Fundamental Biomedical Technologies

Aleš Prokop
Volkmar Weissig *Editors*

Intracellular Delivery III

Market Entry Barriers of Nanomedicines

Springer

Fundamental Biomedical Technologies

Volume 8

Series Editor
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Market Entry Barriers of Nanomedicines

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Intracellular Delivery I: 978-94-007-1247-8
Intracellular Delivery II: ISBN 978-94-017-8895-3

ISSN 1559-7083
Fundamental Biomedical Technologies
ISBN 978-3-319-43523-7 ISBN 978-3-319-43525-1 (eBook)
DOI 10.1007/978-3-319-43525-1

Library of Congress Control Number: 2014939942

Printed on acid-free paper

This Springer imprint is published by Springer Nature
The registered company is Springer International Publishing AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

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Introduction to Volume III

As a continuation of the previous two volumes, this third volume concentrates on commercial aspects. Unfortunately, obtaining data from the industry has proven to be almost impossible. Thus, we tried collecting manuscripts with emphasis on some preclinical and clinical applications.

Our introductory chapter (Prokop-Weissig) introduces problems associated with the translation of research from the bench to the clinic and subsequently market. This chapter was written by us to serve as an introduction to the entire volume. We failed to get some topics covered, e.g., on patenting as well as on the situation of funding in the USA.

The editors would like to acknowledge the effort of some individuals to peer-review manuscripts submitted by other authors as well as by outside reviewers. These are Fyllos Stylianopoulos, Ales Prokop, Shanta Dhar, Mansoor Amiji, Gerard D'Souza, Biana Godin, Vladimir Torchillin, Hideoyoshi Harashima, Pablo Scodeller, Volkmar Weissig, Nicolas Anton, Lars Kuepfer, Karel Petrak, Sjoerd Hak and

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Part I
Introductory Chapters

Chapter 1
Overview of Present Problems Facing Commercialization of Nanomedicines

Aleš Prokop and Volkmar Weissig

Abstract A critical review is attempted to assess the status of nanomedicine entry onto the market. The emergence of new potential therapeutic entities such as DNA and RNA fragments requires that these new "drugs" will need to be delivered in a cell-and organelle-specific manner. Although efforts have been made over the last 50 years or so to develop such delivery technology, no effective and above all clinically approved protocol for cell-specific drug delivery in humans exists as yet. Various particles, macromolecules, liposomes and most recently "nanomaterials" have been said to "show promise" but none of these promises have so far been "reduced" to human clinical practice.

The focus of this volume is on cancer indication since the majority of published research relates to this application; within that, we focus on solid tumors (solid malignancies). Our aim is to critically evaluate whether nanomaterials, both non-targeted and targeted to specific cells, could be of therapeutic benefit in clinical practice. The emphasis of this volume will be on pharmacokinetics (PK) and pharmacodynamics (PD) in animal and human studies.

Apart from the case of exquisitely specific antibody-based drugs, the development of target-specific drug–carrier delivery systems has not yet been broadly successful at the clinical level. It can be argued that drugs generated using the conventional means of drug development (i.e., relying on facile biodistribution and activity after (preferably) oral administration) are not suitable for a target-specific delivery and would not benefit from such delivery even when a seemingly perfect delivery system is available. Therefore, successful development of site-selective drug delivery systems will need to include not only the development of suitable carriers, but also the development of drug entities that meet the required PK/PD profile.

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A. Prokop, V. Weissig (eds.), *Intracellular Delivery III*, Fundamental Biomedical Technologies 8, DOI 10.1007/978-3-319-43525-1_1

In general, human clinical studies are approved only after the expected benefits of targeting have been shown in pre-clinical, *in vivo* animal studies first. Therefore, quantitative data on biodistribution of targeted and non-targeted nanoparticles should be generated as the first step. This should be followed by determining whether an increased presence of nanoparticles in tumors also results in increased concentration of the *free drug* within the tumor space. Any "promise" for reproducing similar data in human clinical studies should be supported by relevant scaling from the animal model used to humans.

For too long now, the same or similar approaches have been used by researchers without success. We believe that new fundamentally different approaches are needed to make cell-specific drug delivery clinical reality. In this volume we want to focus on (a) how nanoparticles could be redesigned from the material-science point of view (for example, redesigning nanoparticles for long-circulating properties, passive (EPR) and active targeting concept); and (b) on the design and properties of drugs that would benefit from cell-specific targeting (examining why active targeting of drug carrier does not necessarily result in drug accumulation in tumor). Further, we will draw attention to (c) the manner pre-clinical animal data should be translated to humans using appropriate scaling, in particular with reference to the differences between mice and men in terms of differing vascular morphology and immunological background.

Successful development of cell-specific drug-delivery systems requires that reliable quantitative pharmacokinetic/pharmacodynamic (PK/PD) data are collected both in animal and human studies. This volume will include (d) information on improved body imaging technologies and on enabling quantitative tools available.

Finally, we address (e) the issue of diminishing academic funding of animal studies and of (f) the current dismal market and proprietary situation in the area of site-specific drug delivery.

Keywords Nanomedicine • Market • Enhanced permeability effect • Targeted delivery • Extracellular matrix components • Imaging • Patenting

Acronyms

μCT	microcomputed tomography
ACA	anticancer agent (functionalized oligomer with attached targeting motif)
Ad-p53	Human Adenovirus Type5 (dE1/E3) expressing Tumor Protein P53 (P53) under a CMV promoter
ADMET	absorption, distribution, metabolism, and excretion – toxicity in pharmacokinetics
AuNC-CS-TPP	Chitosan-coated gold nanocluster – triphenylphosphonium
AuNP-TPP	Triphenylphosphonium gold nanoparticles
BITES	bispecific T-cell engagers

CAFs	cancer associated fibroblasts
CAGR	compound annual growth rate
CBER	Center for Biologics Evaluation and Research
CD3ε	anti-human scFv monoclonal antibody
CT	computed tomography
CTC	circulating tumor cells
DCE-CT	dynamic contrast enhanced computed tomography
DDD	drug discovery and development
DOX	doxorubicin
ECM	extracellular cell matrix
EPR	enhanced permeability retention effect R –endoplasmic reticulum
FA	Folic acid
FMT 3D	fluorescence molecular tomography
FRET	Fluorescence Resonance Energy Transfer
GFP	Green Fluorescence Protein
HA	hyaluronic acid
HPMA	N-(2-hydroxypropyl) methacrylamide
HTS	high throughput screening
HYAL	hyaluronidase
IFP	interstitial fluid pressure
mRNA	messenger RNA
MALDI-IMS	Matrix-assisted laser desorption imaging – ionization mass spectrometry
MHC I	Multihistocompatibility complex I
MHC II	Multihistocompatibility complex II
MRI	magnetic resonance imaging
MSP	mononuclear phagocyte system
NP	nanoparticle
NIRF	near infrared fluorophore
OI	optical imaging
OMICS	a field of study in biology ending in -omics, such as genomics, proteomics or metabolomics
PD	pharmacodynamics
PE-PEG-TPP	phosphatidylethanolamine polyethylene glycol triphenyl phosphonium
PL-TPP	phospholipid triphenyl phosphonium
PEG	polyethylene glycol
PEI-TPP	polyethylene imine triphenyl phosphonium
PET	positron emission tomography
PLGA-PEG-TPP	poly(lactic-co-glycolic acid)- block – polyethylene glycol) triphenylphosphonium
PIT	photo-immunotherapy
PK	pharmacokinetics
PMN	polymorphonuclear leukocyte

RES	reticuloendothelial system
SB	systems biology
SiNP	silica based nanoparticle
TPGS1000-TPP	tocopherol polyethylene glycol 1000 succinate triphenylphosphonium
STPP	stearyl triphenyl phosphonium
SUPR	super enhanced permeability effect
QSAR	quantitative structure activity relationship
T (see Fig. 1) or Tox	toxicology
TAMs	tumor-associated macrophages
TPP	triphenylphosphonium
TSAS	tumor-specific antigen
VW	Volkmar Weissig

1.1 Introduction

It should be stated upfront that the emphasis of this chapter (and Volume) will be on pharmacokinetics (PK) in animal and human studies if available. The focus is on cancer market since it is the most important; as the cancer interest, no doubt, is the fastest growing component of the US market. The majority of literature concerns with this application. We also stress the emphasis on solid tumors (solid malignancies). The controversy of this field is whether targeted (and non-targeted) nanoparticles are of any benefit in clinical practice and how to push towards the market.

The topic of this volume was in part inspired by a statement by Petrák (2005):

> Future efforts will need to be directed to solve, in practical terms, the following fundamental issues:
>
> - The drug–carrier system (including the drug to be delivered) must avoid nonspecific interactions in the vascular compartment (RES).
> - The system should retain its ability to accumulate at the target site(s) (defined in terms of unique anatomical, physiological or disease conditions) and be in a form capable of acting on its pharmacological activity target.
> - Drugs need to be selected, or rather designed, to have the pharmacokinetic properties compatible with the demands of target-selective drug delivery (especially drug retention at the site of delivery and its ability to access its site of molecular action).

According to a report published by BCC Research, the market value of the worldwide nanomedicine industry was $72.8 billion in 2011. The market is estimated to grow at a CAGR of 12.5 % to reach $130.9 billion by the fiscal year 2016. The market for anti-cancer products was valued at $28 billion in the fiscal year 2011 and is anticipated to reach $46.7 billion by the fiscal year 2016. As indicated by Petrák (personal communication) we should note that 10 % rate of inflation would take the market value from $28 to $46 billion in 5 years (i.e., between 2011 and 2016), hence such numbers effectively mean no growth. We don't' know what the market value is today (i.e., in 2016).

Tremendous efforts are underway worldwide, at the bench and in preclinical research, in order to make the big promise of the nano-revolution a reality. However, there is a low number of trials, which reflects neither the massive investments made in the field of nanomedicine nor the general hype associated with the term "nano". This is supported by finding of Weissig in two papers (Weissig et al. 2014; Weissig and Guzman-Villanueva 2015). We believe (BCC Report above) the true promise of nanoscience for drug development still has to materialize.

Soluble cancer drugs present a different kind of problem. When a soluble cancer drug is injected into the patient, it quickly distributes into all the body tissues so that only a small fraction of the drug actually reaches the tumor. Most of the drug enters normal tissues where it kills normal dividing cells, causing the serious side-effects associated with chemotherapy. Another disadvantage to soluble drugs is their rapid elimination from the body through the kidneys. Nanotechnology may offer a solution.

Unfortunately, nanotechnology promises anything but *miniscule effects*, but most of these visions are hypothetical at this point. Most of the present nanotechnologies may come into fruition in 10–20 years. Accordingly, most pitfalls of molecular manufacturing have not yet been explored, because the benefits remain the dominant focus of researchers. We intend to discuss new approaches that would help to realize "dream" of nanomedicine to help the mankind. It is the insufficient innovation which results in a high failure rate in clinic.

We then question the conventional wisdom of definition of nanomedine. We partly redefine nanoparticulate (NP) delivery vehicles, i.e. as to include term "intracellular uptake", although for some applications (i.e. imaging, there is no need to uptake, just to stay firmly at the site). This is in contrast to previously defined nanomedicine or nanopharmaceutical: According to Rivera et al. (2010) nanopharmaceuticals are defined as "pharmaceuticals engineered on the nanoscale, i.e., pharmaceuticals in which the nanomaterial plays a pivotal therapeutic role or adds additional functionality to the previous compound". And, according to the " Medical Standing Committee of the ESF, nanomedicines result from "the science and technology of diagnosing, treating, and preventing disease and traumatic injury, of relieving pain, and of preserving and improving human health, using molecular tools and molecular knowledge of the human body" (ESF–European Science Foundation 2004). The stress on the internalization is important regardless of specific route out of many possible mechanisms. The rapidly proliferating cross-disciplinary area of endocytosis of nanomedicines is at the interface of biology and material science and may bring the next wave of significant technological breakthrough.

As we will see from the assessment discussed below, we have also serious doubts about both targeted and non-targeted carriers, especially in terms of benefits they provide. To resolve all above problems, *we suggest below the following coverage in this volume*. (Interestingly, recently, some other authors considered similar or some additional impediments to drug delivery – in form of nanomedicine. For example, Blanco et al. (2015) highlighted innovative designs, such as the use of nontraditional nanoparticle geometries for improved vascular dynamics, endosomal escape, and multidrug resistance to overcome clinical translations problems. Likewise,

Stylianopoulos and Jain (2015) and separately Bertrand et al. (2014) reviewed our understanding of therapeutic cancer treatments via nanomedicinal approaches, the latter with help of condensed mathematical formulations, discussing most of the impediments as above with a hope that some of the presently developed nanomedicines might become future's blockblusters).

1.2 Extracellular Matrix Manipulation

We would encourage to develop sufficient cell biology understanding of basic principles of cellular uptake and internalization (Hillaireau and Couvreur 2009) and relate them to material science as a basis for nanoparticle (NP) redesign. There is plethora of possible new designs, based on extracellular matrix molecules. Examples are those based on collagen, hyaluronan, and other ECM components.

One recent example features a balance in size: there is a possibility to choose a nanoparticle that is small enough to escape the leaky blood vessels that surround tumors but large enough to avoid rapid clearance from the blood stream via the kidneys. Balancing these two requirements usually results in using nanoparticles that are indeed small enough to accumulate in the vicinity of tumors, but that are really too large to penetrate deeply enough into tumors to have the maximum therapeutic effect. Jain et al. (2015) developed multilayered, or multistage, nanoparticles that partially dissolve once they accumulate around tumors, leaving behind a payload of nanoparticles a mere one-tenth the size of the original delivery vehicle. The remaining 10-nm-diameter nanoparticles, loaded with anticancer drugs, can then diffuse deeply into a tumor's dense interior. The key to the new nanoparticles is a gelatin material that can serve as a substrate for enzymes that are produced at high levels by tumors. Cancer cells use these enzymes to dissolve the extracellular matrix that surrounds organs, enabling these malignant cells to escape into the bloodstream and colonize sites distant from the primary tumor. The researchers took advantage of this enzyme by embedding tiny nanoparticles within the gelatin core of the larger nanoparticles that they designed to be injected into the blood stream.

For this set of experiments, the investigators loaded 100-nm the gelatin nanoparticles with 10-nm quantum dots. While quantum dots are not likely to be used to deliver drugs to tumors, these nanobeacons produce bright optical signals that can be easily monitored as they are released from the larger nanoparticles. Initial experiments using tumors growing in culture showed that the gelatin-degrading enzymes indeed released quantum dots which were able to diffuse farther and more efficiently than the 100 nm particles into the tumors. Subsequent experiments in tumor-bearing mice confirmed these *in vitro* findings, and as a result, the investigators are now planning to repeat these experiments using drug loaded 10-nm particles in place of the quantum dots they used in this study.

Another approach to facilitate the access of nanoparticles deep into tumors is to disrupt a tumor's ability to form the dense extracellular matrix, made of the protein collagen, which keeps nanoparticles in the outer regions of a tumor. Jain et al. used the widely used high-blood pressure medication Losartan to inhibit collagen synthesis. Human clinical studies have shown that Losartan reduces the incidence of cardiac and renal fibrosis by reducing the synthesis of one particular form of collagen (type I). They reasoned that this same inhibitory effect might lead to easier passage of nanoparticles into the deep recesses of a tumor. Consequently, they observed this effect at doses of the drug that were small enough to leave blood pressure unaffected. Their tests showed that Doxil, the first approved nanoparticulate anticancer agent, was more effective at treating dense, fibrotic tumors, such as pancreatic tumors, growing in mice. They also noted in that because long-term Losartan therapy has proven safe in humans, and because many anticancer agents raise blood pressure, administering Losartan with nanoparticles has the strong possibility of benefitting cancer patients.

In addition, many solid tumors develop extensive fibroses, a result of what is termed the desmoplastic reaction. Desmoplasia leads to a significant increase in the production of extracellular matrix (ECM) proteins, as well as extensive proliferation of myofibroblast-like cells. The result is the formation of a dense and fibrous connective tissue that is composed of multiple ECM components, including collagen types I, III, and IV; fibronectin; laminin; hyaluronan (HA); and the glycoprotein osteonectin [also known as secreted protein, acidic and rich in cysteine (SPARC)]. This fibroinflammatory component of the tumor (sometimes called stroma) contributes to an increase in tumor interstitial fluid pressure, blocking perfusion of anticancer therapies to the tumor. Targeting the components of the stromal compartment, in conjunction with cytotoxic agents directed against tumor cells, is gaining attraction as a potential approach to treating patients and overcoming chemoresistance. Hyaluronidase (HYAL), may have the potential to increase penetration of drugs through the stromal compartment and ultimately into tumor cells. With the clinical availability of recombinant HYAL, prospects for targeting HA in the treatment of cancer are improved. Scodeller et al. (2013) have developed new nanoparticle, employing HYAL immobilized on 250 nm silica nanoparticles (SiNP) maintaining specific activity of the enzyme. They noted that tumor volume reduction with SiNP-immobilized HYAL was significantly enhanced compared to non-immobilized HYAL control. In support of the above, prior the Scodeller paper, Whatcott et al. (2011) reported on employment of HYAL itself to facilitate anticancer drug delivery (not in a nano-form). Increased levels of one ECM component—namely, hyaluronan—leads to reduced elasticity of tumor tissue and increased interstitial fluid pressure. Multiple initial reports showed that the addition of hyaluronidase (HYAL) to chemotherapeutic regimens could greatly improve efficacy. Unfortunately, the bovine HYAL used in those studies is limited therapeutically by immunologic responses to treatment. Newly developed recombinant human HYAL has recently been introduced into clinical trials.

1.3 Extending the Blood Nanoparticle Circulation

We next encourage to modify NPs for long circulating properties in order to minimize the MPS uptake (liver, spleen, bone marrow); there are ways of shielding NPs with pre-treatments and surface modifications (stealth particles and liposomes, employing a variety of PEG length and MW, affecting the receptor availability), and charge reversal (negative NP). Pretreatments include e.g. Intralipid, a nutritional supplement approved by FDA. For example, Liu et al. (2013) employed Intralipid for shielding of MSP (more on update in this Volume). Even better results were obtained by Rodriguez et al. (2013) who developed an immune-shielding technology ("self-peptide" shielding or "active stealth strategy"). Foreign particles and cells are cleared from the body by phagocytes that must also recognize and avoid clearance of "self" cells. The membrane protein CD47 is reportedly a "marker of self" in mice that impedes phagocytosis of self by signaling through the phagocyte receptor CD172a. Minimal "Self" peptides were computationally designed from human CD47 and then synthesized and attached to 160 nm nanobeads – virus-size particles – for intravenous injection into NSG (non-obese diabetic – NOD – severe combined immunodefficient IL2rγ^{null}) mice that express a CD172a variant compatible with hCD47. Self peptides delay macrophage-mediated clearance of nanoparticles, which promotes persistent circulation that delay clearance by the liver and spleen and enhances drug delivery to tumors. Recent report states that some of the above ideas are currently being put to the test in the clinic with initial results from anti-cancer clinical trials hopefully to be reported (Sosale et al. 2015).

Another strategy to prolong the circulation is to attach a biomimetic coating derived from membranes isolated from leukocytes (Parodi et al 2013) or from red blood cells (Hu et al. 2011). Likewise, clinical outcomes are not known.

1.4 Passive and Active Targeting

We will discuss drawbacks of passive (EPR) and active targeting now. Often discussed issue is how nanomedicines access the disease site? Here do we recognize passive or active targeting?

Passive targeting of tumors by nanoparticles takes advantage of their endothelial cell lining. The rapid vascularization of solid tumors results in leaky, defective endothelial cells and impaired lymphatic drainage. Nanoparticles ranging from 10 to 100 nm in size then begin to accumulate within tumors because of their ineffective lymphatic drainage. This results in a phenomenon known as the enhanced permeability and retention effect (EPR). Although accumulation in solid tumors is observed, the cellular uptake by neoplastic cells and the subsequent intracellular drug release have been questioned. The EPR effect is purely size- and geometry-dependent mode of action, however. On the other hand, ligand-targeted NPs (active targeting) may prove beneficial in increasing drug exposure due to increased target

cell uptake and target tissue retention compared to ligand-lacking NP. Ligand-targeted approaches are crucial for molecules that need to localize intracellularly for therapeutic activity but are not capable of crossing cellular membranes, such as nucleic acids.

Targeting antibodies or ligands are often selected because of their high specificity and high affinities towards overexpressed antigens on target cells and their ability to trigger receptor-mediated endocytosis after binding. However, the targeting ligands or antibodies do not influence the biodistribution of the nanoparticle: biodistribution still depends on passive targeting to tissues with a leaky vasculature, and targeting ligands only triggers internalization after extravasation into the tumor. The bio-distribution of targeted and non-targeted NPs is often similar. The targeted nanoparticles will show benefits compared to their non-targeted counterparts if they can freely and directly access their targets (PEG chains often impair the ligand-cell interaction). Crommelin and Florence (2013) marked the drug targeting concept as intrinsically biased which has perhaps contributed to the hype surrounding drug targeting. The ideal scenario is if the drug delivery system can reach only one target organ while sparing all other organs. This is rarely the case as most delivery systems reach other organs often at even higher concentrations than the tissue/organ of interest thus undermining the aim of drug targeting. There are many issues that need to be addressed in drug targeting such as the undesirable side effects that may result due to uptake by off-target organs e.g., liver (in case of transferrin-based targeted systems) and kidney (in case of folate-based targeted systems). Thus a critical assessment is needed. They also defined the following: the first-line targeting that can be achieved at the organ level; second-line targeting can be achieved at the cell level, and the third-order targeting at the organelle level. Toxicity is also an important criterion: toxicity, or rather the lack of is very important; one cannot even do human clinical studies if preclinical toxicity is not acceptable (i.e., a high benefit/risk ratio). The critical factors of NP systems for success in clinical application with regard to complement activation and hypersensitivity reactions in particular against polyethylene glycol – PEG (Lehner et al. 2015) are to be assessed.

Very recently, Kobayashi et al. (2014) have developed a "super-enhanced permeability and retention effect" (SUPR) concept induced by photo-immunotherapy (PIT). Photo-immunotherapy (PIT) is a newly developed therapy involving the injection of a conjugate composed of an armed monoclonal antibody and a near infrared phthalocyanine dye. Because it damages cells immediately adjacent to the tumor vasculature, PIT results in marked increases in vascular permeability leading to 12- to 25-fold enhanced nanoparticle delivery into cancer tissue in animals. Again, this effect should be confirmed in humans.

Very small nanodelivery objects, micellar formulations, feature exceptions in tumor accumulation because of their small size. Micelle carriers selectively accumulate in tumor tissue owing to the EPR effect and directly reach the cancer cells in order to attack them. Alternatively, the formulation spontaneously disintegrates while it is retained within the tumor tissue. Disintegrated ACA-bound unimers with the attached drug-payload or released ACA immediately reach and enter cancer cells to kill the cancer cells (Matsumura 2014; also Osada et al. 2009). ACA is an

optimized block copolymer that is typically functionalized with 6-aminocaproic acid, polyaspartate, polyglutamate, etc. and a drug payload.

Very recent, Etrych et al. (2014) reported on micellar DOX formulation (no size given, possibly very small), without targeting, showed selectivity toward solid tumors in mice: (i) drug accumulation in tumors driven by enhanced permeability and retention (EPR) effect, which results in almost 100 times higher concentration of drug in the solid tumor than in normal tissue, (ii) pH-dependent release of drug from polymer-drug conjugate, which releases free drug more efficiently at a lower pH in tumors. This effect might be explained on the basis of hydrophobicity of the carrier (Maeda 2015). The size is thus also an issue. Eventually, a drug concentration at the site would be of help to know. Advanced cytotoxicity has been observed.

A considerable critique of classical EPR was recently brought up by Nichols and Bae (2014). They noted that clinical outcomes from nano-sized drug delivery systems, however, have indicated that EPR is not as reliable as previously thought. Drug carriers generally fail to provide superior efficacy to free drug systems when tested in clinical trials. A closer look reveals that EPR-dependent drug delivery is complicated by high tumor interstitial fluid pressure (IFP), irregular vascular distribution, poor blood flow inside tumors and perhaps absence of lymphatics in experimental models. Furthermore, the animal tumor models used to study EPR differ from clinical tumors in several key aspects that seem to make EPR more pronounced than in human patients. Khawar et al. (2015) also reviewed the evidence that supports a statement that considerable barriers of tumors via various mechanisms exist, which results in imperfect or inefficient EPR and/or targeting effect. Barua and Mitragotri (2014) review focuses on the current understanding of penetration of NPs through biological barriers. Emphasis is placed on transport barriers.

As a way towards a progress in this area, Wong et al. (2015) presented a mathematical model that provides a quantitative framework to guide preclinical trials of new chemotherapeutic delivery vehicles and ultimately to develop design rules that can increase targeting efficiency and decrease unwanted side effects in normal tissue. Likewise, Stapleton devised a linear mixed effect model and verified it on animals *in vivo*. The intra-tumoral relationship between the tumor microcirculation, elevated IFP, and accumulation of liposomes was investigated through experiments. This was accomplished by evaluation of the tumor microcirculation using dynamic contrast enhanced computed tomography (DCE-CT) and measurement of tumor IFP using a novel image-guided robotic needle placement system connected to the micro-CT scanner. Results have important implications for guiding drug delivery using image-based approaches. Elucidating the factors mediating heterogeneous intra-tumoral delivery of nanoparticles can substantially enhance their use in diagnostic and therapeutic applications. As the tumor imaging by means of CT represents a robust and noinvasive way of imaging, it could be potentially used in a clinical setting.

Very recently, a major development was reported in terms of predicting EPR variability to improve the clinical applications of nanomedicines. Miller et al. (2015) reported that a 30-nm magnetic NP (MNP) in clinical use could predict co-localization of TNPs (therapeutic nanoparticles) by magnetic resonance imaging

(MRI). A central question in nanomedicine is whether imaging could be used to identify patients with higher predisposition to TNP accumulation and, in turn, efficacy of understanding how to best exploit EPR effects for clinical applications, how to design better TNPs, and how to alter key physiologic parameters to maximize distributions to and within tumors. Heterogeneous tumor vascularization is a recognized clinical feature that can be detected using various angiography modalities. To progress further clinically, more human-representative disease models, as patient-derived xenografts, genetically engineered autochthonous mouse models, and larger animals, should be used to study EPR effects in metastatic lesions, as suggested by the authors.

1.5 Differences Between Man and Mice

The predictive value of animal models for a given clinical condition is getting increasingly attention. Models in both large and small animal species have value for pharmacology and toxicology, including the first evaluation of adverse side effects and pharmacological efficacy of innovative disease intervention strategies, as well as the selection in a given therapeutic discovery program. Also, such models are helpful in elucidating pathways in physiological or pathological processes. But, progress in the field has made it increasingly clear that animal models have their limitations regarding translational value (van der Meer et al. 2015).

Still not resolved is a fundamental question why cancer trials in men often fail while performing well in mice. The problem is what is the differences between mice (and other animals) and men when moving to upper scale at translational medicine. Perhaps, it is the vascular and immune systems which are different but no real clues are available.

It looks like that a way out is the identification of molecular abnormalities that are not only critical for the life of cancer—but not normal cells—but are also the dominant or the only molecular abnormality within the tumor. Is it possible to identify a common denominator across all of these abnormalities that is not only critical for the survival of cancer cells but is also not present in normal cells? And to push the envelope even further, is it possible that this common denominator is also present in cancer stem cells, so that if targeted, tumor relapse would be limited as well (Kinnaird 2015).

To make it even more complicated and add more doubts, clinical outcomes from nano-sized drug delivery systems, however, have indicated that EPR is not as reliable as previously thought (Nichols and Bae 2014). The number of publications citing EPR has increased exponentially; this flourish of creativity has largely failed to translate into new clinical therapies. Drug carriers generally fail to provide superior efficacy to free drug systems when tested in clinical trials. A closer look reveals that EPR-dependent drug delivery is complicated by high tumor interstitial fluid pressure (IFP), irregular vascular distribution, and poor blood flow inside tumors, typical for mice (see a subchapter above; Nichols and Bae 2014).