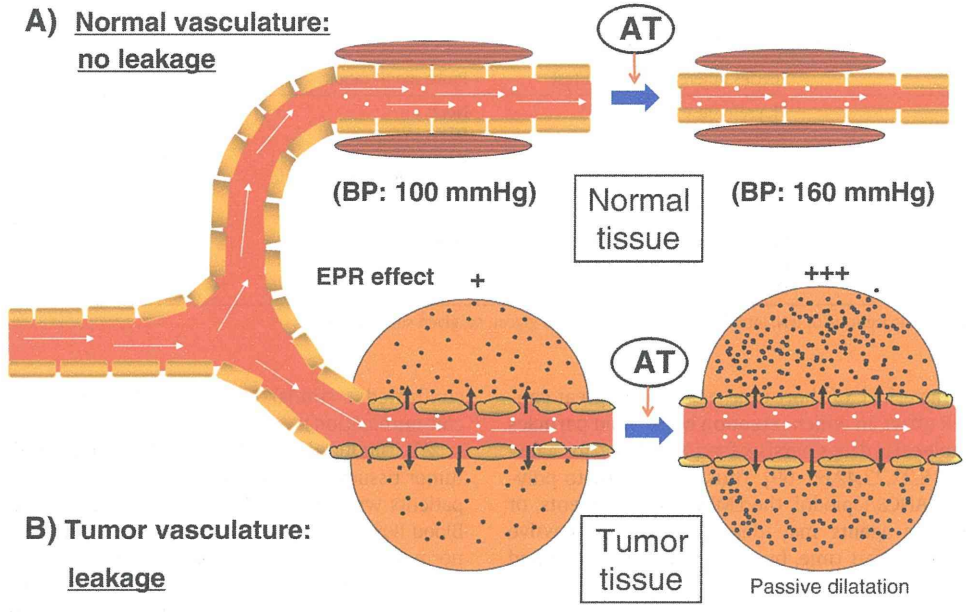

Fig. 6. Diagrammatic representation of the EPR effect and the effect of AT-II-induced hypertension accompanying the enhanced EPR effect and drug delivery in tumor and normal tissue. Under AT-II-induced hypertension (*e.g.* 100→160 mmHg), normal vessels and tumor vessels behaved differently. (A) The normal blood vessels have a smooth muscle cell layer that contracts and tightens the cell–cell junctions, and a narrowing of the vascular diameter results in less drug leakage. In tumors (B), the vascular endothelial cell–cell junctions have a wide gap opening that will be opened further by elevating blood pressure hydrodynamically (B, right). AT-II-induced hypertension thus leads to enhanced tumor-selective delivery of macromolecular drugs due to gap opening as demonstrated in clinical settings [68]. Adapted from refs. [18] and [68].

All these above vascular mediators are completely safe for almost all patients, and thus we encourage clinicians to validate this strategy. More recently, heme oxygenase-1 (HO-1)/carbon monoxide (CO) [76], TGF-β inhibitor [77], TNF-α [78] and heat generated by laser (He/Ne) together with gold nanorod [36] are shown to augment the EPR effect in mouse models.

6. Fluorescence imaging and the EPR effect with fluorescent nanoprobes

For our initial observation of the EPR effect (in vivo), we used Evans blue, which we administered by intravenous (i.v.) injection, and the tumor was selectively stained blue [16,19,22,23,42], which we

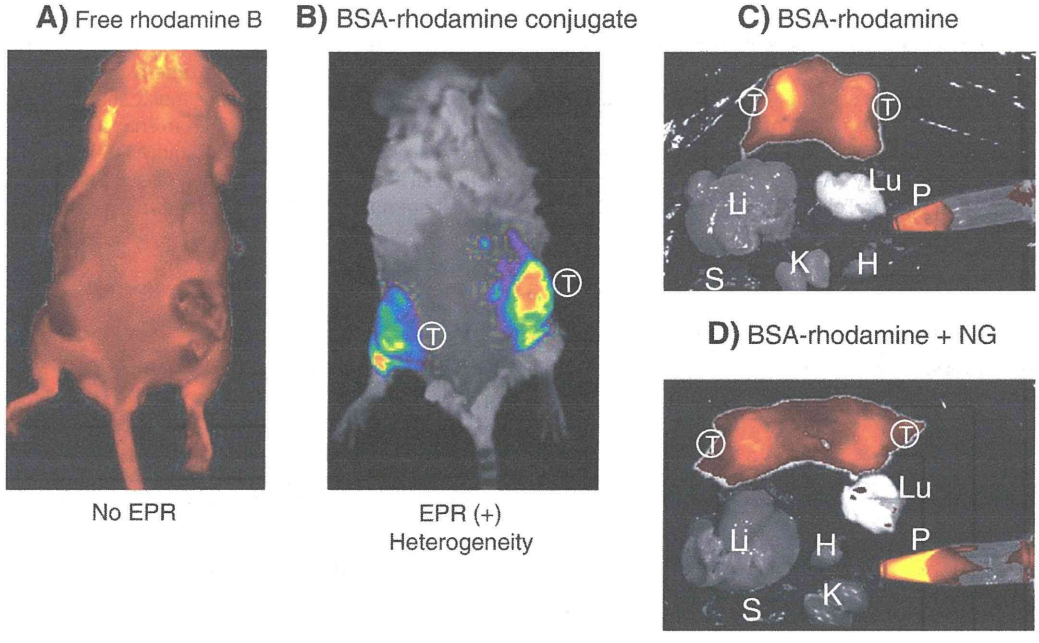

Fig. 7. Fluorescence tumor imaging based on EPR effect. The EPR effect-based uptake of fluorescent nanoprobes in tumors is compared with uptake of parental LMW fluorescent probe in vivo. (A) 24 h after i.v. injection of the LMW fluorescent probe, rhodamine B into S-180 tumor-bearing mice, no distinct tumor image is visible. Whereas injection of tetraethyl rhodamine isothiocyanate (TRITC)-conjugated BSA (67 kDa) resulting in highly tumor selective fluorescence at the same experimental conditions. (C) At 24 h, S-180 tumor-bearing mice were dissected, and each organ was viewed with IVIS® system. Only tumor tissues showed significant fluorescence. (D) Same as C except that nitroglycerin (NG) ointment was applied to the skin, and then the EPR effect and tumor drug delivery were evaluated. In D, the cut surface of tumor tissues shows a more homogeneous tumor uptake of fluorescent drug (TRITC-BSA), and also more fluorescent drug remained in the blood, which indicates that the EPR effect would increase progressively with time. In C and D, fluorescence is not seen in all other normal organs. T, tumor; Li, liver; K, kidney; H, heart; S, spleen; Lu, lung; P, plasma.

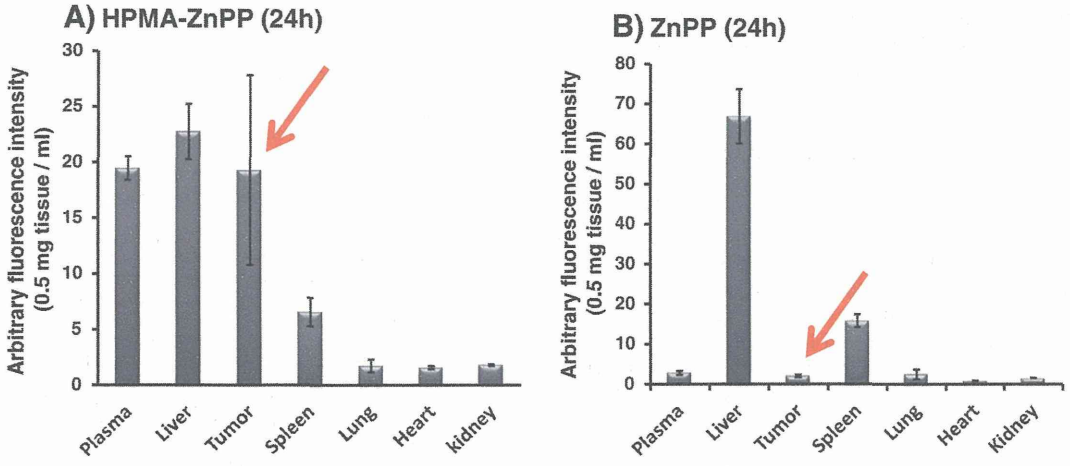

Fig. 8. Tumor selective accumulation of HPMA-conjugate zinc protoporphyrin (HPMA-ZnPP). The specimens were obtained 24 h after iv injection of this conjugate, and fluorescence intensity of each homogenate was measured after extraction of ZnPP with dimethylsulfoxide. Arrows show a great difference in tumor accumulation between (B) free ZnPP vs (A) polymer HPMA conjugated ZnPP.

attributed to the accumulation of blue dye-bound albumin in the tumor. In subsequent studies, we measured various radiolabeled plasma proteins conjugated with radioactive ^{56}Ga via diethylenetriaminepentaacetic acid (DTPA) chelation in solid tumors [16]. Plasma proteins are the most biocompatible macromolecules, and we found that all accumulated in solid tumors more preferentially [16,19,42].

Similarly, when we injected fluorescent macromolecules, we observed tumor-selective staining even 72 hr after i.v. injection. That is, after an i.v. injection of rhodamine isothiocyanate-conjugated bovine serum albumin (BSA) into tumor-bearing mice, we easily and clearly visualized the tumors directly in vivo by using an IVIS imaging system (IVIS, Model Lumina-XR, Hopkinton, MA, a fluorescence imaging system). Fig. 7 illustrates the great difference between tumor imaging obtained with tetramethylrhodamine isothiocyanate (TRITC)-conjugated BSA [MW 67,000] (Fig. 7B) and that obtained with free rhodamine B (MW 479.1) (Fig. 7A), after i.v. injection into tumor-bearing mice. In contrast to TRITC-BSA, free rhodamine B did not produce any appreciable fluorescence of the tumor (Fig. 7A). This finding clearly demonstrates that the EPR effect also operates for macromolecular fluorescent nanoprobes. We found the same result with another polymer, *N*-(2-hydroxypropyl)methacrylamide (HPMA) (13 kDa) conjugated with zinc protoporphyrin (ZnPP); this conjugate formed micelles of about 80 nm in diameter and showed a clear tumor image similar to Fig. 7B (not shown). To validate accumulation of this fluorescent probe more quantitative manner, we extracted HMPA-ZnPP and measured fluorescence intensity after homogenization of each organ and tumor as well as blood plasma. Fig. 8 illustrates that intra-tumor accumulation at 24 h after iv infusion was more than 10 times of other

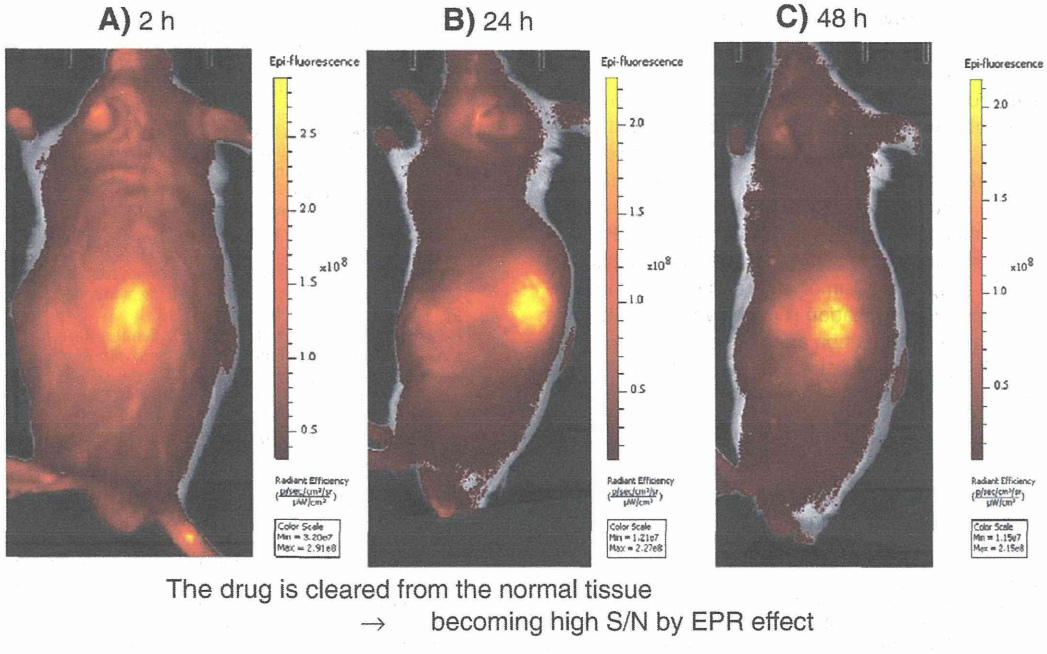

Fig. 9. In vivo tumor imaging by use of indocyaningreen (ICG). ICG was injected i.v. into S-180 tumor-bearing mice and in vivo fluorescent imaging was viewed after 2, 24, and 48 h by IVIS system directly. ICG will bind with albumin to form complex, thus behaving as a macromolecule. As shown in the figures, the contrast of the fluorescent tumor image increased as time passes. That is, nonspecific delivery of the agent to normal tissues was cleared via the lymphatic system and thus improving the contrast of tumor image (cf. 2 h vs 48 h).

vital organ such as the heart, lung, kidney except that in the liver that is the major organ for ZnPP metabolism. Since HPMA-ZnPP generates singlet oxygen upon irradiation, endoscopic light irradiation resulted significant tumor suppression in this mouse model (S-180 tumor) (data not shown). Similarly, TRITC-conjugated transferrin also revealed tumor-selective accumulation in vivo and a distinct tumor image.

In addition, indocyanine green (ICG) showed clear visible tumor image even 2 h after injection (Fig. 9). ICG is routinely used as a probe for evaluating hepatic function. In healthy people, ICG binds albumin and globulin, and it is rapidly liberated in the liver as free dye, which traverses to the bile duct, and excreted into the bile. Rapid plasma clearance of ICG therefore occurs in healthy humans (half-life<20 min). Albumin bound ICG rapidly accumulated in tumor, as early as at 2 h, and time-dependent increase of contrast in tumor image is shown in this model (Fig. 9), which is not seen in normal tissue, and the fluorescence of the normal tissues gradually disappeared because of lymphatic clearance. Namely, the fluorescent nanoprobe was cleared faster from the normal tissue than from the tumor tissue, and thus the contrast of the tumor image improved progressively after 24 and then 48 h. This finding confirms the distinct retention of fluorescent nanoprobes in tumors based on the EPR effect (Figs. 7–9). It is therefore so obvious that radio emitting nuclei or positron emitting or magnetic resonance probes in biocompatible nanoparticles would have a great value similarly for tumor imaging and an important value to offer.

7. Conclusions

Our history of the discovery of EPR effect is briefly reviewed. Comparison of the vascular permeability of tumor tissues as well as inflamed tissue illustrate the relevance of the EPR effect for cancer treatment and diagnosis. Many vascular factors such as bradykinin, NO, prostaglandin and CO were shown to be produced excessively in both inflammation and cancer, and modulation of these factors may potentiate the EPR effect. The defective architecture of tumor vessels and the vascular factors affecting normal tissue surrounding tumors also contribute to macromolecular permeability of the EPR effect as well.

Methods to enhance the EPR effect that utilize nitroglycerin and other NO-releasing agents, ACE inhibitors, and AT-II-induced hypertension, among others, may improve drug delivery to tumors by 2- to 3-fold and thus therapeutic effect as well.

We expect that the use of polymers or nanomedicines to deliver drugs to tumors will provide great advantages not only for delivery of therapeutic agents to obtain better therapeutic effects as well as reduced systemic toxicity; it will be invaluable also for tumor-selective and highly sensitive imaging with fluorescent or other radioloical nanoprobes. Providing detection methods for microtumor nodules would make earlier therapeutic surgical intervention possible. Further, a similar method is applicable for using photosensitizer of nanoparticle, which generates singlet oxygen and tumor detection possible simultaneously under endoscopic light irradiation (not shown). We anticipate a great advancement in tumor detection and treatment at very early stage of tumor before long, by use of nanomedicine, and more cure of cancer would be achieved in one way or the other.

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Cancer Research

Meeting Report

Challenges and Key Considerations of the Enhanced Permeability and Retention Effect for Nanomedicine Drug Delivery in Oncology

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Abstract

Enhanced permeability of the tumor vasculature allows macromolecules to enter the tumor interstitial space, whereas the suppressed lymphatic filtration allows them to stay there. This phenomenon, enhanced permeability and retention (EPR), has been the basis of nanotechnology platforms to deliver drugs to tumors. However, progress in developing effective drugs using this approach has been hampered by heterogeneity of EPR effect in different tumors and limited experimental data from patients on effectiveness of this mechanism as related to enhanced drug accumulation. This report summarizes the workshop discussions on key issues of the EPR effect and major gaps that need to be addressed to effectively advance nanoparticle-based drug delivery. *Cancer Res; 73(8); 2412–7. ©2013 AACR.*

Introduction

The field of nanomedicine, despite being conceptualized as far back as the 1980s, is only now transitioning in a broad sense from academic research to drug development and commercialization. In oncology, unique structural features of many solid tumors, including hypervasculature, defective vascular architecture, and impaired lymphatic drainage leading to the well-characterized enhanced permeability and retention (EPR; ref. 1) effect, are key factors in advancing this platform technology. However, the EPR effect has been measured mostly, if not exclusively, in implanted tumors with limited data on EPR in metastatic lesions. Dextran-coated iron oxide nanoparticles (25–50 nm) have been used clinically for several years (2) to measure permeability and retention noninvasively by MRI (3). Furthermore, tumor response alone is no longer considered a good endpoint, at least from the health authority point of view. This is exemplified by the recent U.S. Food and Drug Administration (FDA) withdrawal of bevacizumab (Avastin) for patients with metastatic breast cancer where impressive tumor responses were seen but bevacizumab showed no improvement in overall survival. Thus, limitations and challenges both in understanding tumor structural features and correlating them with the technology must be addressed and additional critical data need to be generated before nanotechnology-based drug delivery approaches can be fully realized in clinical use in patients with cancer. A one-day workshop was convened at the NIH on October 10, 2012, to specifically address key issues related to understanding of EPR effect and its use to achieve the maximum therapeutic effect with drugs using nanoparticle carriers.

This workshop was organized by the Alliance for Nanotechnology in Cancer and its recently formed public–private partnership consortium, TONIC (Translation of Nanotechnology in Cancer), in response to several questions raised by industry members of TONIC. The main purpose of this meeting was to gain better understanding of the EPR characteristics impacting the use of nanoparticles in the clinic. Experimental evidence of EPR in animal models and humans, clinical relevance of EPR, gaps in knowledge, and ways to address these gaps were all discussed.

Report

The workshop composed of 8 talks covering topics ranging from methods to investigate EPR in preclinical and clinical studies including diagnostic imaging, to the ramifications of EPR for enhanced drug uptake by different tumors and the predictability of preclinical and clinical outcomes. The session opened with an overview of the nanotechnology programs in cancer, funded by the Alliance for Nanotechnology in Cancer (NCI), and was followed by an introduction to TONIC, a corporate partnership model of the public, private, and

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Note: A list of speakers is available as supplementary data for this article at Cancer Research Online (http://cancerres.aacrjournals.org/).

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academic sectors, to accelerate the translation and development of nanotechnology solutions for the early detection, diagnosis, and treatment of cancer. This was followed by scientific presentations relating to the key questions identified at previous TONIC meetings. The discussions at the workshop focused on two key themes, namely, heterogeneity of EPR in tumors and factors that influence EPR effect.

Heterogeneity of EPR in Tumors

EPR exists in tumors and can be exploited for selective delivery of drugs to tumor by nanotechnology. However, there is significant heterogeneity within and between tumor types. It was noted that different tumor types have different pore dimensions in the vasculature and that the maximum pore size changes with the location for a given type of tumor (i.e., primary vs. metastases). In addition, there may be differences in vessel structure within a single tumor type. Thus, to understand whether a tumor is likely to respond to a nanoparticle-based drug that relies on EPR for delivery, an image-guided patient selection or diagnostic approach can potentially prove useful to profile and select tumor types and patients with tumors conducive to such delivery. Hiroshi Maeda (Sojo University, Kumamoto, Japan), who first proposed the EPR effect over 25 years ago (1), suggested a number of ways one can augment the EPR effect. These included increasing the blood pressure during infusion of a nanomedicine or macromolecular drug using angiotensin-II (e.g., blood pressure increase from 100 → 150 mmHg). Other methods involve vascular mediators such as nitroglycerin, ACE inhibitor, or PGE1 agonist (beraprost) and these have been shown to be effective in *in vivo* tumor models resulting in better tumor delivery (2- to 3-fold increase), linked to improved therapeutic effect (4).

Factors Influencing EPR

The following factors influence the EPR effect in tumors: (i) the nature of both the vascular bed and surrounding stroma, the presence or absence of functional lymphatics and interstitial hydraulic conductivity impacting interstitial pressure along with mechanical stresses generated by cancer and stromal cells impacting the extracellular matrix; (ii) tumor size, type, and location (including primary tumor versus metastatic lesions); (iii) extent of macrophage tumor infiltration and the activity of the mononuclear phagocytic system (MPS), which can vary between and within tumor types plus patient characteristics (e.g., age, gender, tumor type, body composition, treatment). These factors lead to accumulation of nanoparticles in both normal tissues and in different sections of the tumor, for example, in the periphery, viable tumor, and necrotic sections; and (iv) co-medications, which may impact, among other things, stroma and blood pressure (hypertension increases tumor blood flow). In addition, several vascular factors (Table 1; ref. 4), such as nitric oxide generators (5) and bradykinin potentiators, that is, ACE inhibitors that lower blood pressure, are known to affect EPR and are relatively safe and inexpensive to combine with a nanoparticle drug (4).

A fundamental limitation in evaluating EPR and the factors that affect EPR is poor understanding of which preclinical tumor models recapitulate patients with solid tumors. The factors affecting delivery of nanoparticles to tumors in preclinical models, such as tumor growth environment, vasculature, functional MPS, etc., appear to vary based on the cancer model [e.g., syngeneic flank xenograft, orthotopic xenograft, genetically engineered mouse model (GEMM)]. Thus, future studies will need to systemically evaluate these factors in preclinical models and in patients with various solid tumors and determine whether the models represent all aspects of the EPR effect.

The observed heterogeneity in EPR may be a contributing factor to the limited impact of nanoparticle-based drugs with reductions in toxicity and gains in overall survival as compared with small-molecule anti-cancer agents. Table 2 summarizes objective data on the survival benefits from nanotherapeutics approved to date. Further understanding and predictability of EPR function in primary tumor and its metastatic sites through the use of imaging studies may aid the development of future, effective nanodrugs. Correlation of EPR activity to clinical responses would likely provide direct clinical data to determine whether tumors with high EPR tumor activity will be more amenable to effective treatment using nanoparticle-based therapies (5). It was noted that the diversity of nanoparticle characteristics and API used is expected to impact the applicability of such correlations across different nanoparticle platforms and products.

The optimal patient selection or diagnostic aid to measure the EPR activity within a patient needs to be further defined. Ideally, this would involve a single imaging agent that is generalizable to all nanoparticles. Given the heterogeneity of nanoparticle-based systems—size, shape, charge characteristics, etc.—a specific diagnostic agent might, however, be required to predict likely response to a particular nanoparticle relying on EPR delivery. The use of contrast agents and MRI to measure the enhanced permeability (EP) component of the EPR effect might be one generic method. Others might include a defined nanoparticle of a fixed size (~100 nm) labeled with an appropriate imaging agent—for example, Cu^{64} for positron emission tomography (PET) or fluorescent marker for near-infrared fluorescence (NIRF). There is precedence for a range of labeled liposomes and iron oxide–loaded nanoparticles for imaging, but there are very few human clinical studies on nanoparticle imaging that can effectively address the prevalence of EPR. In one such study, the biodistribution and pharmacokinetics of $^{[111]}$In-labeled PEGylated liposomes was evaluated in patients with locally advanced cancers. Positive tumor images were obtained in 15 of 17 studies, although levels of tumor liposome uptake varied between and within tumor types (6).

Eva Sevick-Muraca (The University of Texas Health Science Center, Houston, Texas) discussed the use of NIRF to image lymphatic flow and with fluorescent agents to detect cancers. This technique is light based and the fluorescent dye has no half-life and can be repeatedly excited, making it more appropriate for imaging of nanoparticle accumulation over longer timeframes than radioactive imaging agents with short half-lives (7). While NIRF is considered to be a combination product by the FDA and has a maximum tissue penetration of 3 to 5 cm,

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Table 1. Factors affecting the EPR effect of macromolecular drugs in solid tumors (modified after references 4 and 5)

Mediators	Responsible enzymes and mechanisms	Possible application to therapeutic modality and mechanism
Bradykinin	Kallikrein/protease	ACE inhibitors (e.g., enalapril); blocking of kinin degradation elevates local kinin level → more EPR.
NO	iNOS	NO-releasing agents (e.g., nitroglycerin, ISDN, etc.) via denitrase and nitrite reductase to generate NO.
VPF/VEGF	Involved in NO generation	
Prostaglandins	COX-1	Beraprost sodium: PGI_2 agonist works via vascular dilatation and extravasation (5).
Collagenase (MMP)	Activated from proMMPs by peroxynitrite, or proteases	
Peroxynitrite	$NO + O_2$	
Carbon monoxide (CO)	Heme oxygenase (HO)-1	PEG-hemine via induction of HO-1 in tumor → CO generation (15).
Induced hypertension	Using angiotensin II	Slow i.v. infusion → systemic hypertension, vascular extravasation selectively in tumor tissue.
Inflammatory cells and H_2O_2	Neutrophil/NADPH oxidase, etc.	
TGF-β inhibitor		Inducing multiple inflammatory cytokines; NOS, COX, etc.: NO, PGs, etc.
TNF-α		Inducing multiple inflammatory cytokines; NOS, COX, etc.: NO, PGs, etc.
Anticancer agents		
Heat	Vascular dilation	Gold nanoparticle or ferrite nanoparticle using electromagnetic, or laser, or microwave.

such devices are not yet available in hospitals and may not have the right sensitivity at this time to detect the marker agent. The ability to image lymphatic function in the tumor vicinity could also provide a means to assess interstitial pressure imbalances. Efforts are underway to include dual-labeling PET for presurgical imaging and then NIR guidance during surgery (8). It is anticipated that PET will remain a crucial tool for clinical imaging and that the optical imaging counterpart will add value rather than being a replacement.

Ways to enhance the EPR effect in tumors were discussed and included drugs that impacted the vasculature (4)–for example, VEGF-based antagonists leading to vessel normalization, agents causing hypertension and increasing tumor blood flow, and agents that modulate the tumor matrix. Agents that generate nitric oxide [nitroglycerine or ISDN (isosorbide dinitrate)] were also shown to be effective in humans (4, 5). ACE inhibitor (e.g., enarapril), which potentiates the action of bradykinin, is also effective (4). Further work is required to validate the benefits of such agents in the context of exploiting the enhancement of EPR effect in the clinical setting (4, 5). It was suggested that both optimization of the nanoparticle and optimization of the tumor microenvironment were required for optimal delivery. Rakesh K. Jain (Harvard Medical School, Boston, Massachusetts); hypothesized that normalizing the vasculature, extracellular matrix, and lymphatics will lead to better delivery of drugs (9). However, normalized vasculature means that the average pore is smaller and this may require the use of smaller nanoparticles (~20 nm particle size). Overall, the biologic impact of the abovementioned vascular effectors on delivery of nanoparticles of varying composition, shape, and flexibility needs significant further work.

The role of the lymphatics in tumor biology and nanoparticle delivery was discussed. This highlighted the need to consider changes in physiologic status, both in the acute and in long-term functionality of lymphatics in patients with cancer influenced by inflammation, tumor burden, or treatment. This is an area of active research and imaging techniques are being developed that will allow this to be explored in more detail.

In terms of animal tumor models to evaluate the EPR effect, subcutaneous flank tumor xenografts were thought to offer limited value. The vasculature of such models often resembles the vasculature found in very high EPR tumors, for example, renal tumors irrespective of tumor type, and thus probably gives a false impression about the benefit of nanoparticle-based drugs relying on the EPR effect in most tumor settings. The workshop participants felt that better options are provided by metastatic, orthotopic, and GEMM-based models, although these need further characterization and validation. Primary tumor explants may be another option to model delivery to tumor types with high stromal content. Further work is required to understand how to use the preclinical tumor models to investigate drugs relying on the EPR effect for activity and to understand how they reflect the heterogeneity seen in clinical disease. The site of the tumor was also considered to be important, and a more systematic assessment of vasculature architecture versus site of tumor was recommended.

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Table 2. Survival benefits from the FDA-approved nanomedicines to date

Generic drug	Trade name(s)	Indication	Benefit
PEGylated liposomal doxorubicin	Doxil and Caelyx	HIV-related Kaposi's sarcoma	No statistically significant change in overall survival (23 wks) vs. doxorubicin, bleomycin, and vincristine treatment (22.3 wks) for HIV-related Kaposi's sarcoma
		Metastatic ovarian cancer	Statistically significant overall survival improvement (108 wks, $P = 0.008$) vs. topotecan treatment (71.1 wks) for platinum-sensitive patients with ovarian cancer
		Metastatic breast cancer	No statistically significant overall survival change (84 wks) vs. conventional doxorubicin (88 wks) for patients with breast cancer receiving first-line therapy
Liposomal daunorubicin	DaunoXome	HIV-related Kaposi's sarcoma	No statistically significant overall survival change (52.7 wks) vs. doxorubicin, bleomycin, vincristine treatment (48.9 wks)
Poly (styren-*co*-maleic acid)–conjugated naocarzinostatin	SMANCS	Liver cancer, renal cancer	Approved in 1993 in Japan. Far more effective when the EPR is enhanced by increasing the blood pressure in difficult-to-treat tumors, including metastatic liver cancer, cancers of pancreas, gall bladder, etc.

Liver cancer: 5-year survival (%)**

Metastasis	1 seg.+	>	2 seg.
Child A	>90%	~	>50%
Child B	40%		30%

Five-year survival (%) based on the liver function (cirrhosis) by child classification and intrahepatic+ metastasis within one segment or more

Albumin-bound paclitaxel	Abraxane	Metastatic breast cancer	Statistically significant overall survival change (56.4 wks, $P = 0.024$) vs. polyethoxylated castor oil–based paclitaxel treatment (46.7 wks) for patients receiving second-line treatment

NOTE: The polymeric platform methoxy PEG-poly(D,L-lactide) taxol with the trade name Genexol-PM (Sanayang Co.) has been approved in Korea for the treatment of metastatic breast cancer. Adapted from the work of Jain and Stylianopoulos (16).
**, SMANCS data in the table were provided by H. Maeda.

Omid Farokhzad (Harvard Medical School) discussed the advantages of including a targeting agent on the nanoparticle to enhance the retention component and/or enable delivery of drug directly into the tumor cell via internalization of the nanoparticle. The majority of the currently available clinical data on nanoparticle oncology drugs relate to passively targeted liposomal drugs. Recently, several actively targeted nanoparticle products have also entered clinical development, including liposomes and polymeric particles containing payloads ranging from conventional cytotoxic drugs to genes expressing tumor suppressors (10). These particles are targeted to various tumor markers including the transferrin receptor HER-2 and prostate-specific membrane antigen (PSMA) using either protein or small-molecule ligands. Recent data were presented for BIND-014 (11), a docetaxel-encapsulated polymeric nanoparticle targeted to PSMA, which is expressed on the surface of prostate cancer cells and nonprostate solid tumor neovasculature. In preclinical studies, BIND-014 increased the concentration of docetaxel in PSMA-expressing solid tumor xenografts by 5- to 10-fold. In a phase I clinical trial in patients with advanced solid tumors, BIND-014 displayed signals of antitumor efficacy in patients with advanced and metastatic cancer at low doses and in tumors where conventional docetaxel has minimal activity. With progress in polymeric nanoparticle engineering, similar approaches are also being applied to existing and developmental anticancer drugs, including other cytotoxics and molecularly targeted agents such as kinase inhibitors, and it will only be a matter of time before these advances will ultimately impact the treatment of cancer.

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William Zamboni (University of North Carolina, Chapel Hill, North Carolina) characterized the pharmacologic properties of nanoparticles *in vivo* as part of preclinical and clinical studies. He stressed the importance of the MPS, tissue distribution, and potential tumor delivery on the clearance of nanoparticles. There is a bidirectional interaction between monocytes and liposomal agents and potentially other nanoparticle agents (12, 13). Monocytes internalize liposomes, which then releases the drug from the liposome and leads to toxic effects to the monocytes. The tissue distribution and tumor delivery of nanoparticles may involve MPS-mediated and non–MPS-mediated mechanisms where uptake of nanoparticles by circulating MPS cells compared with tumoral macrophages may result in different tumor drug exposure and responses. Dr. Zamboni has developed an ex *vivo* flow cytometry–based, high-throughput screening platform (HTSP) system called PhenoGLO-HTSP to measure the clearance of nanoparticles by the MPS and bidirectional interaction between the MPS and nanoparticles, conjugates, and antibody–drug conjugates. Importantly, this method also predicts nanoparticle pharmacokinetics and pharmacodynamics in humans where the MPS system seems to drive the clearance, efficacy, and toxicity of nanoparticle agents. PhenoGLO-IT can measure MPS function in a blood sample from patients as a method to individualize the dose of nanoparticle agents and/or as a biomarker for predicting pharmacokinetics and pharmacodynamics (response and toxicity) of nanoparticles.

The workshop participants felt that as our understanding of nanoparticle delivery to tumors increases, the emerging nanoformulations should be considered both as a general formulation strategy in drug development and as a selected strategy to improve delivery profiles of existing or failed drugs.

Prospects

During discussions at the conclusion of the symposium, participants recommended the formation of a working group to establish translational and clinical procedures for integrated clinical trials involving nanotherapeutic constructs and accompanying imaging approaches. Such translational studies and clinical trials would enable further understanding and predictability of EPR function in a tumor and its primary or metastatic sites and may be critical for the development of future effective nanodrugs and predictive of antitumor response (14). An additional recommendation from this workshop was to generate a position paper highlighting key translational studies that should be conducted and parameters that should be monitored in nanoparticle drug delivery clinical trials to enable testing of various hypotheses for effective nanoparticle delivery (tumor perfusion, vascular permeability, interstitial penetration, retention, lymphatic function, MPS activity, blood pressure, fluid and solid stresses, others). In coming months, symposium participants will actively pursue these key recommendations and develop the necessary tools required to advance the scientific translation of the nanotechnology platform in the oncology therapeutic area.

Disclosure of Potential Conflicts of Interest

D.C. Blakey has ownership interest (including patents) in AstraZeneca. R.K. Jain is employed (other than primary affiliation; e.g., consulting) as a cofounder and board member of XTuit Pharmaceuticals, and as a board member of *Hambrecht & Quist Healthcare Investors* and *Hambrecht & Quist Life Sciences* Investors; has a commercial research grants from MedImmune, Roche, and Dyax; has ownership interest (including patents) in XTUit Pharmaceuticals; and is a consultant/advisory board member of Enlight Biosciences, SynDevRx, Dyax, Noxxon Pharmaceuticals, and Zyngenia. E.M. Sevick-Muraca has ownership interest (including patents) in NIRFImaging, Inc. W. Zamboni has ownership interest (including patents) in PhenoGLO Technologies. O.C. Farokhzad has ownership interest (including patents) in and is a consultant/advisory board member of BIND Bioscience, Selecta Bioscience, and BLEND. A. Gabizon has a commercial research grant from Janssen Pharmaceuticals. No potential conflicts of interest were disclosed by the other authors.

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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): U. Prabhakar, P. Grodzinski
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