

Releasing Pressure in Tumors: What Do We Know So Far and Where Do We Go from Here? A Review

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Abstract

Tumor interstitial pressure is a fundamental feature of cancer biology. Elevation in tumor pressure affects the efficacy of cancer treatment. It causes heterogenous intratumoral distribution of drugs and macromolecules. It also causes the development of hypoxia within tumor bulk, leading to reduced efficacy of therapeutic drugs and radiotherapy. Tumor pressure has been associated with increased metastatic potential and poor prognosis in some tumors. The formation of increased pressure in solid tumors is multifactorial. Factors known to affect tumor pressure include hyperpermeable tortuous tumor vasculatures, the lack of functional intratumoral lymphatic vessels, abnormal tumor microenvironment, and the solid stress exerted by proliferating tumor cells. Reducing this pressure is known to enhance the uptake and homogenous distribution of many therapies. Pharmacologic and biologic agents have been shown to reduce tumor pressure. These include antiangiogenic therapy, vasodilatory agents, antilymphogenic therapy, and proteolytic enzymes. Physical manipulation has been shown to cause reduction in tumor pressure. These include irradiation, hyperbaric oxygen therapy, hyper- or hypothermic therapy, and photodynamic therapy. This review explores the methods to reduce tumor pressure that may open up new avenues in cancer treatment. *Cancer Res*; 74(10); 2655–62. ©2014 AACR.

Introduction

Elevated pressure in tumor bulk or tumor interstitial pressure (TIP) has been identified as one of the culprits that impedes effective cancer treatment (1). This elevation of TIP can be attributed to several pathophysiologic factors. These include abnormalities in the tumor vasculature, lymphatic vessels and microenvironment, and proliferating tumor cells within a confined space (1).

Pressure within solid tumor can be divided into two components: fluid pressure and solid stress (2). Fluid pressure comprises of capillary and interstitial pressures. Both can be further subdivided into hydrostatic and colloid oncotic pressures. Solid stress is created by the nonfluid element in tumor bulk, which generates the growth-induced stress and the externally applied stress. Growth-induced stress is the pressure exerted by the interactions between the proliferating tumor and stromal cells in the interstitium. Interactions between the growing tumor and its surrounding normal tissues generate the externally applied stress.

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Fluid Pressure

Fluid pressure affects both normal tissue and tumors (1). In normal tissue, the blood vessels are present to supply oxygen and nutrients for cellular metabolism and removal of metabolic wastes. Intravascular pressure increases as the luminal diameter of these vessels decreases from arteries to capillaries. The increased pressure exerted by the blood flow on the capillary wall forces fluid and substances out into the interstitium. This force is called the capillary hydrostatic pressure. The extravasated fluid from the capillary, termed the interstitial fluid, contributes to the interstitial volume, which has a positive effect on interstitial hydrostatic pressure. Plasma proteins is another factor that affects the capillary and interstitial pressures and contributes to the colloid oncotic pressure (COP). In normal circumstances (Fig. 1), plasma proteins remain intravascular due to low permeability of the vessel endothelium. This produces a higher capillary COP relative to that of the interstitial COP.

Interstitial pressures are tightly regulated by reabsorption of extravasated fluid and proteins into the systemic circulation via the postcapillary veins and the lymphatic vessels. An increase in interstitial volume triggers an increase in lymphatic flow to prevent further accumulation of interstitial fluid (3).

Unlike normal tissue, tumor cells proliferate faster than the formation of its vasculatures (Fig. 1). As a tumor grows, the existing blood vessels become insufficient to provide an adequate supply of oxygen and nutrient to accommodate the increased cellular metabolic demand. An increased accumulation of metabolic wastes in tumor interstitium also occur. These two events create an abnormal tumor microenvironment, which triggers an increased production of stromal cells

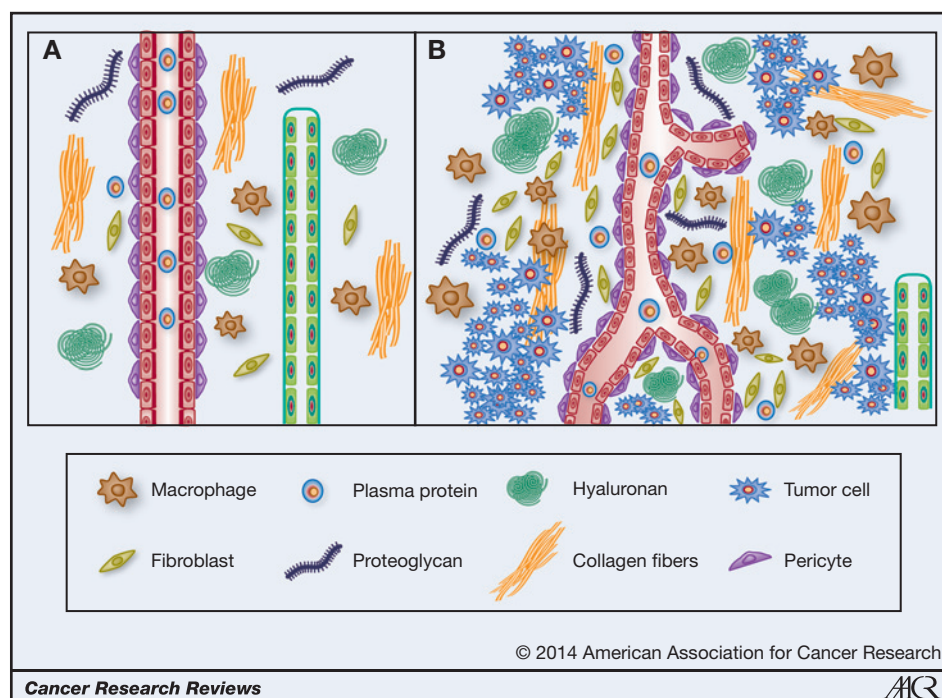


Figure 1. Interstitium in normal tissue versus tumor tissue. A, normal tissue interstitium containing blood vessel, lymphatic vessel, hyaluronan, macrophages, fibroblasts, proteoglycans, and collagen fibers. B, tumor tissue interstitium containing leaky and tortuous tumor vasculature, lacking in lymphatic vessel, higher amount of extravasated plasma proteins, macrophages, fibroblasts, proteoglycans, hyaluronans and collagen fibers, and proliferating tumor cells, all of which contribute to the elevation of TIP.

in the interstitium, including cancer-associated fibroblasts and immune cells such as macrophages (4).

Macrophages and cancer cells are known to upregulate the production of various cytokines, including VEGF, platelet-derived growth factor (PDGF), and TGF- β (1). These cytokines can stimulate angiogenesis to produce new blood vessels to accommodate the increasing metabolic needs. However, overexpression of these cytokines contributes to the formation of abnormal and tortuous tumor vasculatures. These vessels have either incomplete or absent endothelial cell layers and altered basement membrane, making them hyperpermeable. This allows a higher amount of fluid and plasma proteins to extravasate into tumor interstitium, which reduces the hydrostatic microvascular pressure (MVP) and increases TIP.

Similar to tumor angiogenesis, tumor lymphogenesis also produces abnormal nonfunctioning or damaged lymphatic vessels. Overexpression of prolymphogenic molecules such as VEGF-C and VEGF-D has been observed in some tumors (5). These molecules may cause hyperplasia of lymphatic vessels around tumor periphery or formation of immature lymphatics that allow retrograde drainage (6). These abnormalities cause an impairment in their drainage function (6).

Increased vascular permeability combined with abnormal or absent lymphatic vessels in tumor bulk leads to further accumulation of fluid and proteins in the interstitium, contributing to the elevation of TIP.

Solid Pressure

Solid pressure is affected by the nonfluid elements in both normal tissue and tumors (2). In normal tissue, the microenvironment consists of stromal cells, which include myofibroblasts, various cytokines, and the extracellular matrix (ECM). The ECM has various components, such as collagen, proteo-

glycans, and glycosaminoglycans (GAG), which provide structural support for tissue architecture. The ECM components also play a role in cell migration and dictate the cell behavior. They also contain various signaling molecules that can affect cellular gene expression.

The microenvironment in tumors is different than that of normal tissue. There is an increased number of fibroblasts in tumor stroma that stimulates the expansion of ECM and increase the matrix tension. This is due to the synthesis of an abnormally large amount of collagen fibers, hyaluronan, GAGs, proteoglycans, and proteolytic enzymes and its inhibitors (7).

Increased amounts of collagen synthesis, deposition, and cross-linking with other ECM components in the tumor microenvironment (TME) contributes to stiffer and thickened ECM (7). The lack of β_3 -integrins in the stroma has been shown to elevate TIP due to the buildup of thicker collagen fibrils (8). β_3 -Integrin deficiency also causes a reduction of macrophages and inflammatory cytokines, which leads to reduced expression of transcripts for collagen degrading enzymes (8). Collagen also produces ECM fragments that have a potent stimulatory or inhibitory effects on angiogenesis by collaborating with angiogenic factors, including VEGF (7, 9).

Hyaluronan is another component that is increased in TME. It is a type of GAG that is present in most tissues, mainly in the loose connective tissues. Hyaluronan has the ability to cause expansion of the matrix. An increased amount of hyaluronan in TME causes an increase in matrix expansion. It also has the ability to promote the survival of metastatic cancer cells (10).

The stiff, swollen ECM in tumor bulk combined with the proliferating cancer cells increases the growth-induced stress in the tumor, which contributes to the increase in tumor pressure.

The Importance of TIP on Cancer Treatment

Elevated tumor pressure has a negative impact on cancer treatment. High TIP is known to reduce efficacy of cancer therapy via several mechanisms. One mechanism is where high TIP leads to a reduced uptake and heterogeneous distribution of drugs (chemotherapy, targeted therapy), other macromolecules [ref. 11; monoclonal antibodies, immune cells (12)], and oncolytic viral therapy (13) into tumor parenchyma. These macromolecules are normally transported through the interstitial space by convection, a transport process that is dependent on pressure gradients. Increased tumor vessel permeability creates a less steep gradient along the vessels. This leads to a reduction in convective transport across the vessels in tumor bulk. Diffusion then becomes the main transport mechanism for the macromolecules. However, they do so at a slower rate due to a denser tumor ECM. The macromolecules can be reabsorbed back into the circulation before they can accumulate at an optimal level in the tumor to cause an effect.

Another mechanism is due to the high tumor vascular resistance. High tumoral pressure due to proliferating tumor cells causes compression of the tumor vasculature, leading to the increase in vascular resistance (14). This leads to a reduction in tumor blood flow and subsequently reduces the anti-cancer agent transport to tumor cells.

A third mechanism is when abnormal and tortuous tumor vasculature causes blood stasis, which leads to the reduction of oxygen and blood flow in tumors (15). The tumor becomes hypoxic and subsequently ischemic and necrotic. Hypoxia causes an increase in anaerobic metabolism, which creates an acidic TME due to increased lactate concentration. The increase in acidity degrades or deactivates some therapeutic drugs and renders them ineffective. Hypoxia can also cause resistance to radiotherapy. Hypoxia leads to the accumulation of extracellular adenosine, which inhibits antitumor T cells, enabling them to escape immunosurveillance (16). These antitumor T cells can also be inhibited by the increased level of hypoxia-inducible factor-1 α (HIF-1 α ; ref. 16), a transcription factor that is elevated in response to hypoxia that can promote angiogenesis.

It is known that hypoxia promotes cancer metastases through multiple modes. Hypoxia causes DNA modifications in tumor cells and increases the expression of metastatic-related gene products (17). Hypoxia can potentially degrade the ECM, which subsequently reduces cellular adhesions and releases tumor cells into systemic and lymphatic circulations. Another method by which hypoxia contributes to tumor invasion and metastasis is by shedding the MHC class I ligands (18). This also allows tumor cells to escape immune surveillance by various immune cells such as natural killer cells and cytotoxic T cells.

Strategies to Reduce Tumor Pressure

In view of tumor pressure elevation and its substantial effect on cancer treatment, TIP modulation is therefore imperative to improve tumor response to cancer therapy and subsequently the overall treatment outcome. Factors such as the abnormal tumor vasculature, high intratumoral vascular resistance, abnormal lymphatic drainage, and the abnormal ECM com-

ponents contribute to TIP. Targeting these factors pharmacologically and/or physically has been shown to reduce TIP and improve intratumoral drugs distribution and uptake. These agents have a potential for clinical application for cancer treatment; however, they are not without any pitfalls (Table 1).

Strategies Using the Pharmacological and Biological Agents

Targeting the tumor vascular morphology and pressure

Targeting the tumor vasculature can either be done by interfering with the development of the new tumor vasculature or disrupting the existing ones. The agents that target the tumor vasculature can be broadly divided into two categories: vascular targeting agents (VTA) and vascular disrupting agents (VDA). VTAs aim to "normalize" tumor vascular formation (19), whereas VDAs affect the existing tumor vasculatures.

Vascular targeting agents. VTAs aim to "normalize" tumor vasculature (19). This is done by inhibiting the proangiogenic cytokines such as VEGF and PDGF. The "normalization" of tumor vasculature results in reduced vessel diameter, increased pericyte coverage, and normalized basement membrane, accompanied by normalization of its function (19). This "normalized" tumor vasculature becomes less permeable and tortuous with suppression of the erratic sprouting of tumor vessels. This leads to reduced fluid and proteins extravasation into the interstitium, causing TIP reduction. This can potentially lead to an increase in interstitial convection transport and a reduction of outward fluid convection (20).

Overexpression of proangiogenic cytokines such as VEGF is known to cause production of abnormal hyperpermeable tumor vasculature. Targeting VEGF using anti-VEGF antibodies and inhibitors such as bevacizumab (21), DC101 (22), and sorafenib (23) has been shown to reduce TIP. Combination of VEGF-targeting agents with cytotoxic drug (24) or oncolytic viral therapy (25) delays tumor growth and exhibits better therapeutic efficacy. However, there has been evidence that a combination of VEGF and other agents can be detrimental due to increased invasiveness and metastasis in certain types of cancer (26).

Another cytokine that is overexpressed in tumors is the PDGF. PDGF receptor- β (PDGFR- β) is a stromal tyrosine kinase receptor that is frequently upregulated in tumor stroma and blood vessels. It plays an important role in tumor angiogenesis. Phosphorylation of PDGFR- β enhances stromal growth stimulation, activates fibroblasts, and disrupts cell-interstitium interactions. Imatinib, a PDGFR- β -specific tyrosine kinase inhibitor, has been shown to reduce TIP and enhance therapeutic efficacy (27).

Vascular disrupting agents. VDAs target the existing tumor vasculature. They bind to the microtubules causing destruction of the endothelial cells. This results in an increased vascular permeability that leads to an increase in TIP. However, VDAs also cause occlusion of the feeding precapillary vessel and reduction of tumor blood flow, which leads to an eventual tumor necrosis. Development of necrosis will then increase the interstitial hydraulic conductivity and this will potentially

Table 1. Pharmacologic and physical therapies that have been shown to reduce TIP—evidence and disadvantages

Therapy	Evidence	Disadvantages
Pharmacologic therapies		
VTAs	Bevacizumab lowers TIP and plasma protein leakages in KAT-4 xenograft human anaplastic thyroid (21)	Potential increase in invasiveness and metastasis (26) Potential resistance to chemo- and radiotherapy with prolonged treatment (27)
	DC101 reduced TIP in murine mammary carcinoma and several human carcinoma models (22)	Significant toxicity causing agent to be withdrawn from previously approved indication (50) Optimization of "normalization window" yet to be achieved (51)
	Sorafenib reduces TIP in advanced soft tissue sarcomas (23)	
	Imatinib decreases TIP and improves delivery of liposomal doxorubicin in mice-bearing melanoma model (27)	
VDAs	ZD6126 reduces TIP in murine fibrosarcoma and human cervical tumor cell line (28)	Associated dose-limiting toxicities, including neurotoxicity and cardiotoxicity (51)
	Combretastatin A4 disodium phosphate reduces tumor perfusion followed by a decrease in TIP in murine mammary carcinoma (29)	Optimum treatment schedule with combination therapy (53)
	Patupilone (Epothilone B) reduces TIP in murine metastatic melanoma and mammary carcinoma models (30)	
Antimitotics	Taxanes reduces TIP and improves oxygenation in patients with primary invasive breast cancer in combination with doxorubicin (31)	Change in gene status after treatment with taxane that can potentially affect clinical management (53) Dose-limiting toxicity, including neurotoxicity, nephrotoxicity, fatal neutropenic sepsis (62)
Vasodilators	Hydralazine reduces TIP in murine sarcoma by 33% (33)	Reduction in MABP (34) Hypoxic effect (54, 55)
	Cachectin (TNF- α) reduces TIP up to 70% of control values in human melanoma xenografts (34)	Prolonged treatment reduces immunotherapy efficacy (56)
TGF- β inhibitors	Lowers TIP in murine anaplastic thyroid carcinoma model (35)	A balance between the action of anti-TGF therapy that can both promote and suppress tumoral immune response needs to be achieved (57) Pan-TGF- β antibodies to target multiple TGF- β isoforms (57)
Proteases	Hyaluronidase normalizes TIP, and diminished metastatic burden in mice-bearing pancreatic ductal adenocarcinoma when combined with gemcitabine (38)	Lack of clinical data of metastatic potential with proteases treatment
	Collagenase reduces TIP by 45% and doubled intratumoral uptake and distribution of monoclonal antibody in human osteosarcoma xenografts (39)	
Physical therapies		
Irradiation	Reduces TIP in human colon adenocarcinoma xenografts (40)	Not enough evidence to support modulations in vascular, transvascular and interstitial transport (41) Potential local recurrence and metastatic potential (43) Potential reduction in intratumoral treatment efficacy (50)

(Continued on the following page)

Table 1. Pharmacologic and physical therapies that have been shown to reduce TIP—evidence and disadvantages (Cont'd)

Therapy	Evidence	Disadvantages
Hyper-/hypothermic therapy	Local hyperthermia reduces TIP in amelanotic melanoma model with associated delayed tumor growth (41) Milder environmental heat stress reduces TIP in several murine tumor models (42) Externally applied hypothermia reduces TIP in experimental glioma models (43)	Hyperthermia may not work in certain type of cell lines as different cell lines varies in their intrinsic heat sensitivity (51)
Ultrasound therapy	Reduces TIP in parallel with an increase in nanoparticle delivery in epithelial and epithelial-mesenchymal transition tumors treated with ultrasound-induced hyperthermia (44)	Contradictory reports of the efficacy of nanoparticle delivery post ultrasound treatment (44) Optimal treatment scheduling for combination therapy (44)
HBO	Lowers TIP, reduces intratumoral collagen content and causes an increase in intratumoral uptake of [³ H]-5-fluorouracil after a single treatment (45) Repeated HBO treatment lowers TIP, retards tumor growth and has an antiangiogenic effect in experimental mammary adenocarcinoma (46)	Potential effects of promoting tumor growth and recurrence (61) Potential cellular damage and organ dysfunction due to elevated level of reactive oxygen species (61)
PDT	Reduces TIP in murine melanoma model (47)	PDT-mediated cell death requires the presence of oxygen, which is what lacks in solid tumor Self-limiting: induces tumor hypoxia (52) Difficulty to treat large and nonsuperficial solid tumors due to limited laser light penetration depth

decrease TIP. VDAs that have been shown to reduce TIP include ZD6126 (28), combretastatin A-4 (29), and patupilone (30).

Taxane (paclitaxel, docetaxel; ref. 31) is another group of drugs that has the ability to reduce TIP. It has both vascular targeting and disrupting properties. It independently inhibits several proangiogenic factors, including VEGF (31). It binds to microtubules, leading to cellular apoptosis. An increase in cellular apoptosis reduces tumor density and decompresses blood vessels. The increase in tumor vessel diameter reduces MVP and subsequently TIP.

Vasodilators. MVP is a known factor that affects TIP (32). It is influenced by the mean arterial blood pressure (MABP). Any agent that reduces the MABP can potentially reduce TIP, for example, the vasodilators. Hydralazine (33) and cachectin (TNF- α ; ref. 34) are vasodilators that have been shown to reduce TIP. Their vasodilatory effect causes a decrease in vascular resistance followed by an increase in tumor blood flow, which can potentially improve intratumoral transport of macromolecules.

Targeting the tumor lymphatics

The role of TGF- β , a multifunctional cytokine, is well established in promoting tumor angiogenesis and lymphogenesis as well as the ECM formation. TGF- β blockade prevents abnormalization of tumor vasculature and lymphatic vessels (35), which subsequently leads to a reduction in TIP (36). Several preclinical studies of antilymphogenic agents and

their effects on suppression of tumor lymphogenesis and metastasis were reviewed by Duong and colleagues, however, these studies did not investigate the effect of these agents on TIP (37).

Targeting the solid pressure

Proteolytic enzymes have been described to affect MVP and TIP due to degradation of ECM components.

Hyaluronidase is an enzyme that degrades hyaluronan, a component that is significantly increased in tumor ECM. It splits the β -N-acetyl-hexosamine glycosidic bonds in hyaluronan. This subsequently destroys its structural support function in the tumor matrix and leads to a reduction in TIP (38).

Another enzyme, collagenase, has been shown to reduce TIP (39) by cleaving collagen and consequently destroying the collagen lattice in tumors. Collagenase can potentially reduce tumor MVP most likely due to degradation of collagen associated with vascular vessels.

Strategies Using the Physical Methods

Physical methods have been shown to reduce TIP. These include irradiation, hyper- or hypothermic therapy, ultrasound, hyperbaric oxygen (HBO) therapy, and photodynamic therapy (PDT).

Irradiation causes damage to tumor cells and vascular endothelium. This alters the tumor microcirculation by reducing the permeability of the vascular wall to fluid and leads to a

reduction in TIP (40). However, Multhoff and Vaupel concluded in their extensive review that evidence supporting the modulations in vascular, transvascular, and interstitial transport by irradiation, so far has been inconclusive (41).

Induced hyperthermia or hypothermia has been shown to reduce TIP. The induction of hyperthermia to tumors has a similar effect as irradiation. An increase in tumor vascular perfusion and a reduction in tumor hypoxia were also observed, in parallel with the reduction of TIP (42, 43). On the other hand, hypothermia can also reduce TIP, probably due to decreased capillary permeability or cellular metabolism (44).

Ultrasound reduces TIP (45) due to mechanical (cavitation, radiation pressure) and thermal effects. Thermal effects cause damage to tumor cells and ECM, which increase the interstitial hydraulic conductivity, reduce matrix tension, and enhance tumor blood flow.

HBO therapy aims to improve tumoral response to radiation and healing of postradiation tissue injury by increasing oxygen tension and delivery to tissues independent of hemoglobin. It has been shown that HBO therapy significantly lowers TIP (46) and repeated treatment gives a better therapeutic effect (47).

PDT can potentially impair microcirculation in tumors. In a murine melanoma model, PDT causes an initial increase in TIP before showing a decrease to 50% of control value after 24 hours (48). The initial increase of TIP can be explained by the increase in tumor vascular resistance due to vasoconstriction, microembolisation, and partial occlusion of vessels of swollen endothelial, cancer or immune cells treated with PDT. However, progressive microvasculature blockage leads to reduction in MVP and subsequently TIP.

Future Directions and Perspectives

We have discussed TIP effects on the tumor and its associated impact on cancer therapy. Modulation of TIP is therefore a means to improve upon the overall cancer treatment outcomes.

Pharmacologic and targeted therapies have shown their potential as TIP-reducing agents in the clinical setting. However, they are not without their drawbacks. Pharmacologic therapies may work better in smaller rather than larger solid tumors, which tend to have higher TIP (49, 50).

Targeting the TME can be toxic, does not prevent resistance, and requires knowing the optimal biologic dose (51). For example, bevacizumab has recently been withdrawn by the U.S. Food and Drug Administration from its previously approved indication for patients with metastatic HER2-negative breast cancer due to its significant toxicity (51). Treatment with antiangiogenic agents requires an optimal treatment schedule. "Normalization" of tumor vasculature can be transient (52). Prolonged therapy can potentially produce hypoxia due to vascular regression (52). Optimization of this therapeutic window effect requires further investigation especially with combination therapy in different types of cancer (53).

Taxane was shown to change *HER2* gene status in breast cancer after treatment (54). The gene status change can pose challenges to differentiate true amplification of *HER2* versus taxane-induced polyploidization effect on *HER2*. This can affect treatment planning (54).

Vasodilators pose a clinical challenge as a TIP-reducing agent as they can cause significant reduction in MABP (34). Hydralazine is known to selectively induce hypoxia in experimental tumors (55), probably due to stimulation of VEGF production and induction of HIF-1 α (56). Even though low-dose TNF- α can improve antitumor vaccination or adoptive T-cell therapy, prolonged treatment with this agent reduces the immunotherapy efficacy (57).

Clinical translation of successful TGF- β blockade as TIP reduction agent is yet to be seen. It is important to balance out the immune-suppressing and -promoting actions of TGF- β blockade therapy (58).

Preclinically, degrading enzymes have been successful in decreasing TIP while preventing tumor invasiveness and metastatic potential, but clinical studies have yet to be published in this area.

Physical therapies are also not without any disadvantages. TIP-reducing methods include irradiation, hyper- or hypothermic therapy, ultrasound, HBO therapy, and PDT. Irradiation can cause local recurrence and metastasis due to posttreatment tissue hypoxia (52, 59). Irradiation can also cause reduction in intratumoral transport of macromolecules due to an increased intratumoral collagen type 1 level (60). The therapeutic effect of hyperthermic therapy may not be uniform; different cell lines have different intrinsic heat sensitivity (52).

Ultrasound-induced TIP reduction is time-limited and may not fit within the time frame of combination therapies. For example, in preclinical studies, ultrasound-induced TIP reduction ranges from 5 minutes to 6 hours (45).

HBO therapy is relatively safe, but further investigation is required to validate this in different tumor types (61). In addition, combinational HBO treatment scheduling needs to be optimized (45).

PDT as TIP reduction modality is yet to be validated because of its limited laser light penetration depth in large nonsuperficial tumors. It is also self-limiting as it requires oxygen to induce tumor cell death, but at the same time induces hypoxia (52).

In conclusion, pharmacologic and physical therapies have their disadvantages and their application in clinical practice is yet to be validated. Physical methods as a means to reduce TIP have been progressing minimally for the past decade. We believe that physical manipulation of tumor biology has the potential to reduce TIP and subsequently allow better uptake and homogenous distribution of anticancer agents. This method can be performed in a safe manner while improving the overall survival outcome.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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