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Challenges and key considerations of the enhanced permeability and retention (EPR) 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, while the suppressed lymphatic filtration allows them to stay there. This phenomenon - 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.

Introduction

The field of nanomedicine, despite being conceptualized as far back as the 1980's, is only now transitioning in a broad sense from academic research to drug development and

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Disclosure of Potential Conflicts of Interest

D.C.B and **S.T.B** are both employees and share holders of AstraZeneca; **R.K.J.** received research grants from Dyax, MedImmune and Roche; consultant fees from Dyax, Enlight, Noxxon and SynDevRx; owns equity in Enlight, SynDevRx and XTuit, serves on the Board of Directors of XTuit and Board of Trustees of H&Q Healthcare Investors and H&Q Life Sciences Investors; **WZ** is inventor of the PhenoGLO-HTSP^{TM} and PhenoGLO-IT^{TM} technologies; **AG** receives research support from Janssen Pharmaceuticals, and serves in the Board of Directors of Liposome Pharmaceuticals and, **O.C.F.** is a scientific founder and serves on the Scientific Advisory Board and Board of Directors of BIND Biosciences, Selecta Biosciences and Blend Therapeutics.

commercialization. In oncology, unique structural features of many solid tumors including hyper-vasculature, defective vascular architecture, and impaired lymphatic drainage leading to the well characterized enhanced permeability and retention (EPR)[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 in both mice and patients. 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 FDA withdrawal of bevacizumab (Avastin) for metastatic breast cancer patients 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 needs to be generated before nanotechnology based drug delivery approaches can be fully realized in clinical use in cancer patients. 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 utilization 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 (**T**ranslation **o**f **N**anotechnology **i**n **C**ancer), 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 utility 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 comprised of eight 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 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 will prove useful to profile and select tumor types and patients with tumors conducive to such delivery. Maeda (Sojo University, 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 (2–3 fold increase) linked to improved therapeutic effect[2].

Factors influencing EPR

The following factors influence the EPR effect in tumors: 1) 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, 2) tumor size, type and location (including primary tumor versus metastatic lesions), 3) 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, e.g. in the periphery, viable tumor and necrotic sections 4) co-medications, which may impact among other things, stroma and blood pressure (hypertension increases tumor blood flow). Additionally, several vascular factors (Table 1)[2] such as nitric oxide generators[3] and bradykinin potentiators i.e. ACE inhibitors which lower blood pressure, are known to affect EPR and are relatively safe and inexpensive to combine with a nanoparticle drug[2].

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 only accompanied by marginal, if any, gains in overall survival as compared with small molecule anticancer agents. Table 2 summarizes objective data on the survival benefits from nano-therapeutics 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[3]. It was noted that the diversity of nanoparticle characteristics and API utilized 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 nano-particle 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 (~100nm) labeled with an appropriate imaging agent – e.g. Cu^{64} for PET, 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[4].

Eva Sevick-Muraca (UTH, Houston, TX) discussed the use of NIRF to image lymphatic flow and with fluorescent agents, 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[5]. While NIRF is considered to be a combination product by the FDA and has a maximum tissue penetration of 3–5 cm, 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 interstitital pressure imbalances. Efforts are underway to include dual labeling PET for pre-surgical imaging and then NIR guidance during surgery[6]. 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[2]. 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[2,3]. ACE inhibitor (eg. Enarapril) which potentiate the action of bradykinin, is also effective[2]. 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[2,3]. It was suggested that both optimization of the nanoparticle and optimization of the tumor microenvironment was required for optimal delivery. Rakesh Jain (Harvard Medical School, Boston, MA) hypothesized that normalizing the vasculature, extracellular matrix and lymphatics will lead to better delivery of drugs[7]. However normalized vasculature means that the average pore is smaller and this may require the use of smaller nanoparticles (~20nm particle size). Overall, the biological impact of the above mentioned 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 physiological status, both in the acute and long term functionality of lymphatics in cancer patients 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, sub-cutaneous flank tumor xenografts were thought to offer limited value. The vasculature of such models often resembles the vasculature found in very high EPR tumors e.g. renal tumors irrespective of tumor type and thus probably gives a false impression regarding 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 pre-clinical 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 vs. site of tumor was recommended.

Omid Farokhzad (Harvard Medical School, Boston, MA) 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[8]. 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[9], a docetaxel-encapsulated polymeric nanoparticle targeted to PSMA, which is expressed on the surface of prostate cancer cells and non-prostate solid tumor neovasculature. In preclinical studies, BIND-014 increased the concentration of docetaxel in PSMA-expressing solid tumor xenografts by 5–10-fold. In a phase 1 clinical trial in patients with advanced solid tumors, BIND-014 displayed signals of anti-tumor 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.

William Zamboni (UNC, Chapel Hill, NC) characterized the pharmacological properties of nanoparticles in vivo as part of pre-clinical 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[10, 11]. 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 bi-directional interaction between the MPS and nanoparticles, conjugates and antibody-drug conjugates. Importantly, this method also predicts nanoparticle PK and PD in humans where the MPS system appears 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 anti-tumor

response[12]. An additional recommendation from this workshop was to generate a position paper highlighting key translational studies that should be performed 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.

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Table 1

Factors affecting the EPR effect of macromolecular drugs in solid tumors.

Mediators	Responsible enzymes and mechanisms	Possible application to therapeutic modality and mechanism
Bradykinin	Kallikrein/protease	ACE inhibitors (eg. Enalapril®); blocking of kinin degradation elevates local kinin level → more EPR.
NO	iNOS	NO releasing agents (eg. nitroglycerin, ISDN, etc) via denitrase and nitrite reductase to generate NO[2].
VPF/VEGF	Involved in NO generation	
Prostaglandins	Cyclooxygenase (COX) 1	Beraprost sodium: PGI_2 agonist works via vascular dilatation and extravasation[3].
Collagenase (MMPs)	Activated from proMMPs by peroxynitrite, or proteases	
Peroxynitrite	NO + O2	
Carbon monoxide (CO)	Heme oxygenase (HO)-1	PEG-hemine via induction of HO-1 in tumor → CO generation[13]
Induced hypertension	Using angiotensin II	Slow iv infusion → systemic hypertension, vascular extravasation selectively in tumor tissue.
Inflammatory cells and H2O2	Neutrophil/NADPH oxidase, etc	
Transforming growth factor (TGF)-β inhibitor		Inducing multiple inflammatory cytokines; NOS, COX etc: NO, PGs etc.
Tumor necrosis factor (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.

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 weeks) vs. doxorubicin, bleomycin and vincristine treatment (22.3 weeks) for HIV-related Kaposi's sarcoma
		Metastatic ovarian cancer	Statistically significant overall survival improvement (108 weeks, *P*= 0.008) vs. topotecan treatment (71.1 weeks) for platinum-sensitive patients with ovarian cancer
		Metastatic breast cancer	No statistically significant overall survival change (84 weeks) vs. conventional doxorubicin (88 weeks) for breast cancer patients receiving first-line therapy
Liposomal daunorubicin	DaunoXome®	HIV-related Kaposi's sarcoma	No statistically significant overall survival change (52.7 weeks) vs. doxorubicin, bleomycin, vincristine treatment (48.9 weeks)
Poly (styren-co- maleic acid) conjugated naocarzinostatin	SMANCS®	Liver cancer, renal cancer	Approved 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, gallbladder, etc Liver cancer: 5 year survival
			<table><tr><td></td><td>1 seg.+</td><td>></td><td>2 seg</td></tr><tr><td>Child A</td><td>> 90 %</td><td>~</td><td>>50%</td></tr><tr><td>Child B</td><td>40%</td><td></td><td>30%</td></tr></table>
			Five year survival (%) based on the liver function (cirrhosis) by Child classification, and intrahepatic+ metastasis
Albumin-bound paclitaxel	Abraxane®	Metastatic cancer breast	Statistically significant overall survival change (56.4 weeks, *P*= 0.024) vs. polyethoxylated castor oil-based paclitaxel treatment (46.7 weeks) for patients receiving second-line treatment

*The polymeric platform methoxy PEG-poly(D,L-lactide) taxol with the trade name Genexol-PM (Sanayang Co., Seoul, Korea) has been approved in Korea for the treatment of metastatic breast cancer.

Adapted from: [14] Jain, R. K. & Stylianopoulos, T., *Nat. Rev. Clin. Oncol*. (2010) 7, 653; SMANCS data in the table were provided by prof. Hiroshi Maeda.