticancer strategies that combine antiangiogenics with radiotherapy could hold greater promise for clinical development. However, a great emphasis needs to be placed upon the most appropriate scheduling of these agents with respect to radiotherapy to elicit maximum therapeutic benefit. This requires not only a greater understanding of the mechanistic basis for the positive interaction in tumour tissues but also of what may occur in normal tissues, to prevent a therapeutic window of opportunity being lost.

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