Journal of Controlled Release 161 (2012) 175–187

Contents lists available at SciVerse ScienceDirect

Journal of Controlled Release

journal homepage: www.elsevier.com/locate/jconrel

Review

Drug targeting to tumors: Principles, pitfalls and (pre-) clinical progress

Twan Lammers [a,b,c,*], Fabian Kiessling [a], Wim E. Hennink [b], Gert Storm [b,c]

[a] Department of Experimental Molecular Imaging, RWTH – Aachen University, Helmholtz Institute for Biomedical Engineering, Aachen, Germany
[b] Department of Pharmaceutics, Utrecht Institute of Pharmaceutical Sciences, Utrecht University, Utrecht, The Netherlands
[c] Department of Targeted Therapeutics, University of Twente, Enschede, The Netherlands

ARTICLE INFO

Article history:
Received 23 July 2011
Accepted 8 September 2011
Available online 16 September 2011

Keywords:
Cancer
Nanomedicine
Drug delivery
Tumor targeting
EPR
Theranostics

ABSTRACT

Many different systems and strategies have been evaluated for drug targeting to tumors over the years. Routinely used systems include liposomes, polymers, micelles, nanoparticles and antibodies, and examples of strategies are passive drug targeting, active drug targeting to cancer cells, active drug targeting to endothelial cells and triggered drug delivery. Significant progress has been made in this area of research both at the preclinical and at the clinical level, and a number of (primarily passively tumor-targeted) nanomedicine formulations have been approved for clinical use. Significant progress has also been made with regard to better understanding the (patho-) physiological principles of drug targeting to tumors. This has led to the identification of several important pitfalls in tumor-targeted drug delivery, including I) overinterpretation of the EPR effect; II) poor tumor and tissue penetration of nanomedicines; III) misunderstanding of the potential usefulness of active drug targeting; IV) irrational formulation design, based on materials which are too complex and not broadly applicable; V) insufficient incorporation of nanomedicine formulations in clinically relevant combination regimens; VI) negligence of the notion that the highest medical need relates to metastasis, and not to solid tumor treatment; VII) insufficient integration of non-invasive imaging techniques and theranostics, which could be used to personalize nanomedicine-based therapeutic interventions; and VIII) lack of (efficacy analyses in) proper animal models, which are physiologically more relevant and more predictive for the clinical situation. These insights strongly suggest that besides making ever more nanomedicine formulations, future efforts should also address some of the conceptual drawbacks of drug targeting to tumors, and that strategies should be developed to overcome these shortcomings.

Contents

* Corresponding author at: Department of Experimental Molecular Imaging, RWTH - Aachen University, Helmholtz Institute for Biomedical Engineering, Pauwelsstrasse 30, 52074 Aachen, Germany.
E-mail addresses: tlammers@ukaachen.de, t.lammers@uu.nl (T. Lammers).

0168-3659/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jconrel.2011.09.063

1. Introduction

In the past few decades, significant progress has been made in understanding the molecular principles of many different diseases. In the case of cancer, these improved insights into the genetic and (patho-) physiological processes contributing to malignant transformation and tumorigenesis have resulted in the development of several novel (classes of) chemotherapeutic drugs. Such 'molecularly targeted therapeutics', like the growth factor receptor inhibitor Herceptin, the proteasome inhibitor Velcade, the histone deacetylase inhibitor Vorinostat and the antiangiogenic agent Avastin, more selectively interfere with certain 'hallmarks of cancer' [1,2], like with the overexpression of growth factors and growth factor receptors, with the altered balance between apoptosis and anti-apoptosis, with the numerous genetic and epigenetic changes that are present in cancer cells, and with the development of a dense vascular network, needed to provide tumors with oxygen and nutrients. By means of their pharmacologically and/or physiologically more optimal mechanism(s) of action, 'molecularly targeted therapeutics' have been shown to be able to more preferentially kill cancer cells, both in vitro and in vivo, and to improve the balance between the efficacy and the toxicity of systemic anticancer therapy [3–5].

An important but often neglected property that such second-generation chemotherapeutics share with their first generation DNA-damaging counterparts, is that upon intravenous administration, their pharmacokinetics and their tissue distribution often are

Table 1
Barriers limiting the delivery of i.v. applied anticancer agents to tumors.

Barriers to drug delivery to tumors			
Anatomical barriers	Physiological barriers	Chemical barriers	Clinical barriers
Vascular endothelium	Renal filtration	Low solubility	Low efficacy
Perivascular space	Hepatic degradation	Low stability	High toxicity
Cellular membrane	High tumor cell density	Low molecular weight	Need for hospitalization
Nuclear membrane	High interstitial fluid pressure	Large volume of distribution	Frequent administration
Blood brain barrier	Drug efflux pumps	Charge interactions	Low cost-effectiveness

Note that several barriers are inter-related, and that not all barriers apply to all types of (chemo-) therapeutic agents. Table adapted, with permission, from [76].

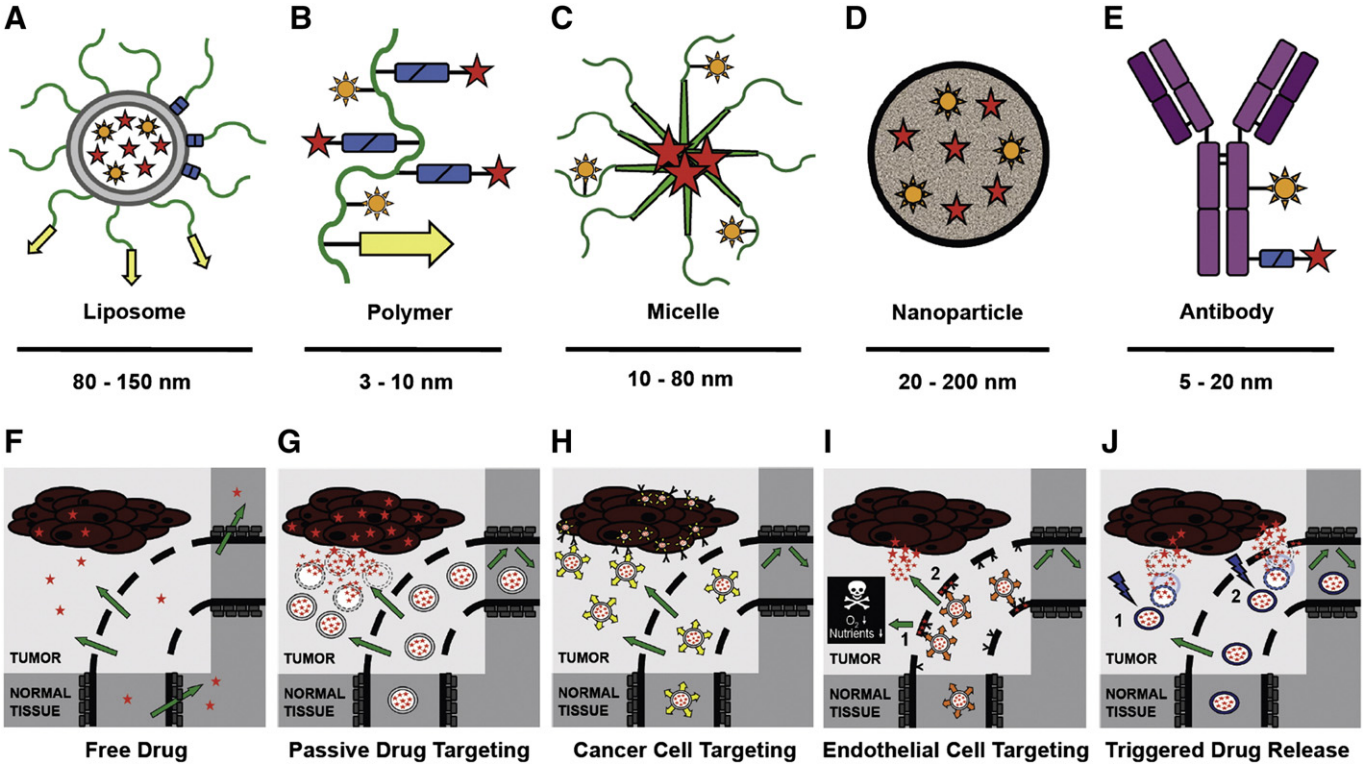

Fig. 1. Systems and strategies used for drug targeting to tumors. A–E: Drug targeting systems. Liposomes and liposomal bilayers are depicted in gray, polymers and polymer-coatings in green, linkers allowing for drug release and for sheddable stealth coatings in blue (rectangles), targeting ligands in yellow (arrows), antibodies and antibody fragment in purple, imaging agents to monitor biodistribution and target site accumulation in orange (suns), and conjugated or entrapped (chemo-) therapeutic agents in red (stars). F–J: Drug targeting strategies. F: Upon the i.v. injection of a low-molecular-weight chemotherapeutic agent, which is often rapidly cleared from the blood, only low levels of the drug accumulate in tumors and in tumor cells, while its localization to normal organs and tissues can be relatively high. G: Upon the implementation of a passively targeted drug delivery system, by means of the EPR effect, the accumulation of the active agent in tumors and in tumor cells can be increased substantially, while its localization to healthy tissues can be attenuated. H: Active drug targeting to internalization-prone cell surface receptors (over-) expressed by cancer cells generally intends to improve the cellular uptake of nanomedicine formulations, and is particularly useful for the intracellular delivery of otherwise poorly internalized macromolecular drugs, such as DNA and siRNA. I: Active drug targeting to receptors (over-) expressed by angiogenic endothelial cells on the one hand aims to increase drug delivery to tumor endothelium, thereby eradicating tumor blood vessels and depriving tumor cells of oxygen and nutrients (I-1). On the other hand, reasoning that tumor endothelial cells are continuously exposed to long-circulating nanomedicines, endothelial cell targeting can likely also be employed to improve the overal accumulation of chemotherapeutic agents in tumors (I-2). J: Stimuli-sensitive nanomedicines, like Thermodox, can be activated (i.e. induced to release their contents) by externally applied physical triggers, like hyperthermia, ultrasound, magnetic fields and light. This can be done either after accumulation at the target site (J-1), or while circulating in the tumor vasculature (J-2). Images adapted, with permission, from [10,42].

far from optimal. Because of their low molecular weight, for instance, the majority of routinely used anticancer agents are rapidly cleared from systemic circulation (e.g. by means of renal filtration), and they do not accumulate well in tumors and in tumor cells. Also, because of their small size and their (generally) high hydrophobicity, drug molecules often have a large volume of distribution, and they tend to accumulate in and cause toxicity towards many different healthy tissues. In addition to this, as outlined in Table 1, a large number of other barriers need to be overcome before an i.v. administered anticancer agent can elicit antitumor efficacy, related e.g. to hepatic and enzymatic degradation, to the high interstitial fluid pressure that is typical of tumors, to cellular and nuclear membranes, and to the presence of drug efflux pumps.

To assist i.v. applied anticancer agents in overcoming such barriers, and to improve their pharmacokinetic profile and their target site accumulation, a large number of nanomedicine formulations have been designed and evaluated over the years. Nanomedicines are submicrometer-sized carrier materials which intend to improve the biodistribution of systemically administered (chemo-) therapeutic drugs. By delivering pharmacologically active agents more selectively to pathological sites ('site-specific drug delivery'), and/or by guiding them away from potentially endangered healthy tissues ('site-avoidance drug delivery'), nanomedicine formulations aim to improve the balance between the efficacy and the toxicity of systemic (chemo-) therapeutic interventions [6–11].

Various different concepts have been envisioned for nanomedicine-mediated drug targeting to tumors over the years, including e.g. passive drug targeting, active targeting to cancer cells, active targeting to endothelial cells and triggered drug delivery (using stimuli-responsive carrier materials). A huge number of preclinical and a significant number of clinical studies have provided insights into the validity of these approaches, and it has been realized that though they might hold true in several cases, there are also situations in which this is not the case. This is due to a series of misconceptions, misunderstandings and pitfalls, which together limit the bench-to-bedside translation of tumor-targeted nanomedicines, as well as their clinical benefit and their use in large numbers of patients.

In the present perspective, we briefly describe the basic principles of drug targeting to tumors, summarize the (pre-) clinical progress made with regard to tumor-targeted nanomedicines, address some of the pitfalls identified along the way, and propose strategies to overcome these shortcomings.

2. Principles of drug targeting to tumors

2.1. Passive targeting

Many different types of nanomedicines have been designed and evaluated for drug targeting to tumors. Prototypic examples of nanomedicine formulations are liposomes, polymers, micelles, nanoparticles and antibodies (Fig. 1A–E). The former four nanomedicines primarily aim to improve the circulation time of the conjugated or entrapped (chemo-) therapeutic drug and, by doing so, to enable it to exploit the pathophysiological fact that solid tumors tend to present with a tortuous and poorly differentiated vasculature, that in contrast to the vasculature in healthy tissues, allows for the extravasation of carrier materials with sizes of up to several hundreds of nanometers (Fig. 1G). Together with the fact that solid tumors tend to lack functional lymphatics, and therefore are unable to eliminate extravasated nanomaterials, this increase in vascular leakiness allows long-circulating nanomedicines to accumulate in tumors over time,

Table 2
Examples of clinically used tumor-targeted nanomedicines.

	Name	Formulation	Indication	Status
Lipoosomes	Myocet	Liposomal doxorubicin	Breast cancer	Approved
	Daunoxome	Liposomal daunorubicin	Kaposi Sarcoma	Approved
	Depocyt	Liposomal cytarabine	Lymphomatus meningitis, leukemia, glioblastoma	Approved[a]
	Doxil/Caelyx	PEGylated liposomal doxorubicin	Breast cancer, ovarian cancer, multiple myeloma, Kaposi sarcoma	Approved
	Thermodox	Temperature-sensitive PEGylated liposomal doxorubicin	Liver cancer, breast cancer	Phase III
	NL CPT-11	PEGylated liposomal irinotecan	Glioma	Phase I
Polymers	Oncaspar	PEG-L-Asparaginase	Leukemia	Approved
	Opaxio	PGA-Paclitaxel	Lung cancer, ovarian cancer	Phase III
	PegAsys/PegIntron	PEG-IFNα2a/-IFNα2b	Melanoma, leukemia	Phase II
	PK1	PHPMA-doxorubicin	Breast cancer, lung cancer, colorectal cancer	Phase II
	ProLindac	PHPMA-oxaliplatin	Ovarian cancer	Phase II
	XMT-1001	Fleximer-bound CPT	Gastric cancer, lung cancer	Phase I
Micelles	Genexol-PM	Micellar paclitaxel	Breast cancer, lung cancer, ovarian cancer	Approved[b]
	Paclical	Micellar paclitaxel	Ovarian cancer	Phase III
	NK105	Micellar paclitaxel	Gastric cancer	Phase II
	NK911	Micellar doxorubicin	Various solid malignancies	Phase II
	NC-6004	Micellar cisplatin	Pancreatic cancer	Phase I/II
	NC-4016	Micellar oxaliplatin	Various solid malignancies	Phase I
Nanoparticles	Abraxane	Albumin-based nanoparticle containing paclitaxel	Breast cancer	Approved
	Docetaxel-PNP	Various solid malignancies	Various solid malignancies	Phase I
	CALAA-01	Cyclodextrin-based nanoparticle containing anti-RRM2 siRNA	Various solid malignancies	Phase I
	Atu027	Lipid-based nanoparticle containing anti-PKN3 siRNA	Various solid malignancies	Phase I
	C-VISA-BikDD	Lipid-based nanoparticle containing BikDD plasmid DNA	Pancreatic cancer	Phase I
	Rexin-G	Retrovirus-based nanoparticle containing dnG1 plasmid DNA	Various solid malignancies	Phase I
Antibodies	Ontak	CD25-targeted diphteria toxin-IL2 fusion protein	T-cell lymphoma	Approved
	Mylotarg	CD33-targeted ozogamycin-gemtuzumab	Leukemia	Approved[c]
	Zevalin	CD20-targeted yttrium-90-ibritumomab tiuxetan	Non-Hodgkin lymphoma	Approved
	Bexxar	CD20-targeted iodine-131-tositumomab	Non-Hodgkin lymphoma	Approved
	SGN-35	CD30-targeted brentuximab vedotin	Non-Hodgkin lymphoma	Phase III
	AGS-5ME	SLC44A4-targeted monomethyl auristatin E	Prostate cancer, pancreatic cancer	Phase I

[a] Approved for lymphomatus meningitis; in Phase III for leukemia; and in Phase I/II for glioblastoma.
[b] Approved in Korea; in Phase II in US and Russia.
[c] Withdrawn in 2010.

by means of a mechanism known as the enhanced permeability and retention (EPR) effect [12,13]. The exploitation of the EPR effect is arguably the most important strategy for improving the delivery of low-molecular-weight chemotherapeutic agents to tumors, and because of the fact that it essentially only relies on the pathophysiological properties of the target tissue, it is generally referred to as 'passive drug targeting'.

In spite of several conceptual drawbacks related to extravasation and penetration (see below; Sections 3.1 and 3.2), the vast majority of nanomedicines developed for drug targeting to tumors rely on the EPR effect. These primarily include long-circulating liposomes, polymers and micelles. Examples of passively targeted nanomedicines approved for clinical use are Myocet (non-PEGylated liposomal doxorubicin), Doxil (Caelyx in Europe; PEGylated liposomal doxorubicin), Daunoxome (non-PEGylated liposomal daunorubicin), Abraxane (albumin-based pacitaxel) and Genexol-PM (paclitaxel-containing polymeric micelles; pre-approved in Korea). Several additional passively tumor-targeted nanomedicines are currently in clinical trials (see Table 2), and a large number of other ones are in early- and late-stage preclinical development [6–11].

2.2. Active targeting to cancer cells

As opposed to passive drug targeting, 'active drug targeting' relies on the use of targeting ligands, like antibodies and peptides, which specifically bind to receptor structures (over-) expressed at the target site [10,14,15]. Active drug targeting is generally implemented to improve target cell recognition and target cell uptake, and not to improve overall tumor accumulation. Examples of targeting ligands routinely used for actively targeting nanomedicine formulations to tumor cells are folate [16], transferrin [17] and galactosamine [18]. To date, however, in spite of significant advances made at the preclinical level with regard to active targeting, only antibody-based nanomedicines, such as Zevalin, Mylotarg, Ontak and Bexxar have been approved for clinical use (see Table 2).

For several reasons (as will be outlined in more detail below; see Section 3.3), no actively targeted liposomes, polymers, micelles and nanoparticles have thus far been approved for clinical use, and only very few are in clinical trials. Among those which are in clinical trials are formulations which really rely on improving cellular uptake for conferring therapeutic efficacy. A prototypic example of this is CALAA-01, i.e. a transferrin receptor-targeted cyclodextrin-based polymeric nanoparticle containing siRNA, which itself is unable to enter cells and which needs to be delivered into the cytoplasm of cancer cells to exert antitumor effects (see Table 2).

The observation that actively targeted 'classical' nanomedicines, such as liposomes, polymers and micelles, have thus far largely failed to demonstrate benefit at the (pre-) clinical level can likely be mostly attributed to the fact that after leaving the highly leaky tumor vasculature, there are quite a number of anatomical and physiological barriers that need to be overcome before antibody- or peptide-targeted formulations can bind to (and enter) cancer cells. These include the presence of pericyte-, smooth muscle cell- and fibroblast-based cell layers between endothelial and tumor cells, the high cellular density within solid malignancies, and the high interstitial fluid pressure that is typical of tumors [11,19]. Therefore, and also because of the binding-site barrier [20], which further limits the penetration of actively targeted nanomedicines into the tumor interstitium, actively targeted nanomedicines tend to have problems finding their target cells, and they generally fail to demonstrate an advantage over passively targeted formulations.

2.3. Active targeting to endothelial cells

To overcome (some of) the abovementioned shortcomings with regard to active drug targeting – i.e. those related to the presence of several additional cell layers between endothelial and tumor cells, to the high tumor cellular density and to the high interstitial fluid pressure in tumors – a number of different endothelial cell-targeted nanomedicine formulations have been designed and evaluated over the years (Fig. 1H). Ligands used to target drugs and/or drug delivery systems to tumor blood vessels include the antibody fragment L19, which uses the EDB domain of the oncofetal protein fibronectin to home to angiogenic vasculature [21,22], and several cyclic and linear derivatives of the oligopeptides RGD and NGR, which bind to angiogenic endothelium through the integrins $\alpha 2b\beta 3$, $\alpha v\beta 3$ and $\alpha 5\beta 1$, and through aminopeptidase-N (CD13), respectively [23–26]. Since tumor vasculature-targeted nanomedicines do not depend on extravasation and penetration across pericyte-, smooth muscle cell- and/or fibroblast-based cell layers, since they encounter their target receptors much more frequently than do cancer cell-targeted nanomedicines (especially if they circulate for prolonged periods of time), and since they do not suffer from the high tumor cell density and the high interstitial fluid pressure that unfavorably affect cancer cell-targeted nanomedicines, it is expected that in general (NB: excluding e.g. nucleic acid-containing formulations), endothelial cell-targeted nanomedicines possess significantly more potential for improving antitumor efficacy than do cancer cell-targeted nanomedicines. This is not only because they are more likely to find, bind and kill their target cells (i.e. endothelial cells; thereby depriving tumors of oxygen and nutrients; Fig. 1H-1), but also because they can be designed to release their contents within the tumor vasculature upon binding to tumor blood vessels, thereby enabling low-molecular-weight drugs to penetrate deep into the tumor interstitium (Fig. 1H-2). Based on these notions, on the significant amount of preclinical evidence that has been obtained with regard to active targeting to tumor blood vessels [21–26], and on the promising findings that have recently been reported in phase I trials for L19-targeted Interleukin-2 [27] and RGD-targeted adenoviruses [28,29], it is expected that the future will see more and more endothelial cell-targeted nanomedicines entering and progressing through clinical trials.

2.4. Triggered drug delivery

Other nanomedicine formulations which are expected to gain more and more momentum in the years to come are systems which can be triggered to release their contents upon exposure to external stimuli, such as heat, light, ultrasound and magnetic fields. Such stimuli-responsive nanomedicines, like Thermodox (i.e. temperature-sensitive PEGylated liposomes containing doxorubicin), in principle hold significant clinical potential, since they are designed to only release the conjugated or entrapped chemotherapeutic drug upon applying locoregionally confined triggers, either upon EPR-mediated passive tumor accumulation (Fig. 1I-1), or already during circulation (Fig. 1I-2), thereby maximizing drug release at the pathological site, while preventing damage to potentially endangered healthy tissues. The downside of such formulations is that they are quite difficult to prepare, at least in such a way that they are really specific with regard to stimuli-responsive drug release; it is often observed that they either already release significant amounts of drug without actually being triggered, or they appear so stable that the triggering conditions required to induce drug release are so severe that they are hardly conformable with physiological processes (i.e. the stimuli themselves become toxic). To overcome these shortcomings, many different efforts are currently being undertaken, both at the academic and at the industrial level. These not only aim to improve the stimuli-responsiveness of tumor-targeted nanomedicines, but also to develop more suitable hardware tools to administer external stimuli more effectively and more selectively to the target tissue. Examples of the former for instance are liposomes and micelles with an optimized composition for heat-triggered drug release at temperatures close to 40 °C. Examples of the latter include fiberoptic catheters, which enable light-exposure in deep-seated tissues, and magnetic resonance-guided high-intensity focused ultrasound, which at the same generates, quantifies and auto-controls heating in deep-seated tissues [30–36].

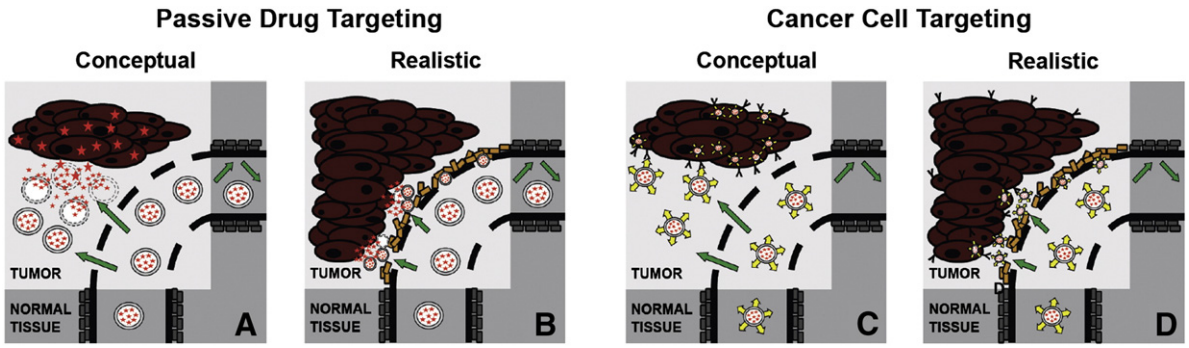

Fig. 2. Conceptual and realistic models for passive and active drug targeting. A–B: In passive drug targeting, it is often mistakenly assumed that all tumor blood vessels are leaky, and that all tumors possess leaky blood vessels. This might be the case in rapidly growing tumor models in rodents, but is definitely not the case in humans, where substantially enhanced vascular leakiness is observed only in certain specific tumors (e.g. in Kaposi sarcoma), and where only certain parts of tumors are hyperpermeable. C–D: Active drug targeting to cancer cells if often mistakenly assumed to be able to increase overall tumor accumulation. This, however, cannot be the case, since nanomedicines enter the tumor interstitium via passive extravasation. After this, especially in physiologically relevant (i.e. slowly growing; comparable to the clinical situation) tumors, they need to cross several pericyte-, smooth muscle- and fibroblast-based cell layers before they are able to bind to cancer cells. Furthermore, even if they are able to reach cancer cell-containing compartments, their penetration deep (−er) into the tumor is limited by the binding-site barrier. Consequently, active cancer cell targeting is considered to be useful only for improving cellular uptake, as well as for targeting certain specific cell types within a solid tumor.

3. Pitfalls in drug targeting to tumors

3.1. Extravasation and the EPR effect

One of the major pitfalls in the field of tumor-targeted drug delivery relates to the fact that the EPR effect is often overrated and/or misinterpreted. Drug delivery scientists generally have an incomplete knowledge of tumor biology and of the anatomical and (patho-) physiological properties of tumors, and therefore tend to neglect the fact that the EPR effect is a highly heterogeneous phenomenon, which varies substantially from tumor model to tumor model, as well as from patient to patient [11,37]. Moreover, even within a single tumor, there are huge differences with regard to vascular permeability, and in many cases, there are parts in which particles as large as 200 nm are able to extravasate, whereas in (the vast majority of) other parts, not even molecules of the size of albumin, i.e. 3–4 nm, are able to enter the interstitium (see Fig. 2A–B). This is because in these areas, either the endothelial lining is intact, or because vascular leakiness is compromised by the presence of a dense peri-vascular lining, constituted e.g. of pericytes, smooth muscle cells and/or fibroblasts.

Apart from this intra- and inter-tumor- and intra- and inter-patient-variability, another important aspect to keep in mind in this regard is that research in animal models, especially in some of the most frequently used animal models (see Section 3.8), can lead to an overestimation of the potential usefulness of passively targeted nanomedicine formulations. This is due to the fact that the EPR effect is undoubtedly much larger in tumors in animal models than in patients, simply because most rodent tumors grow much faster. If a subcutaneously inoculated tumor in a mouse grows to 1 cm (~0.5 g) within 2–4 weeks, this would compare to a ~20 cm and ~1–2 kg tumor in humans, which would take years, and not weeks, to develop. Because of this rapid growth, blood vessels in mouse tumors generally do not develop properly, and they consequently tend to be much more leaky than their human counterparts. This is exemplified by Fig. 3A, in which the tumor accumulation of ~5 nm-sized radiolabeled HPMA copolymers is shown in two different rat tumor models, i.e. in Dunning AT1 and Dunning H prostate carcinomas [38]. The former grow to 1 cm in diameter within 2 weeks, their blood vessels (in red) therefore are poorly differentiated and hardly covered with pericytes (in green), and they consequently show a relatively high degree of EPR-mediated drug targeting (up to 0.5% of the injected dose per gram tumor tissue). The latter, on the other hand, take more than a year to grow to a size of 1 cm, their blood vessels therefore are properly differentiated and densely covered with pericytes and/or smooth muscle cells, and they consequently accumulate radiolabeled HPMA copolymers much less effectively (on average only 0.2% ID per gram tumor tissue).

In spite of the above notion, there are certain human tumors which are very leaky, e.g. because they express high levels of VEGF (which was initially identified as VPF, i.e. vascular permeability factor [39]), and simply also because they are very well-vascularized (thereby increasing the statistical chance of some of them being very leaky). A prototypic example of this is Kaposi sarcoma, which is known to contain many (and many leaky) blood vessels, and which therefore responds relatively well to treatment with passively tumor-targeted chemotherapeutic interventions. This is exemplified by the fact that Doxil works quite well in Kaposi sarcoma: in comparison to the formerly standard combination regimen ABV, i.e. adriamycin (doxorubicin), bleomycin and vincristine, Doxil monotherapy significantly improved response rates, from 25% to 46% [40].

There are several ways to address this shortcoming related to improper extravasation and highly heterogeneous EPR. These for instance include the use of contrast agent-labeled nanomedicines (i.e. theranostics) and appropriate imaging techniques to monitor tumor accumulation, and to thereby preselect patients [41–43]. The obvious aim of such strategies is to only treat patients who show a relatively high level of EPR-mediated drug targeting. In addition, pharmacologically active agents can be used to enhance extravasation (and penetration; see Section 3.2). Prominent examples of such agents are inflammatory mediators, such as tumor necrosis factor-α (TNF-α) and histamine, which have both been shown to be able to improve the extravasation and the penetration of nanomedicines. Both, however, tend to be relatively poorly tolerated when administered systemically, and therefore advanced locoregional setups, such as isolated-limb-perfusion, are needed to exploit their beneficial effects. In addition, they can be given at very low doses, just high enough to permeate tumor blood vessels, but not causing any systemic side effects. If such setups are available and achievable, the combination of extravasation-enhancing pretreatment with nanomedicine treatment can lead to large increases in therapeutic efficacy, as demonstrated e.g. by the pioneering work of Ten Hagen and colleagues [44,45] and Seymour and colleagues [46].

3.2. Penetration

A second important pitfall relates to the (dis-) ability of nanomedicines to properly penetrate tumors. Upon leaving tumor blood vessels, extravasated nanoparticles and macromolecules need to penetrate into and distribute across the interstitium, to reach as many cancer cells as possible. Since they are much larger than

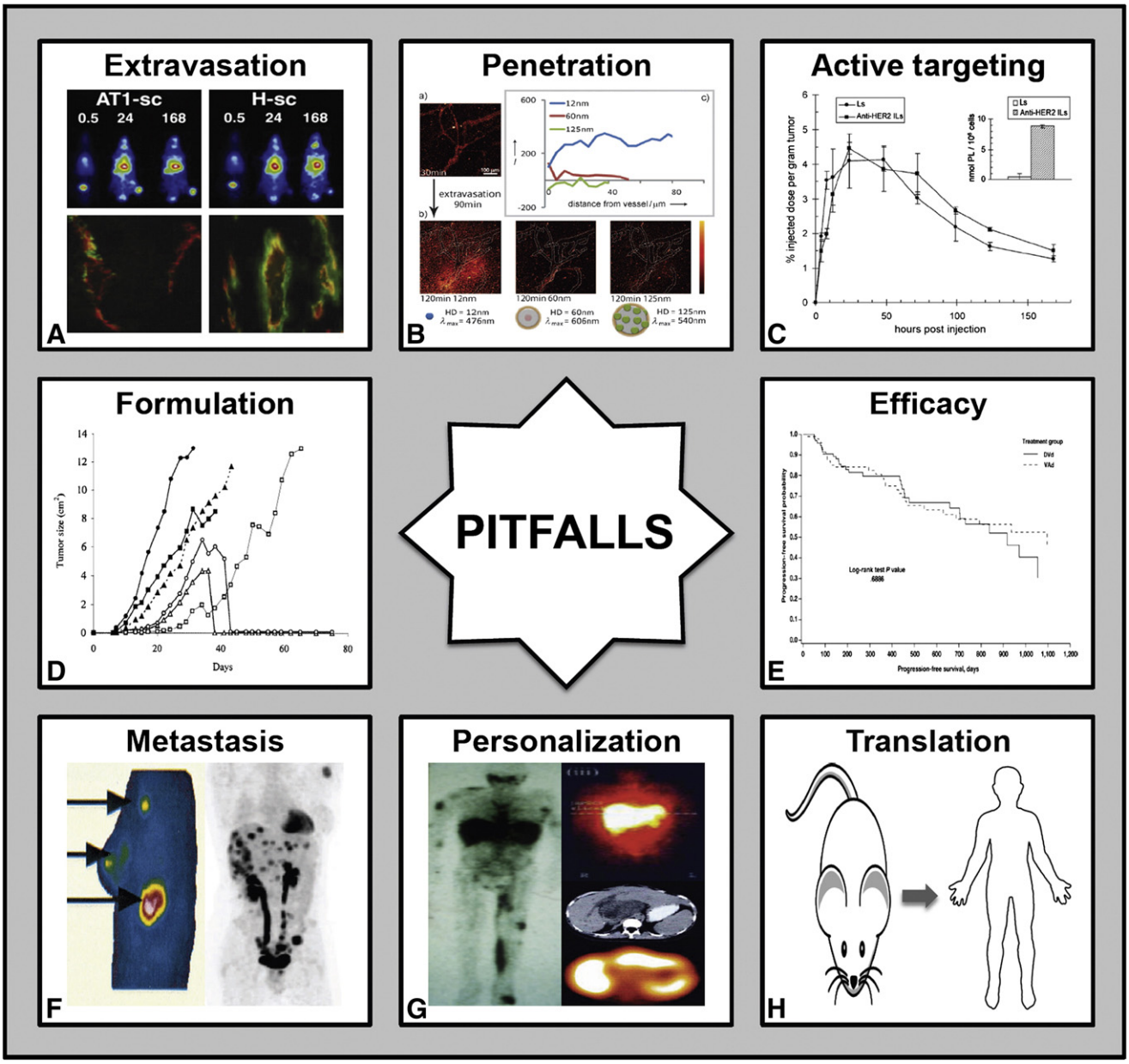

Fig. 3. Pitfalls in drug targeting to tumors. A: The enhanced permeability and retention (EPR) effect is often overrated, and differs substantially between rapidly growing rodent tumors, slowly growing rodent tumors and human tumors. The image shows the accumulation of a radiolabeled 31 kDa HPMA copolymer in Dunning AT1 tumors (which grow to 1 cm in diameter in 2 weeks, have a low degree of pericyte coverage (green) around blood vessels (red) and consequently are quite leaky) and Dunning H tumors (which take more than 1 year to reach 1 cm in diameter, have significant pericyte coverage and consequently are much less leaky). Tumors were implanted in the right hind limbs of the rats. B: Depending on their size, nanomedicines penetrate more or less well into tumors, The image shows the penetration of 12, 60 and 125 nm-sized quantum dots co-injected into the same tumor-bearing mouse 2 h after i.v. injection. The upper left image depicts the presence of the particles within the vasculature at 30 min p.i., and was used to visualize the structures of the tumor blood vessels. C: Active cancer cell targeting is often erroneously assumed to lead to improved tumor accumulation. Because nanomedicine formulations initially accumulate in tumors via EPR, and because they need to penetrate several cell layers before being able to bind to cancer cells, this often is not the case. The image exemplifies this for Her2-targeted liposomes, which as compared to untargeted liposomes, are taken up much more effectively in vitro (top left panel), but do not achieve increased tumor concentrations in vivo (large panel). D: Proper formulation design is critically important to achieve antitumor efficacy in vivo and in patients. The image shows the in vivo efficacy of HPMA copolymers carrying doxorubicin via enzymatically cleavable GFLG linkers (black triangels) and pH-responsive hydrazone linkers (all open symbols) in mice bearing EL4 T cell lymphoma tumors. The efficacy of free doxorubicin is depicted by black squares. E: As opposed to in animal models, in patients, nanomedicine formulations often fail to demonstrate significant therapeutic benefit. They are generally much better tolerated, and tend to have less (and other) side effects, but their ability to improve response rates and survival times is limited. This is exemplified by a Kaplan–Meier plot showing overall survival in patients suffering from multiple myeloma upon treatment with PEGylated liposomal doxorubicin, vincristine and dexamethasone (solid line) versus free doxorubicin, vincristine and dexamethasone (dashed line). F: Nanomedicines are generally designed to target and treat solid tumors. Clinically, however, patients with locally confined tumors can often be curatively treated with surgery and/or radiotherapy, and chemotherapy is only given in an adjuvant setting, to prevent and treat metastasis. Therefore, efforts should also be invested to make nanomedicines (more) effective against metastatic disease. The left image depicts the accumulation of radiolabeled liposomes in a primary breast tumor (middle arrow), in a metastatic lymph node (top arrow) and in the spleen (lower arrow). The right image depicts a patient suffering from a heavily metastasized ovarian carcinoma. G: Only very few efforts have been invested to personalize nanomedicine treatments, whereas this in principle is easily possible. The left image shows a whole-body scintigraphic image (abdominal view) of a Kaposi sarcoma patient with a primary tumor in his lower left leg and several metastatic lesions in his right shoulder region upon the i.v. injection of radiolabeled liposomes. This patient clearly is a good candidate for Doxil treatment. The top image on the right shows highly effective liver targeting in a patient with hepatocellular carcinoma using a hepatocyte-targeted HPMA copolymer carrying doxorubicin. The lower images on the right depict an anatomical CT scan and a functional scintigraphic scan of the same patient, showing that this actively targeted polymer therapeutic only accumulated in healthy liver tissue, and not in the large (dark) tumor mass in the center of the images. On this basis, it could have been predicted that this patient would not respond well to this particular intervention. H: More time and effort should be invested in selecting and generating animal models which are physiologically and clinically more relevant, and able to more confidently predict treatment efficacy in patients. Oftentimes, for practical reasons, rapidly growing subcutaneous tumor models are used, but these do not compare well to the tumors and the pathological conditions nanomedicine formulations are confronted with in the clinic. Images adapted, with permission, from [18,38,46,60,66,100].

conventional (chemo-) therapeutic drugs, however, their penetration is severely hampered, and due to the high tumor cell density and the high interstitial fluid pressure, they often do not cross more than one or two cell layers. This has recently been elegantly exemplified by Bawendi, Jain and colleagues, who used intravital microscopy to show that upon i.v. co-injecting three differently sized quantum dots (12, 60 and 125 nm), only the 12 nm-sized particles were able to properly penetrate, whereas the two larger particles clustered in peri-vascular regions (Fig. 3B) [47]. Similar findings have been reported by Dreher and colleagues, who used five differently sized FITC-labeled dextrans (3.3, 10, 40, 70 and 2000 kDa), and who showed that both the extravasation and the penetration of macromolecules with sizes of up to 70 kDa (i.e. a hydrodynamic diameter of ~6 nm) was reasonable, whereas that of 2000 kDa dextran (~50 nm) was very poor [48]. These insights strongly suggest that attempts should be made to try to tailor the size of nanomedicines to one that enables long-circulation properties, but that at the same time also allows for proper extravasation and penetration.

There are several ways to overcome this penetration barrier. These for instance include the use of inherently unstable or stimuli-sensitive nanomedicines (see above; Section 2.4), which either already in the tumor (micro-) circulation, or relatively fast upon extravasation, release their contents in the peri-vascular space, thereby setting free their low-molecular-weight payload, which can then penetrate deeply into the tumor. In addition, pharmacological treatments can be implemented, including besides the abovementioned extravasation- and penetration-enhancing inflammatory mediators TNF-α and histamine, e.g. also inhibitors of fibrosis and matrix-degrading enzymes. Regarding the former, Miyazono, Kataoka and colleagues have recently shown that a low dose of a transforming growth factor-β (TGF-β) inhibitor, which reduces fibrosis in the tumor microenvironment and the pericyte coverage of tumor blood vessels, can be used to substantially enhance both the extravasation and the penetration of both liposomal and micellar doxorubicin [49], as well as of iron oxide nanoparticles [50]. They demonstrated this in two xenograft models of pancreas cancer, which is notorious for its poor penetration, and also convincingly showed that co-treatment with the TGF-β inhibitor substantially improved the antitumor efficacy of micellar doxorubicin [49]. Regarding the latter, i.e. matrix-modifying agents, Jain and colleagues have shown that by pre-treating tumors with the enzyme collagenase (which degrades collagen; a major matrix component in tumors), caused a 2–3 fold increase in the penetration of antibodies and viral nanoparticles [51–53]. Similarly, pre-treatment of tumors with the hormone relaxin, which changes the structure of collagen, resulted in a 2–3-fold increase in the delivery of antibodies and macromolecules [51,54]. An alternative non-pharmacological means to improve tumor penetration might be based on combinations with radiotherapy, which is known to be able to increase both the extravasation (via inducing the expression of VEGF and FGF) and the penetration (via lowering of the interstitial fluid pressure) of nanomedicines [38,55–58]. A final elegant approach to improve the penetration and the intratumoral distribution of drug delivery systems relies on the development of ~100 nm-sized 'multistage' nanoparticles, which initially profit from their relatively large size to ensure prolonged circulation times, but which upon extravasation are degraded to ~10 nm-sized 'sub-particles' by tumor-associated proteases, such as matrix metalloproteinases, thereby enabling enhanced tumor penetration and improved intratumoral distribution [59].

3.3. Active targeting

A third important pitfall relates to the overestimation and/or the misinterpretation of the potential usefulness of active drug targeting. In many papers in which new tumor-targeted nanomedicines are developed and tested, and in which the in vivo performance of the systems is not as good as initially envisioned, it is stated that in follow-up experiments, ligands will be attached to the surface of the carrier materials, to improve their biodistribution, tumor accumulation and therapeutic efficacy. As exemplified by Fig. 2C–D, however, it in many cases is incorrect to assume that active cancer cell targeting will improve target site accumulation. This is because nanomedicines do not accumulate in tumors to a higher extent upon incorporating ligands which bind to cancer cells, since their primary mode of tumor localization still relies on EPR-mediated passive extravasation. Upon leaving leaky tumor blood vessels and penetrating into the interstitium, they first have to reach the tumor cells, before being able to bind to them. Depending on the tumor model used and/or the human malignancy in question, and on how well they allow for extravasation and penetration, it can be more or less easy to find and bind cancer cells. If the endothelial lining is not very leaky, if there are a lot of pericytes or smooth muscle cells covering tumor blood vessels, if the tumor cell density is high, if there is a dense matrix hindering penetration, and/or if the interstitial fluid pressure is high, it is highly likely that active targeting to cancer cells will not at all lead to any benefit over passive targeting.

In case of rapidly growing tumors in animal models, where several of these (patho-) physiologically phenomena tend to be absent, cancer cells might be located directly behind tumor endothelial cells. In such cases, however, the initial (and overall) tumor accumulation is still based on passive extravasation, and an additional barrier comes into play, i.e. the binding-site barrier [20]. The binding-site barrier is based on the notion that ligand-modified nanomedicines will bind to the first receptors they encounter, and therefore will not penetrate very deeply into the tumor. Theoretically, the only benefit actively targeted nanomedicines may have over passively targeted formulations, is that they might be retained within tumors for a somewhat longer period of time, because their binding to and/or their uptake by cancer cells prevents them from rapidly re-entering systemic circulation. As an additional drawback, however, one should also take into account that the introduction of targeting moieties on the surface of nanocarriers often leads to an increase in immunogenicity and in protein adsorption. This generally has detrimental effects on their circulation time, thereby significantly lowering their ability to passively accumulate in tumors by means of EPR. These considerations explain why the vast majority of nanomedicines (NB: excluding antibody-based formulations; which in spite of their relatively small size and their high target specificity circulate relatively long) approved for clinical use and currently evaluated in clinical trials are passively, rather than actively, targeted.

There is one big advantage of actively (cancer cell-) targeted nanomedicines over passively targeted formulations, and this is that they are taken up by cancer cells much more efficiently. This is exemplified by the inset in Fig. 2C, which present the results obtained by Kirpotin, Park and colleagues, showing that in vitro, Her2-targeted liposomes are taken up >20 times more efficiently than are untargeted liposomes [60]. In vivo, on the other hand, in line with the above considerations, no difference was observed between the two formulations with regard to overall tumor accumulation (i.e. % injected dose per gram tumor; see Fig. 2C). Interestingly, the enhanced uptake by cancer cells did lead to a difference in the in vivo microdistribution of Her-2-targeted liposomes: a significantly higher portion of them was found in cancer cells within tumors, while untargeted liposomes primarily accumulated in stromal cells, and this led to a significant increase in antitumor efficacy [60]. Similar findings have been reported by Davis and colleagues, who showed that overall, transferrin-targeted nanoparticles did not accumulate in tumors to a higher extent than did untargeted nanoparticles, but that they did improve the uptake of the particles by cancer cells in tumors [61].

The notion that active targeting to cancer cells improves cellular uptake within tumors implies that ligand-modified nanomedicines are highly useful systems for transporting agents which themselves are unable to enter cancer cells, such as nucleic acids. This can be

exemplified by taking the recent clinical progress made with CALAA-01 into account (see Table 2). As opposed to the vast majority of other systems developed for siRNA delivery (which are mostly based on cationic polymer- or lipid-containing poly-electrolyte complexes, and which are not very stable, not very specific and not very biocompatible), this formulation is based on a charge-neutral and relatively biocompatible cyclodextrin-based polymeric backbone. Via adamantane inclusion complexes, siRNA (directed against ribonucleotide reductase M2), PEG (for stabilization purposes) and transferrin (for active targeting and uptake by cancer cells) are coupled to the carrier, and it has been convincingly shown that the therapeutic efficacy of this construct is highly dependent on the presence of transferrin [62,63].

The above insights exemplify that in certain specific cases, active targeting to cancer cells is absolutely necessary to make formulations effective, whereas in (the majority of) other cases, it likely won't help at all, and might even have deleterious effects. This is not only because of an altered pharmacokinetic profile, but also because of drawbacks related to the complexity of the formulation, to upscaling and to industrial exploitation (see Section 3.4). Therefore, it is imperative to keep both the requirements of the drug, and the basic principles of passive and active drug targeting in mind, when intending to develop clinically relevant nanomedicine formulations.

3.4. Formulation

Another important pitfall relates to the rational design of the formulations to be developed and tested. To assure proper in vivo functionality, it not only is important to take size and stability into account, but also to try to understand (and incorporate) the complexity of tumor biology. This can be best exemplified by the release mechanism initially envisioned for passively tumor-targeted polymer therapeutics, which were designed to be absolutely stable during their transit in circulation, and to only release the conjugated chemotherapeutic drug upon endocytosis. Several oligopeptide-based linkers were designed for this purpose, as they were found to be perfectly stable in blood, and to liberate active agents only upon incubation with lysosomal enzymes at low pH. A classical example of this is the tetrapeptide linker GFLG, which is cleaved by the lysosomal cysteine protease cathepsin B. PK1, i.e. Prague-Keele-1, the first passively tumor-targeted polymer-drug conjugate to enter clinical trials in 1994 [64], is based on this linker, as is Xyotax (i.e. Opaxio; paclitaxel polyglumex), which entered clinical trials in the mid 2000s. Both of these formulations worked quite well in animal models, but in patients, their therapeutic gain turned out to be very modest [65]. This in spite of reasonably effective passive tumor targeting in approximately 30% of patients treated with PK1 (analyzed using gamma-scintigraphy). In the case of Opaxio, data re-analysis and patient stratification showed that response rates correlated closely to hormone status, with proper responses in pre-menopausal women, but with hardly any responses in post-menopausal women and in men. This disparity was explained by the fact that the activity of cathepsin B closely correlates with estrogen levels, being higher in pre-menopausal women than in other patients. Such biological/physiological insights are highly important for optimal formulation design, especially when intending to treat large cohorts of patients.

A potential means to overcome such shortcomings is to use linkers which do not depend on enzyme activity for releasing conjugated drugs. Using the same type of carrier material, i.e. HPMA copolymers, Ulbrich and colleagues have in the past 10 years provided a significant amount of preclinical evidence demonstrating that this can be achieved using pH-responsive hydrazone linkers. As opposed to GFLG-based linkers, hydrazone bonds are not completely stable in blood at pH 7.4 (with 1–20% release within 24 h, depending on the exact chemical nature of the linker, its flanking groups and the drug), but release the conjugated active agent much faster at lower pH, with in the case of doxorubicin, ~50% release within 5 h at pH 5, and close to 100% release within 2 days. As a result of this less selective, more rapid and more complete release, hydrazone-based polymer-drug conjugates were found to be much more active in inhibiting tumor growth than were GFLG-based conjugates (see Fig. 3D), and strikingly, they were also found to be significantly less toxic [66,67]. Therefore, such enzyme-independent and slightly less stable polymer therapeutics are considered to be particularly suitable formulations for treating large numbers of patients.

A final important consideration with regard to optimizing formulation design relates to the fact that many promising nanomedicines presented in the literature are quite complex, and therefore difficult to synthesize and scale-up by the pharmaceutical industry. Especially systems based on multiple and physicochemically very different components, such as polymers, lipids, antibodies, peptides, drugs and/or imaging agents, are difficult to scale-up, since their production involves many different synthetic and purification steps. This increases the cost, the complexity and the batch-to-batch variance of such formulations, and thereby decreases their commercial attractiveness and their clinical relevance. Only in cases in which multiple components are really necessary to achieve proper in vivo efficacy, as e.g. in the abovementioned case of delivery systems for siRNA (see Section 3.3), it seems to be justified to use multi-component systems. The CALAA-01 formulation, for instance, which is based on a cyclodextrin-based cationic polymer (as a backbone), siRNA (as a drug), adamantane-PEG (for stability and shielding against protein adsorption) and adamantane-PEG-transferrin (for enhancing cellular uptake) is probably the best example for such a complex multi-component system which still has significant clinical relevance [62,63].

Besides for such nucleic acid-containing constructs, however, the most important dogma for forwarding nanomedicine formulations towards industrial exploitation and clinical translation is to ‘keep them simple’. This might at first sound somewhat counter-intuitive, but the fact that the synthetic methods and the materials delivered for clinical testing need to be biocompatible, well-characterized and reproducible (i.e. low batch-to-batch variability), and relatively easy and cost-effective to prepare, underlines the notion that simple and straightforward systems might have much higher chances of success than highly elegant but also highly complex formulations. Taking these insights into account, it is advisable to stick to a phrase coined by Prestwich in the context of tissue engineering, stating that we should “embrace complexicity, engineer versatility, and deliver simplicity” [68]. This statement underlines the importance of I) realizing that biological systems are inherently very complex and variable, that II) carrier materials should be versatile, flexible and broadly applicable, and that III) pharmaceutical products should be as simple and straightforward as possible.

3.5. Efficacy vs. toxicity

Many different nanomedicines have been evaluated and approved for clinical use in the past two decades. In the vast majority of cases, however, and especially in the case of cancer patients, they only turned out to be able to reduce the toxicity of systemic (chemo-) therapeutic interventions, rather than to improve their efficacy. Doxorubicin-containing liposomes, for instance, are well-known to be able to reduce the cardiotoxicity and/or the hematologic side effects associated with anthracycline therapy, but they generally fail to improve response rates and survival times. Only in certain specific cases, such as in patients suffering from Kaposi sarcoma and from cisplatin-responsive ovarian carcinoma, clear-cut improvements in response rates and overall survival times were observed, whereas in all other cases, such as in metastatic breast cancer and in mutlple myeloma, only the incidence and/or intensity of side effects could be reduced [40,69,70]. Regarding Kaposi sarcoma, it should be noted that these tumors possess a dense and highly leaky vasculature, and that likely mostly because of this,

Doxil turned out to be able to not only improve the toxicity but also the efficacy of the intervention: as compared to the formerly standard combination regimen ABV (i.e. adriamycin (doxorubicin), bleomycin and vincristine), which produced a partial response in 31 out of 125 patients (RR = 25%), Doxil achieved 1 complete response and 60 partial responses (RR = 46%) [40]. Regarding ovarian carcinoma, apart from reduced hematogical toxicity, no statistically significant difference could be observed between Doxil and topotecan-based standard treatment (RR = 20 vs. 17%; overall survival 60 vs. 57 weeks; both $p > 0.05$). When evaluating the efficacy of Doxil in cisplatin-responsive patients, however, a significant survival benefit was observed (108 vs. 71; $p = 0.008$) [69].

Comparable clinical observations have been made for polymers, micelles and nanoparticles. Two prototypic doxorubicin-containing polymer therapeutics, for instance, i.e. PK1 and PK2 (i.e. passively and actively liver-targeted HPMA copolymer-bound doxorubicin), have both been shown to be able to substantially reduce drug-related side effects, as exemplified by 5- and 2-fold increases in the maximum tolerated dose (MTD) that could be safely administered to patients, respectively, but they failed to achieve significant increases in response rates [18,64,65]. The same holds true for the micellar doxorubicin formulation NK911, which was tolerated reasonably well (i.e. same MTD as free Dox), but which only induced 1 partial response in 23 patients [71].

Abraxane (i.e. albumin-based nanoparticles containing paclitaxel), on the other hand, did present with quite promising therapeutic efficacy in a large phase III trial. In this trial, more than 400 breast cancer patients were treated either with the combination of Taxol (i.e. 175 mg/m^2 paclitaxel; administered via the castor oil-based solubilizer Cremophor, which is known to cause severe local irritation and inflammation) and corticosteroids (to inhibit Cremophor-associated inflammatory reactions), or with Abraxane alone (i.e. without corticosteroid co-medication; at a dose of 260 mg/m^2). As compared to Taxol, Abraxane significantly improved both the response rate (33 vs. 19%) and the progression-free survival time (23 vs. 17 weeks; $p = 0.024$) of the intervention, and it at the same time also attenuated the toxicity of the systemic taxane treatment: the incidence of grade 4 neutropenias was significantly lower for Abraxane (9 vs 22%), despite the 50% higher dose, and no hypersensitivity reactions were observed, despite the absence of premedication [72].

To overcome the above shortcoming with regard to improving the balance between the efficacy and the toxicity of systemic nano-chemotherapeutic interventions, and to thereby broaden the clinical applicability of tumor-targeted nanomedicines, we and others have in the past 5 years developed several concepts for using nanomedicine formulations to improve the efficacy of combined modality anticancer therapy [73–78]. Convincing and clinically highly relevant evidence has for instance been obtained showing that nanomedicines are highly useful for improving the efficacy of radiochemotherapy and of chemotherapy combinations.

Regarding the former, we have shown that local external beam radiotherapy and polymeric nanomedicines interact synergistically, with radiotherapy improving the tumor accumulation of HPMA copolymers, and with the copolymers improving both the efficacy and the tolerability of radiochemotherapy [38,78]. Using magnetic resonance imaging and γ-scintigraphy, we demonstrated in three different tumor models that pretreating tumors with radiotherapy increases their tumor accumulation (by 25–100%; depending on polymer size and on the tumor model used). These findings were explained by taking into account that radiotherapy increases the production of the permeability-enhancing growth factors VEGF and FGF, that it induces endothelial cell apoptosis, that it reduces the cell density in tumors, and that it lowers the interstitial fluid pressure [55,56]. In addition to this, reasoning that I) the temporal and spatial interaction between i.v. applied weekly chemotherapy and clinically relevant daily radiotherapy is suboptimal, and that II) long-circulating and passively tumor-targeted nanomedicines are able to improve the temporal and spatial parameters of this interaction, we have shown that HPMA copolymers are able to improve both the efficacy and the toxicity of clinically relevant regimens of radiochemotherapy [78]. Both doxorubicin- and gemcitabine-containing copolymers were used for this purpose, and growth inhibition was achieved in an aggressively growing and radio- and chemo-resistant tumor model. These findings are in line with preclinical studies in which Doxil was combined with radiotherapy [57], as well as with the results of a phase I trial in which 12 patients with localized esophageal and gastric cancer were treated with the combination of poly(l-glutamic acid)-bound paclitaxel (i.e. Opaxio/Xyotax) and fractionated radiotherapy, and in which 4 complete responses and 7 partial responses (with reductions in tumor size of more than 50%) were achieved [79]. Together, these insights convincingly show that 'carrier-based radiochemotherapy' might hold significant potential for improving the treatment of advanced solid malignancies.

Regarding chemotherapy combinations, following up on the pioneering efforts by Vicent, Duncan and colleagues [80], we have recently for the first time provided in vivo evidence showing that passively tumor-targeted polymeric drug carriers can be used to deliver two different drugs to tumors simultaneously. To this end, both doxorubicin and gemcitabine were co-conjugated to the same HPMA copolymer, and it was shown that this formulation – which we termed P-Gem-Dox – circulated for prolonged periods of time, that it localized to tumors both effectively and selectively, and that it increased the efficacy of the combination of doxorubicin plus gemcitabine without increasing its toxicity [81]. In addition to this, it was found that P-Gem-Dox more effectively induced apoptosis and reduced angiogenesis than did all relevant control regimens. These findings are in line with the results recently reported by Satchi-Fainaro and colleagues, who co-conjugated the anti-angiogenic agents aminobisphosphonate alendronate and TNP-470 to a single HPMA copolymer [82], as well as with those published by Tardi, Mayer and co-workers, who co-encapsulated optimal ('ratiometric') concentrations of doxorubicin and vincristine, of irinotecan and floxuridine and of daunorubicin and cytarabine into liposomes, and who are currently evaluating the potential of the latter two formulations in patients [83–86].

In addition to such targeted chemotherapy combination regimens, nanomedicines also perform quite well when combined with standard chemotherapy. Abraxane, for instance, has been shown to combine well with bevacizumab and with gemcitabine in patients suffering from metastatic breast cancer [87,88], as did Genexol-PM (i.e. a polymeric micelle formulation of paclitaxel) with cislatin and with carboplatin [89,90]. Similarly, Doxil has been shown to combine very well the microtubule-inhibitor docetaxel in metastatic breast cancer, with the alkylating agent canfosfamide in ovarian carcinoma and with the proteasome inhibitor bortezimob in multiple myeloma [91–93].

Collectively, the above insights and advances convincingly demonstrate that nanometer-sized carrier materials hold significant potential for improving the efficacy of combined modality anticancer therapy. Consequently, they strongly suggest that besides in developing novel and ever more advanced nanomedicine formulations, significant efforts should also be invested in establishing novel and more optimal combination regimens, in order to more optimally exploit the biocompatibility and the beneficial biodistribution of tumor-targeted nanomedicines.

3.6. Metastasis

The sixth important issue to take into account with regard to establishing effective, broadly applicable and clinically relevant carrier materials relates to the fact that virtually all anticancer nanomedicines developed to date are designed to target solid tumors. The vast majority of patients succumbing to cancer, however, eventually die

from metastases, and not from locally confined tumors. Moreover, if tumors are locally confined, they are generally treated with surgery and/or with radiotherapy, and not with chemotherapy. Chemotherapy is often given in such cases, but generally not to be curative as such, but to pre-shrink tumors prior to surgery or radiotherapy (i.e. neo-adjuvant chemotherapy), or to prevent and/or treat metastasis after locally confined therapeutic interventions (i.e. adjuvant chemotherapy). Scientists working on nanomedicine formulations for cancer therapy are urged to keep this notion well in mind, and to try to come up with systems and strategies that enable more effective anti-metastatic treatments. This can e.g. be achieved by evaluating the ability of nanomedicines to deliver chemotherapeutic payloads to sentinel lymph nodes (see Fig. 3F), by involving the immune system, and by developing systems for compartmentalized chemotherapy (which might be particularly useful for treating locally confined ovarian carcinoma metastases; see below).

With regard to involving the immune system, Rihova and colleagues prepared polymeric nanomedicines which possess immunomodulatory properties [94]. They developed a human immunoglobulin- (HuIg-) modified version of PK1, and evaluated it in four end-stage patients in the Czech Republic. Improvements in disease parameters in blood were achieved in several cases, and in all four patients, evidence for an activation of lymphocyte activated killer (LAK) cells and nuclear killer (NK) cells could be observed [95]. At the preclinical level, they obtained similar immunostimulatory effects for a number of other antibody-based polymer therapeutics, e.g. for anti-Thy1.2-, anti-CD71- and B1-targeted versions of PK1, which were all found to be significantly more effective than antibody-free polymer-drug conjugates [96–98]. It is furthermore interesting to note that in addition to producing cures in up to 100% of mice, several of these antibody-targeted polymeric nanomedicines were shown to be able to induce antitumor immunity (i.e. relatively long-lasting immunoprotection), as exemplified by the fact that significant percentages of cured mice were found to be resistant to a re-challenge with a second (lethal) dose of cancer cells [99].

An alternative strategy to (try to) improve the treatment of metastasis using nanomedicine formulations is based on locally confined (i.e. compartmentalized) delivery. In the case of metastatic ovarian carcinoma, for instance, patients could be injected intraperitoneally with actively cancer cell-targeted polymers, liposomes, micelles or nanoparticles, which would then be present in the same compartment (i.e. in the peritoneal cavity) as are the metastases, and which within this compartment can be expected to be able to effectively target and treat metastatic tumor nodules. A similar approach could be envisioned for other abdominally localized and locally metastasized malignancies, such as liver, colorectal and pancreatic carcinomas. Related to this, for the treatment of metastatic liver carcinomas, also the 'local' intra-hepatic artery administration of actively cancer cell-targeted nanomedicines could be considered. Since tumors in general, and liver tumors in particular, mainly rely on arterial blood (and oxygen and nutrient) supply, this would enable the delivery of large amounts of cancer cell-specific nanomedicines to primary liver tumors, as well as to liver metastases.

3.7. Personalization

Like for standard (chemo-) therapeutic drugs, it is increasingly being recognized that also for nanomedicine formulations, attempts should be made to personalize therapeutic interventions. Besides integrating knowledge on genetic polymorphisms, enzyme expression (see above: Section 3.4) and other biomarkers, personalized medicine also involves the establishment of visual methods for predicting and measuring therapeutic responses. Nanomedicines in principle are highly suitable systems for such purposes, since they can be relatively easily modified with imaging agents. By at the same time incorporating both drugs and imaging agents within a single formulation, they can be used to non-invasively monitor the biodistribution and the target site accumulation of the drug and/or carrier material, to visualize drug release, and to assess the therapeutic efficacy of the intervention in real-time [41–43].

Regarding the visualization of the biodistribution and the target site accumulation of such 'theranostic' nanomedicines, it has already been convincingly shown that non-invasive imaging insights are highly useful for assessing the efficacy of drug targeting. In the majority of cases, radionuclides have been used to monitor biodistribution. Harrington and colleagues, for instance, radiolabeled PEGylated liposomes with indium-111, and evaluated their circulation time and their tumor accumulation in 17 patients suffering from various different types of locally advanced malignancy (breast, head and neck, lung, brain and cervical cancer) [100]. They were able to show that PEGylated liposomes circulate for very long periods of time, with an average distribution half-life time ($t1/2\alpha$) of 76 h, and with more than 50% of the injected dose still present in systemic circulation at 48 h p.i., thereby confirming the potential of these systems for passive drug targeting. In addition, they were able to show that radiolabeled liposomes relatively effectively localized to tumors in 15 out of 17 cases, and that tumor uptake depended significantly on tumor type: the highest levels were observed for head and neck cancer (33 ± 16%ID/kg tumor), and the lowest levels for breast cancer (5 ± 3%ID/kg tumor). Interestingly, however, in case of the latter, in spite of relatively low overall tumor accumulation, it was observed that the radiolabeled liposomes effectively accumulated in axillary lymph nodes, which are typically employed by breast cancers to spread throughout the body (see left panel in Fig. 3F). Analogously, as exemplified by the left panel in Fig. 3G, they also showed that indium-111-labeled PEGylated liposomes highly effectively accumulate in Kaposi sarcoma lesions, and that they do so both in primary tumors and in metastatic lesions, thereby explaining – at least in part – why Doxil is so effective in patients suffering from Kaposi sarcoma.

In a comparable setup, Seymour and colleagues visualized the biodistribution and the target site accumulation of liver-targeted HPMA copolymer-bound doxorubicin (PK2) [17]. In PK2, galactosamine, which binds to the asiolaglycoprotein-receptor overexpressed by hepatocytes, is used to direct the polymer-drug conjugates to hepatocellular carcinomas (HCC). The upper right panel in Fig. 3G clearly shows that iodine-123-labeled PK2 highly specifically localized to the liver, and that therefore, organ-specific targeting could be considered highly effective. When looking in more detail at the efficacy of organ targeting, however, using anatomical CT imaging (middle right panel in Fig. 3G) and functional SPECT imaging (lower right panel in Fig. 3G), it was found that the majority of PK2 ended up in healthy liver tissue (i.e. the light gray and white-yellowish parts of the CT and SPECT images, respectively), rather than in the tumor (i.e. the dark area in the center of the CT and SPECT images). Such image-guided insights show that PK2 was not very effective and selective in delivering the conjugated drug to the pathological site, and might explain why it was not very active in improving treatment efficacy in the majority of HCC patients included in this phase I/II trial.

The above examples show that image-guided insights can be used to pre-screen patients assigned to receive e.g. liposome- and polymer-based nanomedicine treatments, in order to identify which tumors are amenable to passive and active drug targeting and which are not, and to thereby predict which patients are likely to respond to such targeted therapeutic interventions and which are not. In addition to this, if minute amounts of radiolabeled nanomedicines could be added in during every (second or third) cycle of drug treatment, it would furthermore be possible to non-invasively visualize the efficacy of the intervention in real-time, e.g. by tracking the size of (and the accumulation of nanomedicines in) metastasic lesions. This could assist in deciding whether or not to (dis-) continue therapy, and whether or not to adjust drug doses. Therefore, theranostic nanomedicines and non-invasive imaging techniques might contribute substantially to realizing the potential of personalized medicine.

3.8. Translation

A final important pitfall with regard to realizing the potential of nanomedicines and drug targeting to tumors relates to the fact that the animal models routinely used in preclinical trials are far from being representative for the clinical situation. As already outlined above, tumors which grow to ~1 cm in diameter within 2–3 weeks in rodents are very different from tumors developing in patients, which generally take several years to grow to sizes beyond 1 cm in diameter; therefore, blood vessels in human tumors tend to be much less leaky than blood vessels in animal models (see Section 3.1). Also, tumors growing subcutaneously are anatomically and physiologically very different from tumors growing in their native (orthotopic) environment, and also very different from the metastatic lesions that (nanomedicine) formulations are often confronted with in early phase clinical trials. Furthermore, many in vivo efficacy experiments are merely performed with human xenograft tumors in immunodeficient nude mice, thereby excluding the possibility to assess the involvement of the immune system in positively or negatively affecting therapeutic outcome. Because of such biological, physiological and immunological differences, it can be argued that many agents which work well in animal models, might not at all work in patients.

To overcome this shortcoming, to better predict how well nanomedicines (and also standard chemotherapeutic drugs) will work in patients, and to thereby improve the time- and cost-effectiveness of clinical translation, it would be worthwhile to consider establishing a well-defined panel of animal models – comparable to the NCI-60 set of cancer cell lines – to test (and head-to-head compare) the efficacy of all formulations which are close to being evaluated in patients. Depending on the tumor type to be treated, such a panel could for instance include 1 representative subcutaneous xenograft model, 1 representative subcutaneous syngeneic model, 1 orthotopic model, 1 metastatic model and ideally also 1 transgenic model. By attempting to (pre-) define which rodent tumor models are representative for the clinical situation, by validating these choices via standard chemotherapeutic interventions (i.e. those which are currently used clinically), and by evaluating how well novel (nanomedicine) formulations are able to improve therapeutic outcome in such models, it should in principle be possible to much better predict how well new therapeutic entities might eventually perform in patients, to more reliably compare novel to established treatments, and to thereby substantially facilitate the time- and cost-effectiveness of clinical translation.

4. (Pre-) clinical progress

In the past few decades, many different nanomedicines have been evaluated in (pre-) clinical trials. Initially, several types of doxorubicin-containing liposomes were tested, leading to the approval of Doxil in the US for the treatment of Kaposi sarcoma in 1995, and of Caelyx in Europe in 1997 (NB: both are similar, i.e. PEGylated liposomal doxorubicin). In the years that followed, Doxil/Caelyx was also approved for metastatic breast cancer, for ovarian cancer and for multiple myeloma, as was Myocet (i.e. non-PEGylated liposomal doxorubicin) for metastatic breast cancer, and DaunoXome (i.e. non-PEGylated liposomal daunorubicin) for Kaposi sarcoma. Furthermore, DepoCyt, i.e. non-PEGylated liposomal cytarabine, was approved in 1999 for the local intrathecal treatment of lymphomatous menginitis, i.e. a serious complication associated with brain cancer. In addition, DepoCyt is currently being tested in phase III trials for leukemia and in phase I/II clinical trials for glioblastoma (see Table 2). Other promising liposomal nanomedicines in clinical trials are Thermodox, a thermo-sensitive liposomal doxorubicin formulation which releases the drug at temperatures >39 °C and which is currently being tested in phase III trials in hepatocellular carcinoma patients together with radiofrequency ablation; NL CPT-11, i.e. nanoliposomal irinotecan, which is in phase I trials for glioma; and several liposomal formulations carrying two different types of chemotherapeutics, such as cytarabine and daunorubicin, and irinotecan and floxuridine [84–86]. At the preclinical level, many other liposomal nanomedicines are being evaluated, containing various different types of drugs, as are several image-guided formulations, used for instance to confirm EPR-mediated passive drug targeting to tumors, or to validate drug release from Thermodox-like triggerable formulations upon MR-guided high-intensity focused ultrasound [32–36].

Similarly, a large number of polymer-drug conjugates, micelles, nanoparticles and antibody-drug conjugates have been evaluated clinically. Several PEGylated proteins, such as Oncaspar for the treatment of leukemia, have already been approved, while other polymer therapeutics, such as Opaxio and ProLindac, are in late-stage clinical trials. Genexol-PM, a micellar formulation of paclitaxel, has been (pre-) approved in Korea for breast and lung cancer, and is currently undergoing phase II trials in the US and Russia for similar indications. Paclical, another micellar formulation of paclitaxel, has recently received FDA orphan drug designation, and is in phase III trials for ovarian carcinoma. In the nanoparticle field, most progress has thus far been made with Abraxane, which is a co-condensate of paclitaxel and albumin, and which has been shown to be able to improve the efficacy and reduce the toxicity of systemic taxane treatment. The majority of other nanoparticles in clinical trials, including the abovementioned multi-component CALAA-01 formulation, aim to deliver nucleic acid-based therapeutics, such as siRNA and plasmid DNA (see Table 2). Finally, a number of antibody-drug conjugates have been approved for clinical use, including e.g. Ontak, Zevalin and Bexxar (NB: Mylotarg was withdrawn in 2010, because of insufficient efficacy and unacceptable toxicity), carrying either bacterial toxins or radionuclides as therapeutic moieties.

The above advances clearly demonstrate that significant progress has been made in the targeted nanomedicine field, and that more progress is to be expected. Through the large number of clinical trials performed to date, however, as well as through several follow-up studies in which already approved formulations have been combined with other treatment modalities, such as with standard chemotherapy and/or with radiotherapy, it has become clear that tumor-targeted nanomedicines – as do standard chemotherapeutic drugs – perform particularly well when integrated in combined modality anticancer therapy. Therefore, in the years to come, besides making ever more carrier materials, and on attempting to better understand the biological and (patho-) physiological principles of drug targeting to tumors, efforts should also focus on establishing rational combination regimens, in order to more optimally exploit the biocompatibility and the beneficial biodistribution of nanomedicines.

5. Conclusion

Many different systems and strategies have been evaluated for drug targeting to tumors over the years. Several of these formulations have managed to gain FDA and/or EMA approval, and are routinely used in the clinic. The majority of them, however, have failed either in late-stage preclinical or in early-stage clinical trials. This might be due to several reasons, most prominently to the overinterpretation and/or the misunderstanding of some of the basic concepts used in tumor-targeted drug delivery, as well as to the fact that some of the formulations tested were not effective enough in monotherapy regimens, or too complex to be upscaled by the industry. Furthermore, also the lack of image-guided insights to personalize nanomedicine-based therapeutic interventions, and the notion that in many cases, inappropriate animal models were used at the preclinical level, might have hindered the clinical translation of tumor-targeted nanomedicines. Therefore, in future efforts, it is highly important to not only make ever more nanomedicine materials, but also to strive for a better understanding of the biological and the (patho-) physiological principles of drug targeting, to in-depth investigate the pitfalls in tumor-targeted drug delivery, and to come up with strategies to overcome these shortcomings.

Acknowledgments

The authors gratefully acknowledge financial support by the European Commission's Framework Programme 6 (MediTrans: Targeted Delivery of Nanomedicine) by the DFG and by Hightech.NRW (ForSaTum).

References

[1] D. Hanahan, R.A. Weinberg, The hallmarks of cancer, Cell 100 (2000) 57–70.
[2] D. Hanahan, R.A. Weinberg, Hallmarks of cancer: the next generation, Cell 144 (2011) 646–674.
[3] D.S. Krause, R.A. Van Etten, Tyrosine kinases as targets for cancer therapy, N. Engl. J. Med. 353 (2005) 172–187.
[4] R.M. Sharkey, D.M. Goldenberg, Targeted therapy of cancer: new prospects for antibodies and immunoconjugates, CA Cancer J. Clin. 56 (2006) 226–243.
[5] I. Collins, P. Workman, New approaches to molecular cancer therapeutics, Nat. Chem. Biol. 2 (2006) 689–700.
[6] V.P. Torchilin, Drug targeting, Eur. J. Pharm. Sci. 11 (2000) S81–S91.
[7] T.M. Allen, P.R. Cullis, Drug delivery systems: entering the mainstream, Science 303 (2004) 1818–1822.
[8] D. Peer, J.M. Karp, S. Hong, O. Farokhzad, R. Margalit, R. Langer, Nanocarriers as an emerging platform for cancer therapy, Nat. Nanotechnol. 2 (2007) 751–760.
[9] M.E. Davis, Z.G. Chen, D.M. Shin, Nanoparticle therapeutics: an emerging treatment modality for cancer, Nat. Rev. Drug Discov. 7 (2008) 771–782.
[10] T. Lammers, W.E. Hennink, G. Storm, Tumour-targeted nanomedicines: principles and practice, Br. J. Cancer 99 (2008) 392–397.
[11] R.K. Jain, T. Stylianopoulos, Delivering nanomedicine to solid tumors, Nat. Rev. Clin. Oncol. 7 (2010) 653–664.
[12] H. Maeda, Y. Matsumura, A new concept for macromolecular therapeutics in cancer chemotherapy: mechanism of tumoritropic accumulation of proteins and the antitumor agent Smancs, Cancer Res. 46 (1986) 6387–6392.
[13] H. Maeda, J. Wu, T. Sawa, Y. Matsumura, K. Hori, Tumour vascular permeability and the EPR effect in macromolecular therapeutics: a review, J. Control. Release 65 (2000) 271–284.
[14] T.M. Allen, Ligand-targeted therapeutics in anticancer therapy, Nat. Rev. Cancer 2 (2002) 750–763.
[15] F. Marcucci, L. Lefoulon, Active targeting with particulate drug carriers in tumor therapy: fundamentals and recent progress, Drug Discov. Today 9 (2004) 219–228.
[16] S. Wang, P.S. Low, Folate-mediated targeting of antineoplastic drugs, imaging agents, and nucleic acids to cancer cells, J. Control. Release 53 (1998) 39–48.
[17] Z.M. Qian, H. Li, H. Sun, K. Ho, Targeted drug delivery via the transferrin receptor-mediated endocytosis pathway, Pharmacol. Rev. 54 (2002) 561–587.
[18] L.W. Seymour, D.R. Ferry, D. Anderson, S. Hesslewood, P.J. Julyan, R. Poyner, J. Doran, A.M. Young, S. Burtles, D.J. Kerr, Cancer Research Campaign Phase I/II Clinical Trials Committee, hepatic drug targeting: phase I evaluation of polymer-bound doxorubicin, J. Clin. Oncol. 20 (2002) 1668–1676.
[19] C.H. Heldin, K. Rubin, K. Pietras, A. Ostman, High interstitial fluid pressure - an obstacle in cancer therapy, Nat. Rev. Cancer 4 (2004) 806–813.
[20] M. Juweid, R. Neumann, C. Paik, M.J. Perez-Bacete, J. Sato, W. van Osdol, J.N. Weinstein, Micropharmacology of monoclonal antibodies in solid tumors: direct experimental evidence for a binding site barrier, Cancer Res. 52 (1992) 5144–5153.
[21] L. Borsi, E. Balza, M. Bestagno, P. Castellani, B. Carnemolla, A. Biro, A. Leprini, J. Sepulveda, O. Burrone, D. Neri, L. Zardi, Selective targeting of tumoral vasculature: comparison of different formats of an antibody (L19) to the ED-B domain of fibronectin, Int. J. Cancer 102 (2002) 75–85.
[22] D. Neri, R. Bicknell, Tumour vascular targeting, Nat. Rev. Cancer 5 (2005) 436–446.
[23] W. Arap, R. Pasqualini, E. Ruoslahti, Cancer treatment by targeted drug delivery to tumor vasculature in a mouse model, Science 279 (1998) 377–380.
[24] R. Pasqualini, E. Koivunen, R. Kain, J. Lahdenranta, M. Sakamoto, A. Stryhn, R.A. Ashmun, L.H. Shapiro, W. Arap, E. Ruoslahti, Aminopeptidase N is a receptor for tumor-homing peptides and a target for inhibiting angiogenesis, Cancer Res. 60 (2000) 722–727.
[25] K. Temming, R.M. Schiffelers, G. Molema, R.J. Kok, RGD-based strategies for selective delivery of therapeutics and imaging agents to the tumour vasculature, Drug Resist. Updat. 8 (2005) 381–402.
[26] A. Mitra, J. Mulholland, A. Nan, E. McNeill, H. Ghandehari, B.R. Line, Targeting tumor angiogenic vasculature using polymer-RGD conjugates, J. Control. Release 102 (2005) 191–201.
[27] M. Johannsen, G. Spitaleri, G. Curigliano, J. Roigas, S. Weikert, C. Kempkensteffen, A. Roemer, C. Kloeters, P. Rogalla, G. Pecher, K. Miller, A. Berndt, H. Kosmehl, E. Trachsel, M. Kaspar, V. Lovato, R. González-Iglesias, L. Giovannoni, H.D. Menssen, D. Neri, F. de Braud, The tumour-targeting human L19-IL2 immunocytokine: preclinical safety studies, phase I clinical trial in patients with solid tumours and expansion into patients with advanced renal cell carcinoma, Eur. J. Cancer 46 (2010) 2926–2935.
[28] K.J. Kimball, M.A. Preuss, M.N. Barnes, M. Wang, G.P. Siegal, W. Wan, H. Kuo, S. Saddekni, C.R. Stockard, W.E. Grizzle, R.D. Harris, R. Aurigemma, D.T. Curiel, R.D. Alvarez, A phase I study of a tropism-modified conditionally replicative adenovirus for recurrent malignant gynecologic diseases, Clin. Cancer Res. 16 (2010) 5277–5287.
[29] P. Nokisalmi, S. Pesonen, S. Escutenaire, M. Särkioja, M. Raki, V. Cerullo, L. Laasonen, R. Alemany, J. Rojas, M. Cascallo, K. Guse, M. Rajecki, L. Kangasniemi, E. Haavisto, A. Karioja-Kallio, P. Hannuksela, M. Oksanen, A. Kanerva, T. Joensuu, L. Ahtiainen, A. Hemminki, Oncolytic adenovirus ICOVIR-7 in patients with advanced and refractory solid tumors, Clin. Cancer Res. 16 (2010) 3035–3043.
[30] K. Kono, T. Ozawa, T. Yoshida, F. Ozaki, Y. Ishizaka, K. Maruyama, C. Kojima, A. Harada, S. Aoshima, Highly temperature-sensitive liposomes based on a thermosensitive block copolymer for tumor-specific chemotherapy, Biomaterials 31 (2010) 7096–7105.
[31] C. Oerlemans, W. Bult, M. Bos, G. Storm, J.F.W. Nijsen, W.E. Hennink, Polymeric micelles in anticancer therapy: targeting, imaging and triggered release, Pharm. Res. 27 (2010) 2569–2589.
[32] R. Deckers, C.T. Moonen, Ultrasound triggered, image guided, local drug delivery, J. Control. Release 148 (2010) 25–33.
[33] T. Tagami, M.J. Ernsting, S.D. Li, Efficient tumor regression by a single and low dose treatment with a novel and enhanced formulation of thermosensitive liposomal doxorubicin, J. Control. Release 152 (2011) 303–309.
[34] A.H. Negussie, P.S. Yarmolenko, A. Partanen, A. Ranjan, G. Jacobs, D. Woods, H. Bryant, D. Thomasson, M.W. Dewhirst, B.J. Wood, M.R. Dreher, Formulation and characterisation of magnetic resonance imageable thermally sensitive liposomes for use with magnetic resonance-guided high intensity focused ultrasound, Int. J. Hyperthermia 27 (2011) 140–155.
[35] M. de Smet, E. Heijman, S. Langereis, N.M. Hijnen, H. Grüll, Magnetic resonance imaging of high intensity focused ultrasound mediated drug delivery from temperature-sensitive liposomes: an in vivo proof-of-concept study, J. Control. Release 150 (2011) 102–110.
[36] T. Tagami, M.J. Ernsting, S.D. Li, Optimization of a novel and improved thermosensitive liposome formulated with DPPC and a Brij surfactant using a robust in vitro system, J. Control. Release 154 (2011) 290–297.
[37] Y.H. Bae, K. Park, Targeted drug delivery to tumors: Myths, reality and possibility, J. Control. Release 153 (2011) 198–205.
[38] T. Lammers, P. Peschke, R. Kühnlein, V. Subr, K. Ulbrich, J. Debus, P.E. Huber, W.E. Hennink, G. Storm, Effect of radiotherapy and hyperthermia on the tumor accumulation of HPMA copolymer-based drug delivery systems, J. Control. Release 117 (2007) 333–341.
[39] H.F. Dvorak, L.F. Brown, M. Detmar, A.M. Dvorak, Vascular permeability factor/vascular endothelial growth factor, microvascular hyperpermeability, and angiogenesis, Am. J. Pathol. 146 (1995) 1029–1039.
[40] D.W. Northfelt, B.J. Dezube, J.A. Thommes, B.J. Miller, M.A. Fischl, A. Friedman-Kien, et al., Pegylated-liposomal doxorubicin versus doxorubicin, bleomycin, and vincristine in the treatment of AIDS-related Kaposi's sarcoma: results of a randomized phase III clinical trial, J. Clin. Oncol. 16 (2005) 2445–2451.
[41] D. Sun, Nanotheranostics: integration of imaging and targeted drug delivery, Mol. Pharm. 7 (2010) 1879.
[42] T. Lammers, F. Kiessling, W.E. Hennink, G. Storm, Nanotheranostics and image-guided drug delivery: current concepts and future directions, Mol. Pharm. 7 (2010) 1899–1912.
[43] T. Lammers, S. Aime, W.E. Hennink, G. Storm, F. Kiessling, Theranostic Nanomedicines, Acc. Chem. Res. (in press) doi:10.1021/ar200019c.
[44] T.L. Ten Hagen, A.L. Seynhaeve, A.M. Eggermont, Tumor necrosis factor-mediated interactions between inflammatory response and tumor vascular bed, Immunol. Rev. 222 (2008) 299–315.
[45] A.L. Seynhaeve, S. Hoving, D. Schipper, C.E. Vermeulen, G. de Wiel-Ambagtsheer, S.T. van Tiel, A.M. Eggermont, T.L. Ten Hagen, Tumor necrosis factor alpha mediates homogeneous distribution of liposomes in murine melanoma that contributes to a better tumor response, Cancer Res. 67 (2007) 9455–9462.
[46] T. Seki, F. Carroll, S. Illingworth, R. Cawood, V. Subr, K.D. Fisher, L.W. Seymour, Tumour Necrosis Factor-Alpha Increases Extravasation of Virus Particles into Tumour Tissue by Activating the Rho A/Rho Kinase Pathway, J. Control Release. 156 (2011) 381–389.
[47] Z. Popovic, W. Liu, V.P. Chauhan, J. Lee, C. Wong, A.B. Greytak, N. Insin, D.G. Nocera, D. Fukumura, R.K. Jain, M.G. Bawendi, A nanoparticle size series for in vivo fluorescence imaging, Angew. Chem. Int. Ed Engl. 49 (2010) 8649–8652.
[48] M.R. Dreher, W. Liu, C.R. Michelich, M.W. Dewhirst, F. Yuan, A. Chilkoti, Tumor vascular permeability, accumulation, and penetration of macromolecular drug carriers, J. Natl. Cancer Inst. 98 (2006) 335–344.
[49] M.R. Kano, Y. Bae, C. Iwata, Y. Morishita, M. Yashiro, M. Oka, T. Fujii, A. Komuro, K. Kiyono, M. Kaminishi, K. Hirakawa, Y. Ouchi, N. Nishiyama, K. Kataoka, K. Miyazono, Improvement of cancer-targeting therapy, using nanocarriers for intractable solid tumors by inhibition of TGF-beta signaling, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 3460–3465.
[50] M. Kumagai, M.R. Kano, Y. Morishita, M. Ota, Y. Imai, N. Nishiyama, M. Sekino, S. Ueno, K. Miyazono, K. Kataoka, Enhanced magnetic resonance imaging of experimental pancreatic tumor in vivo by block copolymer-coated magnetite nanoparticles with TGF-beta inhibitor, J. Control. Release 140 (2009) 306–311.
[51] E. Brown, T. McKee, E. diTomaso, A. Pluen, B. Seed, Y. Boucher, R.K. Jain, Dynamic imaging of collagen and its modulation in tumors in vivo using second-harmonic generation, Nat. Med. 9 (2003) 796–800.
[52] J. Choi, K. Credit, K. Henderson, R. Deverkadra, Z. He, H. Wiig, H. Vanpelt, M.F. Flessner, Intraperitoneal immunotherapy for metastatic ovarian carcinoma: resistance of intratumoral collagen to antibody penetration, Clin. Cancer Res. 12 (2006) 1906–1912.
[53] T.D. McKee, P. Grandi, W. Mok, G. Alexandrakis, N. Insin, J.P. Zimmer, M.G. Bawendi, Y. Boucher, X.O. Breakefield, R.K. Jain, Degradation of fibrillar collagen in a human melanoma xenograft improves the efficacy of an oncolytic herpes simplex virus vector, Cancer Res. 6 (2006) 2509–2513.
[54] J.Y. Perentes, T.D. McKee, C.D. Ley, H. Mathiew, M. Dawson, T.P. Padera, L.L. Munn, R.K. Jain, Y. Boucher, In vivo imaging of extracellular matrix remodeling by tumor-associated fibroblasts, Nat. Methods 6 (2009) 143–145.

[55] P.A. Netti, L.T. Baxter, Y. Boucher, R. Skalak, R.K. Jain, Time-dependent behavior of interstitial fluid pressure in solid tumors: implications for drug delivery, Cancer Res. 55 (1995) 5451–5458.
[56] F. Samaniego, P.D. Markham, R. Gendelman, Y. Watanabe, V. Kao, K. Kowalski, J.A. Sonnabend, A. Pintus, R.C. Gallo, B. Ensoli, Vascular endothelial growth factor and basic fibroblast growth factor present in Kaposi's sarcoma (KS) are induced by inflammatory cytokines and synergized to promote vascular permeability and KS lesion development, Am. J. Pathol. 152 (1998) 1433–1443.
[57] C.L. Davies, L.M. Lundstrom, J. Frengen, L. Eikenes, O.S. Bruland, O. Kaalhus, M.H. Hjelstuen, C.R. Brekken, Radiation improves the distribution and uptake of liposomal doxorubicin (caelyx) in human osteosarcoma xenografts, Cancer Res. 64 (2004) 547–553.
[58] T. Lammers, V. Subr, K. Ulbrich, W.E. Hennink, G. Storm, F. Kiessling, Polymeric nanomedicines for image-guided drug delivery and tumor-targeted combination therapy, Nano Today 5 (2010) 197–212.
[59] C. Wong, T. Stylianopoulos, J. Cui, J. Martin, V.P. Chauhan, W. Jiang, Z. Popovic, R.K. Jain, M.G. Bawendi, D. Fukumura, Multistage nanoparticle delivery system for deep penetration into tumor tissue, Proc. Natl. Acad. Sci. U.S.A. 108 (2011) 2426–2431.
[60] D.B. Kirpotin, D.C. Drummond, Y. Shao, M.R. Shalaby, K. Hong, U.B. Nielsen, J.D. Marks, C.C. Benz, J.W. Park, Antibody targeting of long-circulating lipidic nanoparticles does not increase tumor localization but does increase internalization in animal models, Cancer Res. 66 (2006) 6732–6740.
[61] C.H. Choi, C.A. Alabi, P. Webster, M.E. Davis, Mechanism of active targeting in solid tumors with transferrin-containing gold nanoparticles, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 1235–1240.
[62] M.E. Davis, The first targeted delivery of siRNA in humans via a self-assembling, cyclodextrin polymer-based nanoparticle: from concept to clinic, Mol. Pharm. 6 (2009) 659–668.
[63] M.E. Davis, J.E. Zuckerman, C.H. Choi, D. Seligson, A. Tolcher, C.A. Alabi, Y. Yen, J.D. Heidel, A. Ribas, Evidence of RNAi in humans from systemically administered siRNA via targeted nanoparticles, Nature 464 (2010) 1067–1070.
[64] P.A. Vasey, S.B. Kaye, R. Morrison, C. Twelves, P. Wilson, R. Duncan, A.H. Thomson, L.S. Murray, T.E. Hilditch, T. Murray, S. Burtles, D. Fraier, E. Frigerio, J. Cassidy, Cancer Research Campaign Phase I/II Committee, Phase I clinical and pharmacokinetic study of PK1 [N-(2-hydroxypropyl)-methacrylamide copolymer doxorubicin]: first member of a new class of chemotherapeutic agents-drug-polymer conjugates, Clin. Cancer Res. 5 (1999) 83–94.
[65] R. Duncan, Development of HPMA copolymer-anticancer conjugates: clinical experience and lessons learnt, Adv. Drug Deliv. Rev. 61 (2009) 1131–1148.
[66] K. Ulbrich, T. Etrych, P. Chytil, M. Jelinkova, B. Rihova, HPMA copolymers with pH-controlled release of doxorubicin: in vitro cytotoxicity and in vivo antitumor activity, J. Control. Release 87 (2003) 33–47.
[67] K. Ulbrich, V. Subr, Structural and chemical aspects of HPMA copolymers as drug carriers, Adv. Drug Deliv. Rev. 62 (2010) 150–166.
[68] G.D. Prestwich, Engineering a clinically-useful matrix for cell therapy, Organogenesis 4 (2008) 42–47.
[69] A.N. Gordon, J.T. Fleagle, D. Guthrie, D.E. Parkin, M.E. Gore, A.J. Lacave, Recurrent epithelial ovarian carcinoma: a randomized phase III study of pegylated liposomal doxorubicin versus topotecan, J. Clin. Oncol. 19 (2001) 3312–3322.
[70] M.E. O'Brien, N. Wigler, M. Inbar, R. Rosso, E. Grischke, A. Santoro, R. Catane, D.G. Kieback, P. Tomczak, S.P. Ackland, F. Orlandi, L. Mellars, L. Alland, C. Tendler, CAELYX Breast Cancer Study Group, Reduced cardiotoxicity and comparable efficacy in a phase III trial of pegylated liposomal doxorubicin HCl (CAELYX/Doxil) versus conventional doxorubicin for first-line treatment of metastatic breast cancer, Ann. Oncol. 15 (2004) 440–449.
[71] Y. Matsumura, T. Hamaguchi, T. Ura, K. Muro, Y. Yamada, Y. Shimada, K. Shirao, T. Okusaka, H. Ueno, M. Ikeda, N. Watanabe, Phase I clinical trial and pharmacokinetic evaluation of NK911, a micelle-encapsulated doxorubicin, Br. J. Cancer 91 (2004) 1775–1781.
[72] W.J. Gradishar, S. Tjulandin, N. Davidson, H. Shaw, N. Desai, P. Bhar, M. Hawkins, J. O'Shaughnessy, Phase III trial of nanoparticle albumin-bound paclitaxel compared with polyethylated castor oil-based paclitaxel in women with breast cancer, J. Clin. Oncol. 23 (2005) 7794–7803.
[73] F. Greco, M.J. Vicent, Combination therapy: opportunities and challenges for polymer-drug conjugates as anticancer nanomedicines, Adv. Drug Deliv. Rev. 61 (2009) 1203–1213.
[74] A. Dicko, L.D. Mayer, P.G. Tardi, Use of nanoscale delivery systems to maintain synergistic drug ratios in vivo, Expert Opin. Drug Deliv. 7 (2010) 1329–1341.
[75] T. Lammers, G. Storm, F. Kiessling, Nanomedicine formulations for combination therapies, Nano Rev. 1 (2010) 5705.
[76] T. Lammers, Improving the efficacy of combined modality anticancer therapy using HPMA copolymer-based nanomedicine formulations, Adv. Drug Deliv. Rev. 62 (2010) 203–230.
[77] T. Lammers, V. Subr, K. Ulbrich, P. Peschke, P.E. Huber, W.E. Hennink, G. Storm, F. Kiessling, HPMA-based polymer therapeutics improve the efficacy of surgery, of radiotherapy and of chemotherapy combinations, Nanomedicine (Lond.) 5 (2010) 1501–1523.
[78] T. Lammers, V. Subr, P. Peschke, R. Kühnlein, W.E. Hennink, K. Ulbrich, M. Heilmann, F. Kiessling, P.E. Huber, G. Storm, Image-guided and passively tumour-targeted polymeric nanomedicines for radiochemotherapy, Br. J. Cancer 99 (2008) 900–910.
[79] T. Dipetrillo, L. Milas, D. Evans, P. Akerman, T. Ng, T. Miner, D. Cruff, B. Chauhan, D. Iannitti, D. Harrington, H. Safran, Paclitaxel poliglumex (PPX-Xyotax) and concurrent radiation for esophageal and gastric cancer: a phase I study, Am. J. Clin. Oncol. 29 (2006) 376–379.
[80] M.J. Vicent, F. Greco, R.I. Nicholson, A. Paul, P.C. Griffiths, R. Duncan, Polymer therapeutics designed for a combination therapy of hormone-dependent cancer, Angew. Chem. Int. Ed. 44 (2005) 4061–4066.
[81] T. Lammers, V. Subr, K. Ulbrich, P. Peschke, P.E. Huber, W.E. Hennink, G. Storm, Simultaneous delivery of doxorubicin and gemcitabine to tumors in vivo using prototypic polymeric drug carriers, Biomaterials 30 (2009) 3466–3475.
[82] K. Miller, R. Erez, E. Segal, D. Shabat, R. Satchi-Fainaro, Targeting bone metastases with a bispecific anticancer and antiangiogenic polymer-alendronate-taxane conjugate, Angew. Chem. Int. Ed. 48 (2009) 2949–2954.
[83] S.A. Abraham, C. McKenzie, D. Masin, R. Ng, T.O. Harasym, L.D. Mayer, M.B. Bally, In vitro and in vivo characterization of doxorubicin and vincristine coencapsulated within liposomes through use of transition metal ion complexation and pH gradient loading, Clin. Cancer Res. 10 (2004) 728–738.
[84] P.G. Tardi, R.C. Gallagher, S. Johnstone, N. Harasym, M. Webb, M.B. Bally, L.D. Mayer, Coencapsulation of irinotecan and floxuridine into low cholesterol-containing liposomes that coordinate drug release in vivo, Biochim. Biophys. Acta 1768 (2007) 678–687.
[85] L.D. Mayer, T.O. Harasym, P.G. Tardi, N.L. Harasym, C.R. Shew, S.A. Johnstone, E.C. Ramsay, M.B. Bally, A.S. Janoff, Ratiometric dosing of anticancer drug combinations: controlling drug ratios after systemic administration regulates therapeutic activity in tumor-bearing mice, Mol. Cancer Ther. 5 (2006) 1854–1863.
[86] P. Tardi, S. Johnstone, N. Harasym, S. Xie, T. Harasym, P. Harvie, D. Bermudes, L. Mayer, In vivo maintenance of synergistic cytarabine:daunorubicin ratios greatly enhances therapeutic efficacy, Leuk. Res. 33 (2009) 129–139.
[87] V. Roy, B.R. LaPlant, G.G. Gross, C.L. Bane, F.M. Palmieri, North Central Cancer Treatment Group, Phase II trial of weekly nab (nanoparticle albumin-bound)-paclitaxel (nab-paclitaxel) (Abraxane) in combination with gemcitabine in patients with metastatic breast cancer (N0531), Ann. Oncol. 20 (2009) 449–453.
[88] C. Lobo, G. Lopes, O. Baez, A. Castrellon, A. Ferrell, C. Higgins, E. Hurley, J. Hurley, I. Reis, S. Richman, P. Seo, O. Silva, J. Slingerland, K. Tukia, C. Welsh, S. Glück, Final results of a phase II study of nab-paclitaxel, bevacizumab, and gemcitabine as first-line therapy for patients with HER2-negative metastatic breast cancer, Breast Cancer Res. Treat. 123 (2010) 427–435.
[89] D.W. Kim, S.Y. Kim, H.K. Kim, S.W. Kim, S.W. Shin, J.S. Kim, K. Park, M.Y. Lee, D.S. Heo, Multicenter phase II trial of Genexol-PM, a novel Cremophor-free, polymeric micelle formulation of paclitaxel, with cisplatin in patients with advanced non-small-cell lung cancer, Ann. Oncol. 18 (2007) 2009–2014.
[90] H.J. Kim, K.H. Kim, J. Yun, S.H. Kim, H.J. Kim, S.C. Lee, S.B. Bae, C.K. Kim, N.S. Lee, K.T. Lee, D.J. Kim, S.K. Park, J.H. Won, D.S. Hong, H.S. Park, Phase II clinical trial of Genexol® (Paclitaxel) and Carboplatin for patients with advanced non-small cell lung cancer, Cancer Res. Treat. 43 (2011) 19–23.
[91] J.A. Sparano, A.N. Makhson, V.F. Semiglazov, S.A. Tjulandin, O.I. Balashova, I.N. Bondarenko, N.V. Bogdanova, G.M. Manikhas, G.P. Oliynychenko, V.A. Chatikhine, S.H. Zhuang, L. Xiu, Z. Yuan, W.R. Rackoff, Pegylated liposomal doxorubicin plus docetaxel significantly improves time to progression without additive cardiotoxicity compared with docetaxel monotherapy in patients with advanced breast cancer previously treated with neoadjuvant-adjuvant anthracycline therapy: results from a randomized phase III study, J. Clin. Oncol. 27 (2009) 4522–4529.
[92] I. Vergote, N.J. Finkler, J.B. Hall, O. Melnyk, R.P. Edwards, M. Jones, J.G. Keck, L. Meng, G.L. Brown, E.M. Rankin, J.J. Burke, R.V. Boccia, C.D. Runowicz, P.G. Rose, Randomized phase III study of canfosfamide in combination with pegylated liposomal doxorubicin compared with pegylated liposomal doxorubicin alone in platinum-resistant ovarian cancer, Int. J. Gynecol. Cancer 20 (2010) 772–780.
[93] R.Z. Orlowski, A. Nagler, P. Sonneveld, J. Blade, R. Hajek, A. Spencer, J. San Miguel, T. Robak, A. Dmoszynska, N. Horvath, I. Spicka, H.J. Sutherland, A.N. Suvorov, S.H. Zhuang, T. Parekh, L. Xiu, Z. Yuan, W. Rackoff, J.L. Harousseau, Randomized phase III study of pegylated liposomal doxorubicin plus bortezomib compared with bortezomib alone in relapsed or refractory multiple myeloma: combination therapy improves time to progression, J. Clin. Oncol. 25 (2007) 3892–3901.
[94] B. Rihova, M. Kovar, Immunogenicity and immunomodulatory properties of HPMA-based polymers, Adv. Drug Deliv. Rev. 62 (2010) 184–191.
[95] B. Rihova, J. Strohalm, J. Prausova, K. Kubackova, M. Jelinkova, L. Rozprimova, M. Sirova, D. Plocova, T. Etrych, V. Subr, T. Mrkvan, M. Kovar, K. Ulbrich, Cytostatic and immunomobilizing activities of polymer-bound drugs: experimental and first clinical data, J. Control. Release 91 (2003) 1–16.
[96] B. Rihova, J. Strohalm, K. Kubackova, M. Jelinkova, O. Hovorka, M. Kovar, D. Plocova, M. Sirova, M. Stastny, L. Rozprimova, K. Ulbrich, Acquired and specific immunological mechanisms co-responsible for efficacy of polymer-bound drugs, J. Control. Release 78 (2002) 97–114.
[97] M. Kovar, T. Mrkvan, J. Strohalm, T. Etrych, K. Ulbrich, M. Stastny, B. Rihova, HPMA copolymer-bound doxorubicin targeted to tumor-specific antigen of BCL1 mouse B cell leukemia, J. Control. Release 92 (2003) 315–330.
[98] M. Kovar, J. Tomala, H. Chmelova, L. Kovar, T. Mrkvan, R. Joskova, Z. Zakostelska, T. Etrych, J. Strohalm, K. Ulbrich, M. Sirova, B. Rihova, Overcoming immunoescape mechanisms of BCL1 leukemia and induction of CD8+ T-cell-mediated BCL1-specific resistance in mice cured by targeted polymer-bound doxorubicin, Cancer Res. 68 (2008) 9875–9883.
[99] M. Sirova, J. Strohalm, V. Subr, D. Plocova, P. Rossmann, T. Mrkvan, K. Ulbrich, B. Rihova, Treatment with HPMA copolymer-based doxorubicin conjugate containing human immunoglobulin induces long-lasting systemic anti-tumour immunity in mice, Cancer Immunol. Immunother. 56 (2007) 35–47.
[100] K.J. Harrington, G. Rowlinson-Busza, K.N. Syrigos, R.G. Vile, P.G. Uster, A.M. Peters, J.S. Stewart, Pegylated liposome-encapsulated doxorubicin and cisplatin enhance the effect of radiotherapy in a tumor xenograft model, Clin. Cancer Res. 6 (2000) 4939–4949.