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Article

Impact of the Enhanced Permeability and Retention (EPR) Effect and Cathepsins Levels on the Activity of Polymer-Drug Conjugates

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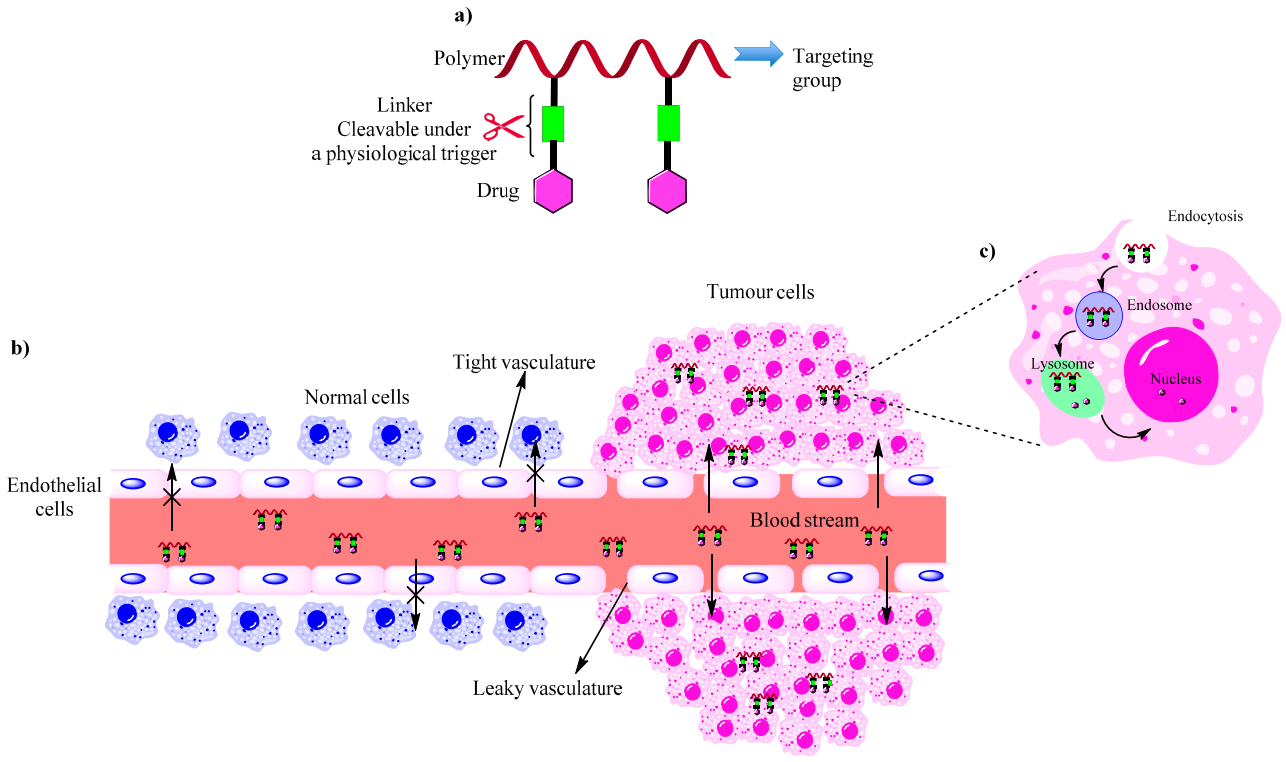
Abstract: Polymer-drug conjugates have demonstrated clinical potential in the context of anticancer therapy. However, such promising results have, to date, failed to translate into a marketed product. Polymer-drug conjugates rely on two factors for activity: (i) the presence of a defective vasculature, for passive accumulation of this technology into the tumour tissue (enhanced permeability and retention (EPR) effect) and (ii) the presence of a specific trigger at the tumour site, for selective drug release (e.g., the enzyme cathepsin B). Here, we retrospectively analyse literature data to investigate which tumour types have proved more responsive to polymer-drug conjugates and to determine correlations between the magnitude of the EPR effect and/or expression of cathepsin B. Lung, breast and ovarian cancers showed the highest response rate (30%, 47% and 41%, respectively for cathepsin-activated conjugates and 31%, 43%, 40%, across all conjugates). An analysis of literature data on cathepsin content in various tumour types showed that these tumour types had high cathepsin content (up to 3835 ng/mg for lung cancer), although marked heterogeneity was observed across different studies. In addition, these tumour types were also reported as having a high EPR effect. Our results suggest that a pre-screening of patient population could bring a more marked clinical benefit.

Keywords: polymer-drug conjugate; enhanced permeability and retention effect; cathepsin; tumour microenvironment

1. Introduction

Polymer-drug conjugates (PDCs) are nano-sized drug delivery systems, in which one or more chemotherapeutic agent is covalently linked to a water-soluble polymer (Figure 1a) [1,2]. The main rationale for PDCs stems from their ability to passively accumulate into the tumour tissue by means of the enhanced permeability and retention (EPR) effect (Figure 1b) [3]. This is a unique feature of tumour vasculature, which makes the tumour vasculature hyper-permeable to macromolecules compared to normal vasculature. As a result, PDCs have been shown to: (a) selectively target tumour tissues; (b) be less toxic and display an extended half-life compared to the parent free drug (e.g., maximum tolerated dose (MTD) for *N*-(2-hydroxypropyl)methacrylamide (HPMA) copolymer-doxorubicin (320 mg/m^2) is four-fold higher than for the parent compound doxorubicin (60 mg/m^2)) [4].

Figure 1. (**a**) Schematic representation of a polymer-drug conjugate (PDC); (**b**) EPR effect facilitating passive tumour targeting of PDCs; and (**c**) Lysosomotropic delivery of PDC.



Nineteen PDCs have undergone/are undergoing clinical evaluation (Table 1 and Figure 2). Clinical trials have shown evidence of tumour responses to PDC to various degrees, and some conjugates (e.g., poly-L-glutamic acid (PGA)-paclitaxel) have progressed to Phase III trials [5,6]. In spite of several advantages of PDCs over the relevant parent drugs, such as a more favourable toxicity profile, superior

quality of life and a significant survival rate [7], to date, no such conjugate has entered the market place, which suggests that system design and/or patient selection are still suboptimal.

When looking at possible reasons for the slow progression of the development of this technology, various factors need to be considered. First, PDCs are intrinsically more complex systems than low molecular weight drugs, for instance they have an inherent degree of variability due to the polydispersity of the carrier. In addition, and of greater significance, the anti-tumour activity of PDCs relies on two factors: (a) passive tumour accumulation via the EPR effect; (b) drug release following a biological stimulus (e.g., enzyme or pH) (Figure 1c). Failure to identify the correct patient/tumour type population, which displays a sufficient level of EPR effect and a sufficient level of enzymes, would negatively bias the results obtained with this technology.

Many studies have reported evidence for the EPR effect in a variety of solid tumours [8–11] but the factors that affect the magnitude of such an effect are still unclear. Recent studies have considered different *in vivo* tumour models and these have reported that both the *size* and tumour *type* can affect the magnitude of the EPR effect [12,13]. A discussion group constituted by world experts of the EPR effect has looked at the heterogeneity of such a phenomenon and has identified its key influencing factors, mainly: (a) the nature of the vascular bed and stroma, including the presence or absence of lymphatics; (b) tumour size, type and location; and (c) patient characteristics such as age, gender, body composition, and treatment [10,13,14]. With regards to expression of the various activating enzymes, again the situation is heterogeneous and unclear. It is well known that enzymes that have been exploited for activation of PDCs, such as cathepsin B, are expressed in tumour tissues [2,13,15–17]. However, it has been recently noted that various factors, such as hormone levels, can affect the abundance of such enzymes. For instance, cathepsin B levels are higher in pre-menopausal women than in post-menopausal women as the former have higher oestrogen levels than the latter [5,18,19].

Table 1. Current clinical status of polymer-drug conjugates (PDCs) and types of release triggers.

Release Trigger	Code/Product Name	Composition	Linker/Spacer	Status	Reference
Enzymatic					
Cathepsins	FCE28068/PK1	HPMA copolymer-doxorubicin	Amide/Peptide [d]	Phase II	[4,20]
Cathepsins	FCE28069/PK2	HPMA copolymer-doxorubicin-galctosamine	Amide/Peptide [d]	Phase I	[21]
Cathepsins	DE-310	Carboxymethyldextran-exatecan	Amide/Peptide [d]	Phase I	[22]
Cathepsins	Delimotecan (MEN 4901/T-0128)	Carboxymethyldextran-T2513	Triglycine	Phase I	[23]
Esterases/Acid hydrolysis [a] Cathepins [b]	CT-2106/PGA-CPT	PGA-camptothecin	Ester	Phase I	[24]
Esterases/Acid hydrolysis [a] Cathepins [b]	CT-2103/PGA-PTX XYOTAX™/ OPAXIO®	PGA-paclitaxel	Ester	Phase III	[5,6,19, 25–34]
Acid hydrolysis/ Cathepsins [c]	AP5280	HPMA copolymer-carboplatinate	Aminomalonate/ Peptide [d]	Phase I/II	[35]

Table 1. *Cont.*

Release Trigger	Code/Product Name	Composition	Linker/Spacer	Status	Reference
Acid hydrolysis/ Cathepsins [c]	AP5346/ProLindac®	HPMA copolymer-DACH oxiplatinate	Aminomalonate/ Peptide [e]	Phase I	[36]
Hydrolysis/ Esterases	PNU166945/ HPMA-PTX	HPMA copolymer-paclitaxel	Ester	Phase I discontinued	[37]
Hydrolysis/ Esterases	PNU166148// HPMA-CPT/MAG-CPT	HPMA copolymer-camptothecin	Ester	Phase I discontinued	[38–40]
Hydrolysis/ Esterases	EZN246/ PEG-CPT/Pegamotecan/ Prothecan™	PEG-camptothecin	Ester	Phase II discontinued	[41–43]
Hydrolysis/ Esterases	PEG-PTX	PEG-paclitaxel	Ester	Phase I discontinued	[44]
Hydrolysis/ Esterases	EZN-2208/ PEG-SN-38	PEG-SN-38	Glycinamidoester	Phase II	[45,46]
Hydrolysis/ Esterases	NKTR-102	PEG-Irinotecan	Glycinamidoester	Phase II/III	[47–50]
Hydrolysis/ Esterases	NKTR-105	PEG-Docetaxel	-	Phase I	[51]
Hydrolysis/ Esterases	XMT-1001	PHF-camptothecin	Succinamidoester	Phase I	[52]
Esterases	CRLX101/IT-101	Cyclodextrin-camptothecin	Glycinamidoester	Phase II	[53]
Non-enzymatic					
pH-sensitive	ONCOFID-PTM	HA-paclitaxel	Hydrazone	Phase I/II	[54]
	AD-70, DOX-OXD	Oxidised dextran-doxorubicin	Schiff's base	Phase I discontinued	[55]

Notes: [a] Mediates the cleavage of the active drug from the polymeric backbone; [b] Mediates the cleavage of the biodegradable polymeric backbone; [c] Mediates the cleavage of the peptide linker/spacer; [d] Spacer-Gly-Phe-Leu-Gly; [e] Spacer-Gly-Gly-Gly. HPMA, *N*-(2-Hydroxypropyl) methacrylamide; PGA, Poly-L-glutamic acid; PEG, Polyethylene glycol; PHF, Poly(1-hydroxyl-methylethylene hydroxyl-methyl-formal); HA, Hyaluronic acid.

In this paper, we retrospectively analyse data obtained from the literature concerning clinical trials carried out on PDCs in order to determine whether there is a connection between the clinical responses of various tumour types and the levels of enzyme expression/magnitude of the EPR effect in such tumour types. First, we analysed clinical data within the literature to identify the tumour types for which marked tumour responses were observed. Then, we documented the content of cathepsin B that has been reported for the various tumour types. Finally, we determined the extent of the EPR effect, which has been, reported for these various tumour types.

Figure 2. Chemical structures of the PDCs discussed in this article. (**a**) PDCs for which the release of the drug is triggered via an enzymatic mechanism; and (**b**) PDCs for which the release of the drug is triggered by a non-enzymatic mechanism. Note: The chemical structure of NKTR-105 is not available.

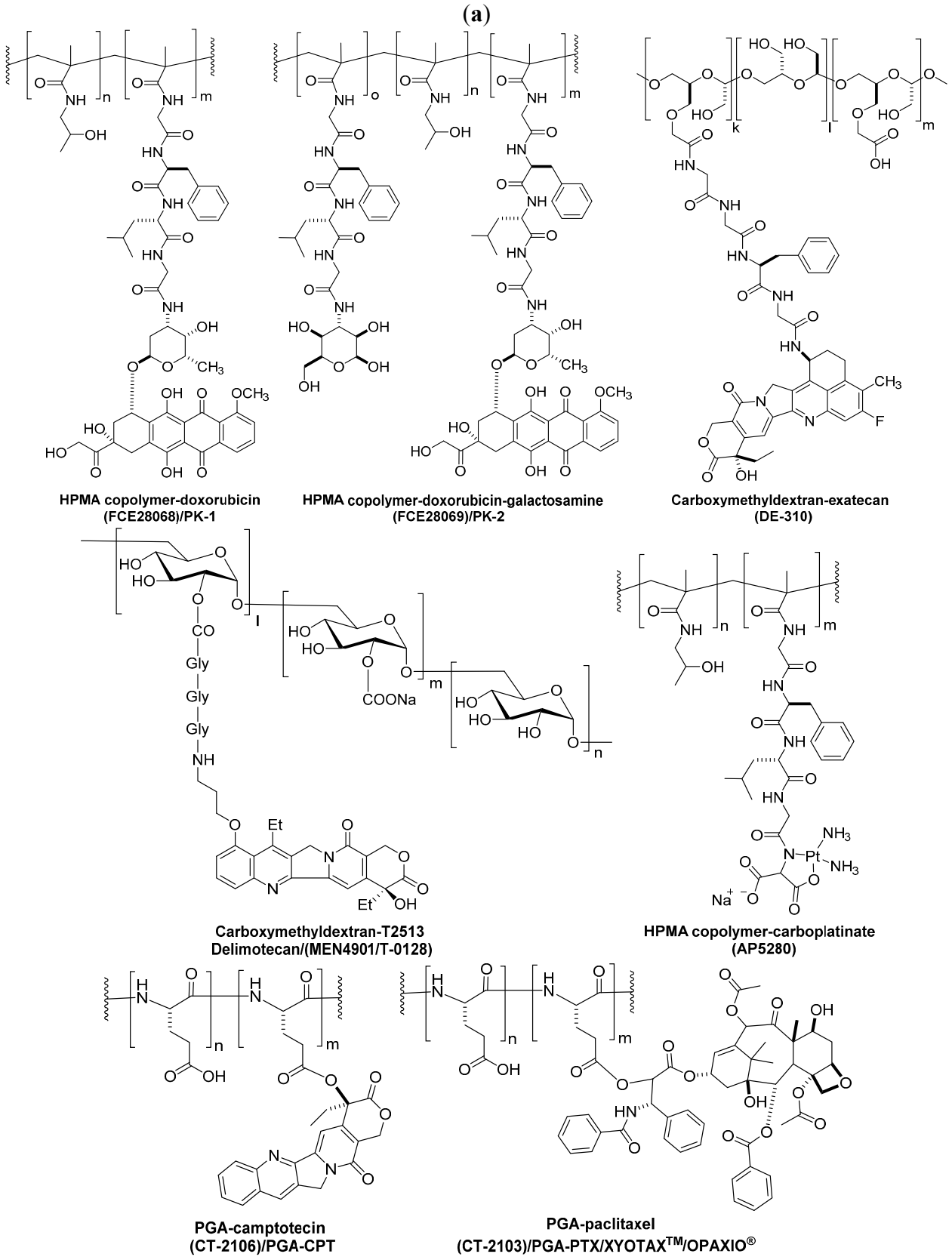
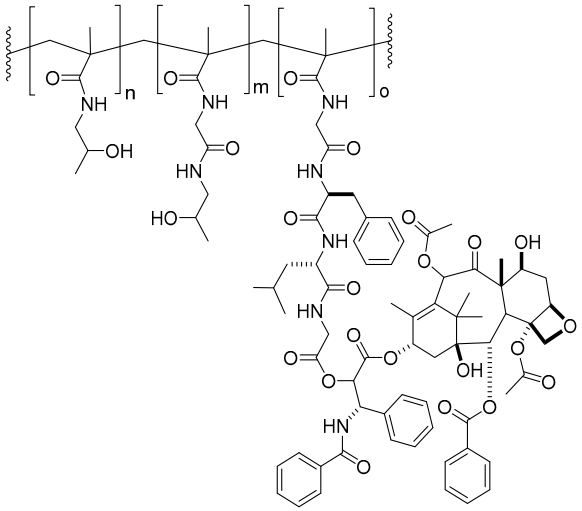
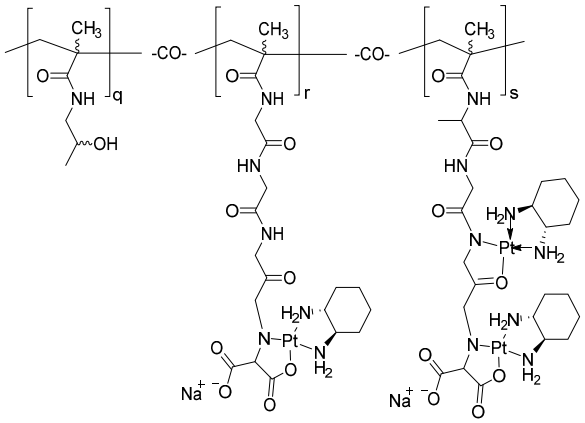


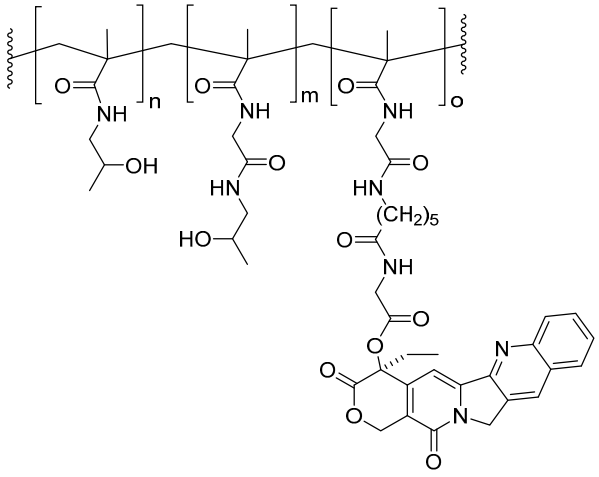
Figure 2. *Cont.*



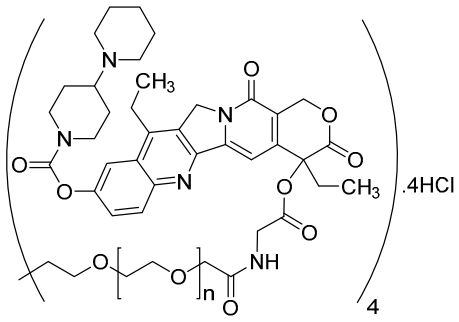
HPMA copolymer-paclitaxel (PNU16695)/HPMA-PTX



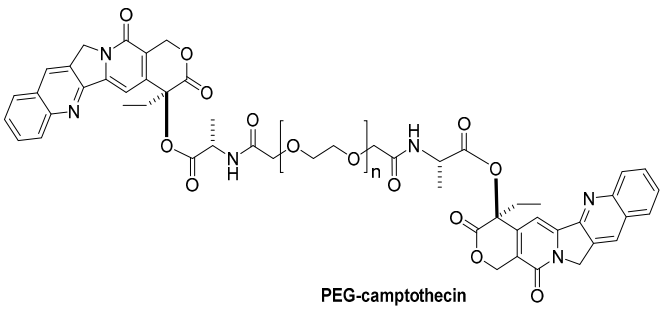
HPMA copolymer-DACH carboplatinate (AP5346)/ProLindac



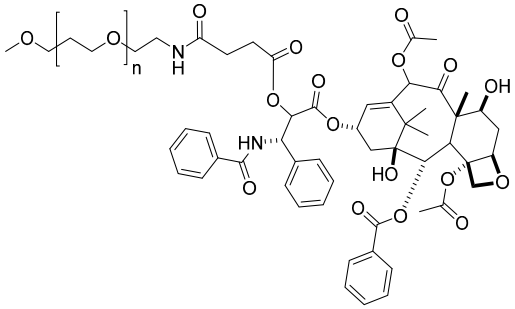
HPMA copolymer-camptothecin (PNU166148)/HPMA-CPT/MGA-CPT



PEG-Irinotecan (NKTR-102)

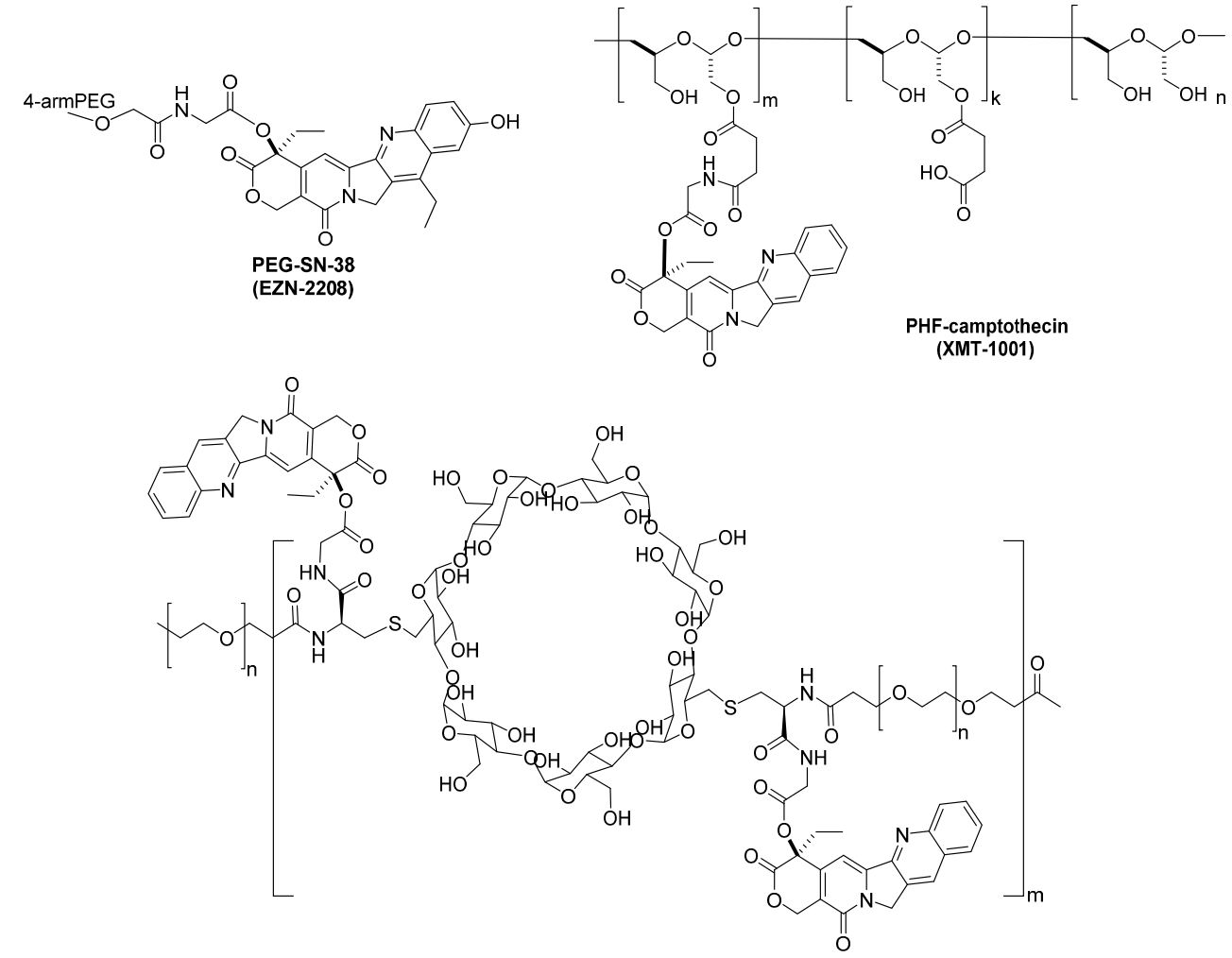


PEG-camptothecin (EZN246)/PEG-CPT/Pegamotecan/Prothecan™

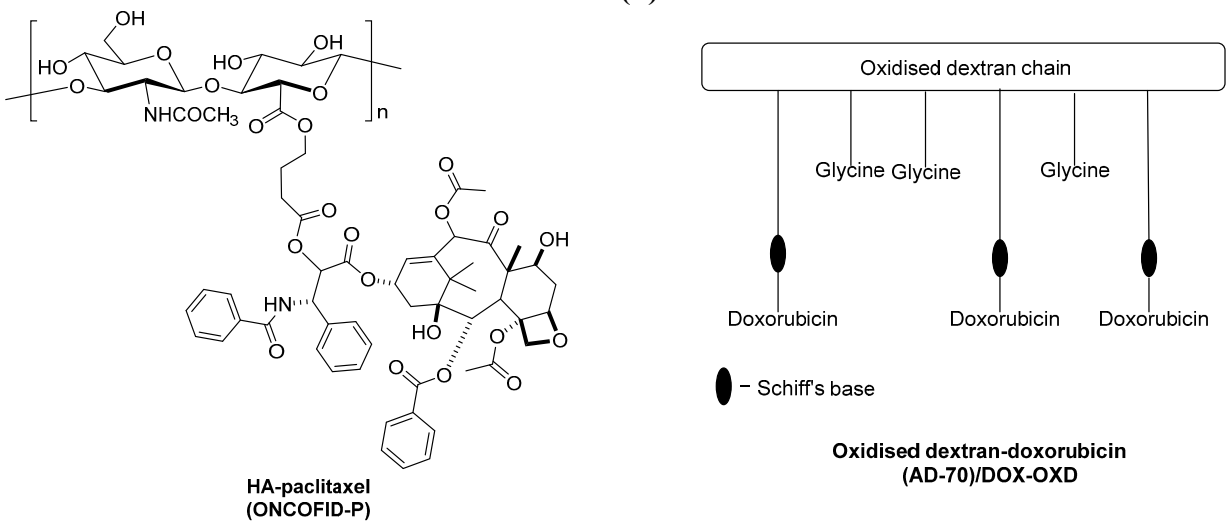


PEG-paclitaxel (PEG-PTX)

Figure 2. *Cont.*



(b)



2. Experimental Section

The data presented in this paper were collected via systematic literature searches using search engines such as PubMed, Science Direct and Web of Science. No date restrictions were applied to the searches.

2.1. Terminology

This paper reports data collected from various sources that are derived from both clinical and pre-clinical studies. Different studies used different terminologies when referring to the various cancer types, with the terminologies ranging from technical/very specific (e.g., glioblastoma) to more general (e.g., brain cancer). In addition, in some cases, the effects of the tumours on adjacent organs are reported (e.g., gastroesophageal cancer). To ensure that a consistent terminology is used within this paper, clinical tumour types and preclinical models were grouped under general umbrella terms summarised in Table 2.

Table 2. Summary of the terminology used in this article to identify various tumour types/tumour models.

Tumour Type (Terminology Used in This Article)	**Tumour Type (Terminology Used in the Original Reference)**
Breast	Breast, mammary gland, MCF-7, MDA-MB-231, BT 20 and DU4476-cell lines, Walker 256-murine model, MX-1, MAXF 449-human xenograft.
Colon and/or rectum	Colon, rectum, colorectal, anus, C26 NL-17, HT-29, LS174T-human xenograft.
Head/Neck and brain	Brain, glioblastoma, head/neck, thyroid, salivary gland, follicular, papillary, tongue, maxillary sinus, parotid gland, IMR-32, SK-N-SH, SK-N-DZ- human xenograft.
Lung	Non-small cell lung cancer, small cell lung cancer, bronchial, Meta-7-murine model, H522, COR L23-human xenograft.
Oesophagus, stomach and intestine	Oesophageal, cardioesophageal, stomach, gastroesophageal, gastrointestinal, intestine, small bowel, peritoneal carcinosis, OCUM-2MLN-human xenograft.
Ovary	Ovarian, Oca-1-murine model, A2780 cell line/human xenograft, SK-OV-3, OVCAR-3-human xenograft.
Pancreas	Pancreatic, PAXF 546-human xenograft.
Skin	Melanoma, basal cell, histosarcoma, B16F10, A431-murine model, MEXF 276-human xenograft.
Urinary	Bladder, urinary tract, urethal, urothelial, urachus.
Others: The term indicates the tumour types which were either studied in a very low sample size ($n < 3$) and/or those for which low responses were observed.	Adrenal, adenoid cystic, adenocarcinoma (unknown primary), bone (ewing sarcoma, osteosarcoma), cervix (uterine, leiomysarcoma uteri, ME180-human xenograft), fibrosarcoma (S-180, Meth A-murine model), gall bladder, kidney, leiomysarcoma, liver (cholangiocarcinoma, ampullary, bile duct, L1210-murine model, VX-2 carcinoma), lymphoma, mesothelioma, neuroendocrine, prostrate, sarcoma (unknown), soft tissue sarcoma, squamous cell sarcoma (unknown origin), solid tumours (unknown), unknown primary tumours, MAC 15A-murine model, MAC 26-murine model, RXF 486, RXF 1220, ME 180-human xenograft.

2.2. Clinical Status of the PDCs

The data on the current clinical status of the PDCs, reported in Table 1, were gathered from articles obtained using the key word "polymer-drug conjugate*". The clinical data related to each individual conjugate, which are reported in the supplementary information, were gathered using the name of the individual conjugate.

The percentage tumour response reported for each conjugate, per tumour type, was determined considering all of the clinical responses observed (*i.e.*, number of (partial response (PR) + complete response (CR) + stable disease (SD) + minor responses (MR)), as well as the total number of patients evaluated per tumour type using the following formula:

Response to conjugate A in tumour X (%) = (Total clinical responses observed for conjugate A in tumour X/ Number of patients evaluated for conjugate A in tumour X) × 100

The results obtained for each conjugate for each tumour type (reported in supplementary information, Tables S1 to S19) were then further processed in order to obtain an overall response to all PDCs per tumour type.

The overall percentage tumour response for each tumour type was determined using the following formula:

Overall response in tumour X (%) = Σ[(Response to conjugate A in tumour X (%) × number of patients with tumour X in which conjugate A was tested) + (Response to conjugate B in tumour X (%) × number of patients with tumour X in which conjugate B was tested) + (Response to conjugate *n* in tumour X (%) × number of patients with tumour X in which conjugate *n* was tested)]/Total number of patients with tumour X

2.3. Cathepsin Level

The data on cathepsin levels in various tumour types were collected via literature searches using the following key words and their combinations: "cathepsin level*", "cathepsin content*", "tumour/tumor types".

2.4. EPR Effect

The data related to the EPR effect in different tumour types were retrieved via literature searches using the following key words (and their combinations): "enhanced permeability and retention effect", "EPR effect", "tumour/tumor*", "drug accumulation", "biodistribution" and the names of the individual conjugates. In addition, original research articles quoted in reviews related to the EPR effect, which were not identified from the general search, were also considered.

Priority was given to articles related to PDCs, however, as the EPR effect is a phenomenon that applies to any macromolecular system, clinical and preclinical data relating to other such systems (e.g., liposomes and micelles) were also included.

3. Results and Discussion

Many clinical studies have been carried out on PDCs (e.g., OPAXIO) [26]. The main clinical outcomes have been summarized in extensive reviews [55–57], but, as yet, a detailed analysis of which tumour types are proving more responsive to this type of drug delivery technology is still missing. A number of studies have already attempted to identify correlations between tumour types and enzyme content or the magnitude of the EPR effect, but this approach has been largely carried out in preclinical models and systematic assessments of these factors and clinical activities are missing, which is surprising since the latter are two key pre-requisites for the activity of PDCs.

In an attempt to provide a more comprehensive study that links clinical outcomes, enzyme content, and the EPR effect, we initially documented all the PDCs that have been explored in clinical trials, alongside the triggers that have been used to promote drug release in each (Table 1). Of the 19 conjugates that have been tested in patients, the vast majority (17) rely, at least partially, on the presence of enzymes for drug release (Table 1). Of the various enzymes exploited as triggers, cathepsins, a family of lysosomal proteases, are the most widely targeted (eight of the conjugates reported in Table 1 rely on cathepsins action for drug release). This is not surprising, as cathepsins have been reported to be linked with cancer progression. In particular, cathepsin B has been connected with tumour invasion [58].

3.1. Effect of Tumour Type on Clinical Response

To assess the clinical responses observed in different tumour types, we analysed clinical data for PDCs retrieved from our literature searches. Although the purpose of phase I, II and III clinical trials and their patient selection criterias are different, the objective responses (*i.e.*, partial response (PR), complete response (CR), stable disease (SD) and minor responses (MR)) are considered as the end point in all such studies [59]. Therefore for each conjugate, evidence of a clinical response (PR, CR SD and MR) in the various tumour types has been summarised in detail in Tables S1–S19 in the supplementary information. A representative data set with clinical responses for the top three highest responsive tumour types for each conjugate has been provided in Table 3. For example, in the case of HPMA copolymer-doxorubicin (PK1; FCE28068), the top three highest responsive tumour types are lung, breast, colon and/or rectum, with tumour responsive rate of 57%, 53%, and 4%, respectively calculated from both phase II clinical trials and I. Likewise, the top three highest responsive tumour types based on the calculation from all the available clinical phase trials have been summarised for the other eighteen PDCs.

Table 3. Clinical data for the top three tumour types for the nineteen PDCs that are released by both cathepsins and change in pH.

Tumour Type	No. of Patients per Tumour Total [a] [Ph I/Ph II/Ph III]	Clinical Responses [b] Total (Ph I/Ph II/Ph III)						Tumour Response Rate [c] (%) Total [Ph I/Ph II/Ph III]
		No. of SD	No. of PR	No. of MR	No. of CR	No. of OS	No. of NR	
HPMA copolymer-doxorubicin (PK1; FCE28068) [4,20]								
Lung	31[(2/29(21) */-]	8[-/8/-]	5[2/3/-]	-	-	-	10[0/10/-]	57[100/52/-]
Breast	20[3/17(14) */-]	5[-/5/-]	3[-/3/-]	1[1/-/-]	-	-	8[2/6]	53[33/57/-]
Colon and/or rectum	24[8/16/-]	-	-	1[1/-/-]	-	-	23[7/16/-]	4[4/-/-]
HPMA copolymer-doxorubicin-galactosamine (PK2; FCE28069) [21]								
Liver	25[25/-/-]	-	2[2/-/-]	1[1/-/-]	-	-	22[22/-/-]	12[12/-/-]
Colon and/or rectum	6[6/-/-]	-	-	-	-	-	6[6/-/-]	0
Carboxymethyldextran-exatecan; DE-310 [22]								
Adenocarcinoma (Unknown primary)	2[2/-/-]	1[1/-/-]	-	-	1[1/-/-]	-	0	100[100/-/-]
Pancreas	3[3/-/-]	2[2/-/-]	1[1/-/-]	-	-	-	0	100[100/-/-]
Urinary	1[1/-/-]	1[1/-/-]	-	-	-	-	0	100[100/-/-]
Delimotecan; MEN 4901/T-0128 [23]								
Head/Neck and brain	2[2/-/-]	-	1[1/-/-]	-	-	-	1[1/-/-]	50[50/-/-]
Colon and/or rectum	7[7/-/-]	-	1[1/-/-]	-	-	-	6[6/-/-]	14[14/-/-]
Mesothelioma	3[3/-/-]	-	-	-	-	-	3[3/-/-]	0
Poly-L-glutamic acid-camptothecin; PGA-CPT; CT-2106 [24]								
Breast	4[4/-/-]	1[1/-/-]	-	-	-	-	3[3/-/-]	25[25/-/-]
Skin	14[14/-/-]	2[2/-/-]	-	-	-	-	12[12/-/-]	14[14/-/-]
Bone	1[1/-/-]	-	-	-	-	-	1[1/-/-]	0
Poly-L-glutamic acid-paclitaxel; PGA-PTX; CT2103; XYOTAX; OPAXIO® [5,6,19,24–33]								
Breast	18[-/18/-]	2[-/2/-]	4[-/4/-]	4[-/4/-]	-	-	8[-/8/-]	56[-/56/-]
Ovary	99[-/99/-]	32[-/32/-]	10[-/10/-]	-	-	-	57[-/57/-]	42[-/42/-]
Mesothelioma	3[3/-/-]	-	-	1[1/-/-]	-	-	2[2/-/-]	33[33/-/-]

Table 3. *Cont.*

Tumour Type	No. of Patients per Tumour Total [a] [Ph I/Ph II/Ph III]	Clinical Responses [b] Total (Ph I/Ph II/Ph III)						Tumour Response Rate [c] (%) Total [Ph I/Ph II/Ph III]
		No. of SD	No. of PR	No. of MR	No. of CR	No. of OS	No. of NR	
HPMA copolymer-carboplatin; HPMA-carboplatin; AP5280 [35]								
Lung	4[4/-/-]	2[2/-/-]	-	-	-	-	2[2/-/-]	50[50/-/-]
Ovary	2[2/-/-]	1[1/-/-]	-	-	-	-	1[1/-/-]	50[50/-/-]
Colon and/or rectum	12[12/-/-]	2[2/-/-]	-	-	-	-	10[10/-/-]	17[17/-/-]
HPMA copolymer-platinate; HPMA-Pt; AP5346 [36]								
Cervix	1[1/-/-]	1[1/-/-]	-	-	-	-	0	100[100/-/-]
Oesophagus, stomach and intestine	1[1/-/-]	1[1/-/-]	-	-	-	-	0	100[100/-/-]
Skin	5[5/-/-]	1[1/-/-]	1[1/-/-]	-	-	-	3[3/-/-]	40[40/-/-]
Polyethylene-camptothecin; PEG-CPT; EZN246; Pegmaotecan; Prothecan™ [41–43]								
Bone	1[1/-/-]	-	-	1[1/-/-]	-	-	0[0/-/-]	100[100/-/-]
Oesophagus, stomach and intestine	42[7/35/-]	14[-/14/-]	5[-/5/-]	3[2/1/-]	-	-	20[5/15/-]	52[29/57/-]
Unknown primary	5[5/-/-]	-	-	1[1/-/-]			4[4/-/-]	20[20/-/-]
Multiarm-polyethylene-SN38; EZN-2208 [45,46]								
Urinary	1[1/-/-]	1[1/-/-]	-	-	-		0[0/-/-]	100[100/-/-]
Oesophagus, stomach and intestine	3[3/-/-]	2[2/-/-]	-	-	-		1[1/-/-]	67[67/-/-]
Breast	3[3/-/-]	2[2/-/-]	-	-	-		1[1/-/-]	67[67/-/-]
PHF-CPT; MER-1001; XMT-1001 [52]								
Skin	2[2/-/-]	1[1/-/-]	-	-	-	-	1[1/-/-]	50[50/-/-]
Lung	7[7/-/-]	3[3/-/-]	-	-	-	-	4[4/-/-]	43[43/-/-]
Solid tumours (Unspecified)	8[8/-/-]	3[3/-/-]	-	-	-	-	5[5/-/-]	38[38/-/-]
Cyclodextrin-camptothecin; CRLX101; IT-101 [53]								
Lung	27[27/-/-]	16[16/-/-]	-	-	-	-	11[11/-/-]	59[59/-/-]
Solid tumours *	35[35/-/-]	28[28/-/-]	-	-	-	-	7[7/-/-]	80[80/-/-]

Table 3. *Cont.*

Tumour Type	No. of Patients per Tumour Total [a] [Ph I/Ph II/Ph III]	Clinical Responses [b] Total (Ph I/Ph II/Ph III)						Tumour Response Rate [c] (%) Total [Ph I/Ph II/Ph III]
		No. of SD	No. of PR	No. of MR	No. of CR	No. of OS	No. of NR	
HA-paclitaxel (ONCOFID-P™) [54]								
Urinary	15[15/-/-]	-	-	-	9[9/-/-]	-	6[6/-/-]	60[60/-/-]
Oxidized dextran-Dox; OXD-DOX (AD-70) [55]								
Colon and/or rectum	6[6/-/-]	1[1/-/-]	-	-	-	-	5[5/-/-]	17[17/-/-]
Oesophagus, stomach and intestine	2[2/-/-]	-	-	-	-	-	2[2/-/-]	0
Lung	2[2/-/-]	-	-	-	-	-	2[2/-/-]	0
HPMA copolymer-paclitaxel; HPMA-PTX; PNU166945 [37]								
Solid tumours [1]	12[12/-/-]	2[2/-/-]	1[1/-/-]	-	-	-	9[9/-/-]	25[25/-/-]
HPMA copolymer-camptothecin; HPMA-CPT; PNU166148 [38–40]								
Solid tumours [2]	40[40/-/-]	5[5/-/-]	-	1[1/-/-]	-	-	34[34/-/-]	15[15/-/-]
Polyethylene glycol-paclitaxel; PEG-PTX [44]								
Solid tumours [3]	13	NA					NC	NC
Multi-arm-polyethylene glycol-pacliaxtel; PEG-PTX; NKTR-102 [47–49]								
Solid tumours [4]	125[32/68/-]	28[-/28/-]	24[7/17/-]	6[6/-/-]	2[2/-/-]	-	65[8/23/-]	48[47/66/-]
Multi-arm-polyethylene glycol-docetaxel; NKTR-105 [51]								
NA	17	NA	NA	NA	NA	NA	NC	NC

Notes: [a] Indicates total number of patients considered for the clinical evaluation; [b] Clinical responses includes the total of SD, Stable disease; PR, Partial response; MR, Minor response; CR, Complete response; OS, Overall survival; NR, No response; [c] Tumour response rate (%) is the added responses (SD + PR + MR + CR + OS) per tumour type. NA, Not available, NC, Not calculated. * Indicates number of patients evaluated for the tumour responses. The term "Solid tumours" has been mentioned wherever the clinical responses per tumour type have not been reported in the respective study. [1] Solid tumours includes (number of patients is denoted in the brackets for those that are available in the study)-Ovary (4); Breast (2); Colon and/or rectum (2); Lung (1) and others (3); [2] Solid tumours includes-Colon and/or rectum (18); Ovary (2); Oesophagus, stomach and intestine (4); Unknown primary (4); Head/Neck and brain (2); Lung (6); Kidney (3); Adrenal (1); Cervix (1); Bone (1); Mesothelioma (1); Prostate (1) and Sarcoma (3); [3] Solid tumours includes-Colon and/or rectum (3); Breast (2); Neuroendocrine (2); Lung (1); Prostate (1) and Others (4); [4] Solid tumours includes-Ovary; Breast; Adrenal; Oesophagus, stomach and intestine; Lymphoma; Lung; Cervix; Head/Neck and brain; Urinary and Breast.

Finally, summary graphics assembling the percentage responses per tumour type for all conjugates were compiled (Figure 3 and Table 4). Figure 3 and Table 4 shows the percentage responses observed for cathepsin-activated conjugates. Breast, ovary and lung cancer were the cancer types in which the highest response rates were observed (>30%). However, the responses observed for the various conjugates in each tumour type were variable, for example, the HPMA copolymer-oxaliplatin conjugate (AP5346/ProLindac) showed no responses in breast cancer while the HPMA copolymer-doxorubicin conjugate (PK1) showed 53% response in that tumour. This variability might be due to the fact that the latter relies solely on cathepsins for drug release, and hence has a more specific activation than the former, for which both cathepsins and acid hydrolysis play a role in the release of oxaliplatin. This finding however can also be affected to some extent by the nature of the therapeutic agent.

Figure 3. Graphical representation of the tumuor response rates for PDCs (release mediated only by cathepsin).

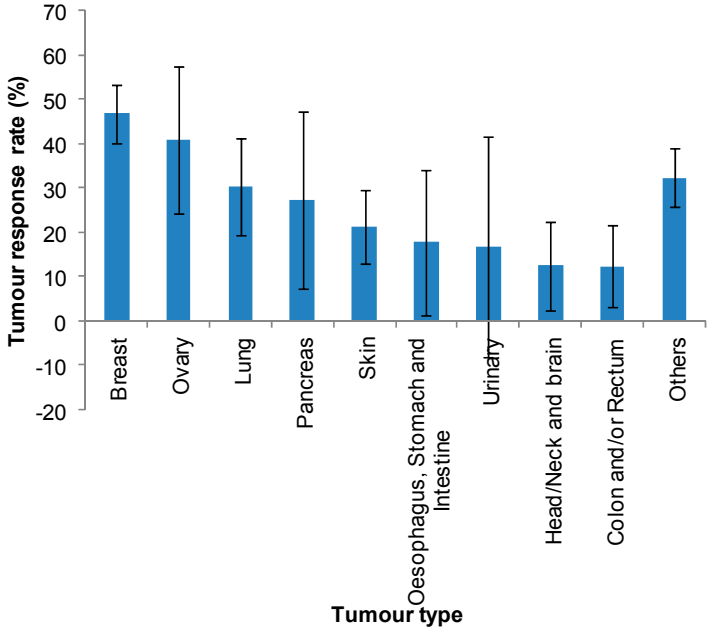


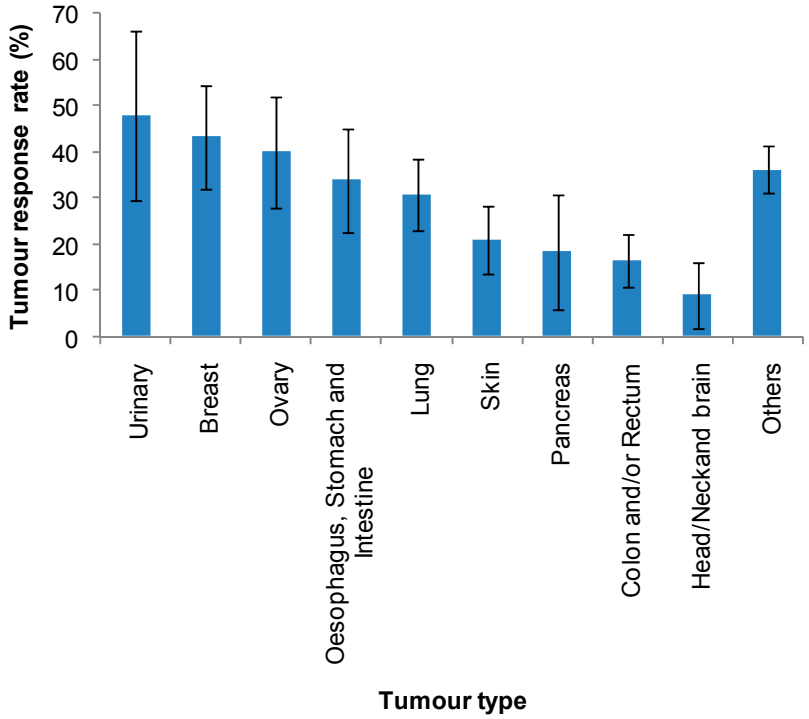
Table 4. Tumour response rates for PDCs (release mediated only by cathepsin).

Tumour Type / PDC	Tumour response rate (%)								
	Breast	**Ovary**	**Lung**	**Pancreas**	**Skin**	**Oesophagus, Stomach and Intestine**	**Urinary**	**Head/Neck and Brain**	**Colon and/or Rectum**
PK1	53	0	56	0	-	0	0	0	4
PK2	-	-	-	-	-	-	-	-	0
DE-310	-	100	67	100	33	0	100	-	67
MEN4901	-	-	0	-	0	0	0	50	14
CT-2106	25	-	0	0	14	-	-	-	0
CT-2103	56	42	30	-	-	21	-	0	0
AP5280	-	50	50	0	0	0	-	0	16
AP5346	0	25	0	0	40	100	0	0	-
Normalised Average *	47	41	30	27	21	18	17	13	12
SD	26	37	29	45	19	40	50	22	24
SE	13	17	11	20	8	16	25	10	9

Notes: * Averages were normalised to take into account of the number of patients evaluated for each conjugated, see experimental section; "-" indicates that the conjugate was not evaluated against the particular tumour type.

Figure 4 and Table 5 show the result for all conjugates, independent of their activation mechanism. The results are similar to that observed for cathepsin-activated PDCs, with breast, ovary and lung cancer still achieving >30% responses. However, urinary cancer and cancers of the oesophagus, stomach and intestines showed a high response as well (48% and 34%, respectively).

Figure 4. Graphical representation of the tumuor response rates for PDCs (release mediated both by cathepsin and pH).



3.2. Cathepsin Levels in Different Tumour Types

Having assessed which tumour types showed the highest responses to PDCs, we investigated which tumour types had been reported to express the highest levels of cathepsins.

Cathepsins are lysosomal cysteine proteases involved in the bulk degradation of intracellular and endocytosed proteins. Cathepsin B is a large lysosmal protease that is generally over expressed in tumour tissues and plays a very important role in the release and activation of PDCs. Several studies have indicated that its co-existing isoenzymes such as cathepsin D, L, H, S and X are also overexpressed in tumour tissues and are thought to play a role in proteolysis [60–63]. Some studies have been carried out to elucidate precisely which isoenzyme contributes to drug release. For example, extensive studies carried out in the 80s have identified cathepsin B as the primary enzyme responsible for doxorubicin release from the HPMA copolymer-Dox conjugate, when a Gly-Phe-Leu-Gly (GFLG) linker was used. However, the release mechanism for other conjugates has not been investigated so extensively. Also, it is known that various isoenzymes can contribute to drug release from peptidyl linkers, for example, both cathepsin L and cathepsin B were found to contribute to the release of daunorubicin when GPLG and GPPL linkers were used [64]. Similarly, both cathepsin B and cathepsin H contributed to the release of 5-FU, albeit to a different extent [65]. The level of expression of these cathepsins in tumour

tissues (especially cathepsin B) is very crucial for the selective release of the drug from the PDC within the tumour environment (Table 1). Also, several studies have indicated that cathepsin expression in tumours varies greatly [63] which is affected by various parameters including gender, age and hormone levels [14,18]. Therefore, to determine if there is a correlation between the level of cathepsin expression in a tumour type, and the clinical response to cathepsin-activated PDCs, data on cathepsin levels is presented for clinical as well as for *in vivo* and *in vitro* samples of different tumour types to obtain reliable results (Table 6). The information on tumour grades and other parameters that are related to the cathepsin's expression were limited in the original studies, hence these details have not been considered in our present study.

Table 5. Tumour response rates for PDCs (release mediated both by cathepsin and pH).

Tumour type / PDC	Tumour response rate (%)								
	Urinary	**Breast**	**Ovary**	**Oesophagus, Stomach and Intestine**	**Lung**	**Skin**	**Pancreas**	**Colon and/or Rectum**	**Head/ Neck and Brain**
PK1	0	53	0	0	56	-	0	4	0
PK2	-	-	-	-	-	-	-	0	-
DE-310	100	-	100	0	67	33	100	67	-
MEN4901	0	-	-	0	0	0	-	14	50
CT-2106	-	25	-	-	0	14	0	0	-
CT-2103	-	56	42	21	30	-	-	0	0
AP5280	-	-	50	0	50	0	0	16	0
AP5346	0	0	25	100	0	40	0	-	0
PNU166945 [a]									
PNU166148 [a]									
EZN246	-	0	0	47	14	0	0	0	0
PEG-PTX					NC				
EZN2208	100	67	0	-	50	-	33	20	-
NKTR-102	-	-	-	1	-	-	-	-	-
NKTR-105					NC				
XMT-1001	-	0	33	33	43	50	11	18	-
IT-101	-	-	-	-	59	-	-	-	-
ONCOFID-P	60	-	-	-	-	-	-	-	-
AD-70	0	-	-	0	0	0	-	17	0
Normalised average *	48	43	40	34	31	21	18	16	9
SD	48	30	34	36	26	21	35	20	19
SE	18	11	12	13	8	7	12	6	7

Notes: * Averages were normalised to take into account of the number of patients evaluated for each conjugated, see experimental section; "-" indicates that the conjugate was not evaluated against the particular tumour type; [a] The clinical responses for this conjugate are considered under the tumour type "others" as the responses per specific tumour type has not been mentioned in the original references [36–39]. NC: Not considered.

Table 6. Cathepsin levels found in clinical and preclinical sample of different tumour types (clinical studies highlighted in grey).

Tumour Type	Type of Cathepsin (CAT)	Clinical (C)/ Pre-Clinical (PC)/*In vitro* (IV)	Sample Size (*n*)	Cathepsin Content	Reference
Lung	CAT B	C	105	10.65 ng/mL	[66]
	CAT B	C	17	448 ng/mg of protein	[67]
	CAT B	PC	159	High *	[68]
	CAT D	C	17	1304 ng/mg of protein	[67]
	CAT S	C	60	4.2 ± 0.22 ng/mg of protein	[69]
	CAT H	C	123	172 ± 86 ng/mg of protein	[70]
	CAT L	C	105	26.16 ng/mL	[66]
	CAT L	C	17	3835 ng/mg of protein	[68]
Head/Neck and brain	CAT B	C	84	High *	[71]
	CAT B	C	47	High *	[72]
	CAT B	C	32	High *	[73]
	CAT B	PC	NA	High *	[74]
	CAT B	PC	NA	High *	[75]
	CAT B	PC	11	High *	[76]
	CAT B	PC	NA	High *	[77]
	CAT D	PC	7	1300 ng/mg of protein	[78]
	CAT S	PC	11	Low *	[76]
	CAT H	PC	7	1500 ng/mg of protein	[79]
	CAT L	PC	11	Low *	[76]
Oesophagus, stomach and intestine	CAT B	C	25	325.9 ng/mg of protein	[80]
	CAT B	C	175	10.83 ± 1.8 ng/mL	[81]
	CAT B	PC	NA	Low *	[67]
	CAT L	C	25	43.6 ng/mg of protein	[80]
Colon and/or rectum	CAT B	C	72	13.38 ng/mL	[81]
	CAT B	C	108	168 ± 86 ng/mg of protein	[82]
	CAT B	C	60	253.5 ng/mg of protein	[82]
Colon and/or rectum	CAT B	C	74	55 ± 5 hg/mg of protein	[83]
	CAT B	PC	40	High *	[84]
	CAT X	C	77	17.4 ng/mL	[85]
	CAT H	C	74	7 ± 1 ng/mg of protein	[83]
	CAT L	C	74	50 ± 10 ng/mg of protein	[83]
	CAT L	C	60	274 ng/mg of protein	[82]
Breast	CAT B	C	30	74 ng/mg of protein	[86]
	CAT B	PC (DU4475)	4	High *	[86]
	CAT B	IV (BT20)	NA	Low *	[87]
	CAT D	C	57	High *	[88]
	CAT X	IV (MCF-7)	NA	2.5 ng/mL	[89]
	CAT X	IV (MDA-MB-231)	NA	37 ng/mL	[89]

Table 6. *Cont.*

Tumour Type	Type of Cathepsin (CAT)	Clinical (C)/ Pre-Clinical (PC)/*In vitro* (IV)	Sample Size (*n*)	Cathepsin Content	Reference
Ovary	CAT L	C	318	16.1 ± 5.1 ng/mL	[90]
Pancreas	CAT B	PC	NA	High *	[91]
Urinary	CAT B	PC	7	High *	[67]
Others					
Liver	CAT B	C	28	13.46 ng/mL	[81]

Notes: * The cathepsin levels are expressed as high and low (rather than a specific value) for those entries in which definite quantitative values were not available in the original study; NA, Not applicable; "*n*" = number of human/animal subjects.

Two tumours, lung and breast, which have shown the highest percentage response clinically to PDCs activated by cathepsins, also showed a generally high content of this class of enzyme (in some studies, as high as 3835 ng/mg for lung cancer). This correlation is as expected, as the presence of a trigger is essential for the activation of a prodrug, but has not been widely reported from analysis of experimental data. It should also be noted that the content of cathepsins reported in various studies concerning the same tumour type were highly variable. This finding suggests that heterogeneity might be also present in the tumour samples of the patients that have undergone treatment with PDCs and this could potentially explain why only a fraction of the patients enrolled in the clinical studies responded to the treatment [92,93]. Differences in the content of cathepsins are particularly marked for breast cancer. For example, the content of cathepsin B was reported to be "high" in the study from Bremer *et al.* [86] but "low" from that of Hulkower *et al.* [87]. It is also key to highlight that, as mentioned previously, cathepsins level have been found to be affected by oestrogen levels and therefore an additional element of variability is added depending on whether the sample is taken from a post-menopausal or pre-menopausal woman [5,18].

3.3. Magnitude of the EPR Effect in Different Tumour Types

A second, but at least equally important, factor for activity of PDCs is the extent of the EPR effect in a tumour. To establish if there was a correlation between the magnitude of the EPR effect and the tumour's response to PDCs, data on drug accumulation of different drug delivery technologies such as PDCs and liposomes in clinical tumour samples as well as in preclinical samples were collected as summarised in Table 7. Although this comparison holds a limitation with respect to the heterogeneity of systems (different nanoconstructs and different phases of clinical research) and heterogeneous tumour types, pooling the results from such heterogeneous data was necessary due to the limited clinical studies on EPR effect with PDCs. Different methodologies have been used in different studies to evaluate the extent of passive accumulation in the tumour both qualitatively and quantitatively. For example, in some studies analogues of conjugates containing radioisotopes were used with imaging techniques where the results were expressed qualitatively (*i.e.*, expressed as whether drug accumulation was or was not observed) and quantitatively (expressed as % ID/Kg of drug uptake in tumour). Similarly with the staining technique using Evan's blue dye the results were expressed both qualitatively

and quantitatively (expressed as % dose/g/tumour). A few studies involved the comparison between the free drug accumulation and the nano particle accumulation in which case the results were expressed in terms of degree of their accumulation. Some studies have indicated that the extent of the EPR effect is also dependent on the tumour sizes [3,13,94] but due to the lack of information on the tumour sizes in most of the studies, this factor was not considered in our evaluation.

EPR-mediated accumulation was generally observed in all the tumour types where the highest percentage responses were reported (namely breast, lung and ovary). Studies on liposomal drug accumulation have shown poor EPR effects in liver and pancreatic tumours [95,96]. Colorectal cancer was another tumour type where poor EPR effect has been reported in some studies ([11,20] and Table 7). This observation is probably mainly due to the fact that large tumours in liver, pancreas and prostate possess hypovascular properties with low drug accumulation potential. However, it should be remembered that the smaller tumours possess high vascular density and can exhibit profound EPR effect [97].

This observation also correlates with a recent study on validation of the tumour models for EPR activity, where high drug accumulation has been reported for both large and small lung tumours, and a low drug accumulation has been reported for the large breast tumours [13]. Disparities observed across the various studies are might be due to the heterogeneity within and between the tumour types (size and histological differences) and the diversity of the nanopharmaceutical characteristics [14].

Table 7. Extent of passive accumulation of macromolecular system in different tumour types in both clinical and pre-clinical samples, clinical studies highlighted in grey.

Tumour Type	Clinical (C)/ Pre-Clinical (PC)	Sample Size (*n*)	Macromolecule System Used	Remarks	Reference
Breast	C	2	PDC	1.8%–5.9% dose of PDC uptake in tumour	[20]
	C	5	Liposome	5.3% ± 2.6% ID/Kg of drug uptake in tumour #	[97]
	C	6	Liposome	4–16 fold higher drug accumulation in tumour than the free drug	[98]
	PC (MX-1, Human xenograft)	10	Protein-conjugate	33% higher drug accumulation in tumour than the free drug	[99]
	PC (MAXF 449, Human xenograft)	NA	PDC	1.0%–0.1% dose/g/tumour drug accumulation	[13]
	PC (Mouse)	NA	PDC	5.29% dose/g for HMW 3.18% dose/g for LMW	[100]
	PC (MX-1)	NA	PDC	207 fold higher tumour exposure than the free drug (SN-38)	[101]
	PC (Walker 256, Rat)	NA	Protein-conjugate	7 fold higher drug accumulation in tumour than the free drug	[102]
	PC (Mouse)	NA	Nanoparticle	Drug accumulation observed in tumour *	[103]

Table 7. *Cont.*

Tumour Type	Clinical (C)/ Pre-Clinical (PC)	Sample Size (*n*)	Macromolecule System Used	Remarks	Reference
Pancreas	C	NA	Liposomes	Low drug accumulation observed in tumour *	[95]
	PC (Mouse)	40	Micelle	3 fold higher drug accumulation in tumour than the free drug	[104]
Lung	C	6	PDC	No drug accumulation observed in tumour *	[20]
	C	4	Liposome	18.3% ± 5.7% ID/Kg of drug uptake in tumour #	[97]
	C	3	Liposome	4–16 fold higher drug accumulation in tumour than the free drug	[98]
	C	15	Liposome	Higher drug accumulation observed in tumour *	[105]
	PC (Meta-7, Mouse)	NA	PDC	3.5%–4.7% dose/g/tumour drug accumulation	[13]
	PC (COR L23, Human xenograft)	NA	PDC	4.7%–12.2% dose/g/tumour drug accumulation	[13]
	PC (B16, Mouse)	NA	Liposomes	10.6% ± 0.2% ID/g of tumour	[106]
	PC (B16F10, Mouse)	NA	PDC	8.82% dose/g for HMW 3.23% dose/g for LMW	[100]
	PC (B16, Mouse)	NA	Micelle	2–3 fold higher drug accumulation in tumour than the free drug	[107]
	PC (B16, Mouse)	NA	PDC	6–12 fold higher drug accumulation in tumour than the free drug	[28]
	PC (B16, Mouse)	3	PDC	Higher drug accumulation observed in tumour *	[108]
Lung	PC (A431, Mouse)	NA	Protein-conjugate	24 fold higher drug accumulation in tumour than the free drug	[109]
	PC (B16, Mouse)	NA	PDC	30–63 fold higher drug accumulation in tumour than the free drug	[110]
	PC (B16, Mouse)	NA	PDC	16.3 fold higher drug accumulation in tumour than the free drug	[111]

Table 7. *Cont.*

Tumour Type	Clinical (C)/ Pre-Clinical (PC)	Sample Size (*n*)	Macromolecule System Used	Remarks	Reference
Ovary	C	3	Liposome	4–16 fold higher drug accumulation in tumour than the free drug	[98]
	PC (Mouse)	NA	PDC	Drug accumulation observed in tumour *	[28]
	PC (Oca-1, Mouse)	NA	PDC	5 fold higher drug accumulation in tumour than the free drug	[112]
	PC (Oca-1, Mouse)	NA	PDC	28–38 times higher drug accumulation in tumour than the free drug	[113]
	PC (A2780, Human xenograft)	NA	PDC	Drug accumulation observed in tumour *	[114]
	PC (OVCAR-3, Human xenograft)	NA	PDC	45 fold higher drug accumulation in tumour than the free drug	[115]
Oesophageal, stomach and intestine	PC (Mouse)	NA	PDC	Drug accumulation observed in tumour *	[116]
	PC (OCUM-2MLN, Human xenograft)	NA	Micelle	Drug accumulation observed in tumour *	[117]
Colon and/or rectum	C	5	PDC	No drug accumulation observed in tumour *	[20]
	C	10	PDC	64 fold higher drug accumulation in tumour than the free drug	[118]
	PC (HT29, Human xenograft)	4	Liposome	1.7 fold higher drug accumulation for 0.6 mol % PEG-conjugate in tumour than the free drug	[119]
	PC (C26 NL-17, Mouse)	NA	Liposome	Higher drug accumulation observed in tumour *	[120]
	PC (Mouse)	NA	Micelle	Drug accumulation observed in tumour *	[121]
Colon and/or rectum	PC (LS174T, Human xenograft)	NA	PDC	160 fold higher drug accumulation in tumour than the free drug	[122]
	PC (LS174T, Human xenograft)	NA	Liposomes	6.3% ± 2.9% ID/g of tumour	[106]
	PC (Mouse)	NA	PDC	Drug accumulation observed in tumour *	[123]
	PC (LS174T, Human xenograft)	NA	PDC	Drug accumulation observed in tumour *	[124]
	PC (HT29, Human xenograft)	NA	PDC	Drug accumulation observed in tumour *	[125]
	PC (HT29, Human xenograft)	111	Nano crystal (3H-PTX)	Low drug accumulation observed in tumour *	[126]

Table 7. *Cont.*

Tumour Type	Clinical (C)/ Pre-Clinical (PC)	Sample Size (*n*)	Macromolecule System Used	Remarks	Reference
Head/Neck and brain	C	6	PDC	2.2% ± 2.1% dose at 2–3 h 1.3% ± 0.4% dose at 24 h 0.5% ± 0.3% dose at 8 days uptake in tumour	[4]
	C	10	Liposome	13–19 times higher accumulation in tumour as compared to the normal brain tissue	[127]
	C	5	Liposome	7–13 times higher accumulation in tumour as compared to the normal brain tissue	[127]
	C	7	Liposome	33.0% ± 15.8% ID/Kg of drug uptake in tumour #	[97]
Others					
Adenocarcinoma (Unknown)	PC (MAC 26, Mouse)	NA	PDC	6.9%–10.8% dose/g/tumour drug accumulation	[13]
Adenocarcinoma (Unknown)	PC (MAC 15A, Mouse)	NA	PDC	8.2%–12.6% dose/g/tumour drug accumulation	[13]
Cervix	PC (ME180, Human xenograft)	NA	Liposome	Drug accumulation observed in tumour *	[128]
Fibrosarcoma	PC (S-180, Mouse)	NA	Polymer conjugates	Drug accumulation observed in tumour *	[129]
	PC (S-180, Mouse)	NA	Micelle	13 fold higher drug accumulation in tumour than the free drug	[130]
	PC (S-180, Mouse)	NA	PDC	Drug accumulation observed in tumour *	[131]
	PC (S-180, Mouse)	NA	Protein-conjugate	Drug accumulation observed in tumour*	[10]
Fibrosarcoma	PC (Meth A, Mouse)	NA	PDC	Drug accumulation observed in tumour *	[132]
	PC (S-180, Mouse)	NA	Protein-conjugate	4 fold higher drug accumulation in tumour than the free drug	[8]
	PC (S-180, Mouse)	NA	PDC	Drug accumulation observed in tumour *	[133]

Table 7. *Cont.*

Tumour Type	Clinical (C)/ Pre-Clinical (PC)	Sample Size (*n*)	Macromolecule System Used	Remarks	Reference
Liver	C	31	Liposomes	Low drug accumulation observed in tumour *	[95]
	C	3	Liposomes	Low drug accumulation observed in tumour *	[96]
	PC (Mouse)	NA	Micelle	4 fold higher drug accumulation in tumour than the free drug	[134]
	PC (VX-2, Rabbit)	NA	Polymer-protein conjugate (SMANCS-Lipidol)	Drug accumulation observed in tumour *	[135]
	PC (VX-2, Rabbit)	NA	Polymer-protein conjugate (Lipidol)	Drug accumulation observed in tumour *	[136]
	PC	NA	PDC	Drug accumulation observed in tumour *	[137]
Prostate	PC (Human xenograft)	NA	^{89}Zr-DFO-mAlb (Polymer-protein conjugate)	Drug accumulation observed in tumour *	[138]
	PC (Human xenograft)	NA	PDC	Drug accumulation observed in tumour *	[139]
	PC (Rat)	NA	PDC	Drug accumulation observed in tumour *	[140]
	PC (Rat)	NA	PDC	Drug accumulation observed in tumour *	[141]

Notes: * The quantitative data on accumulation in tumour is not available in the original research paper; # related free drug uptake is not stated in the original study; "*n*" = number of human/animal subjects.

Two decades since the first PDC entered the clinical evaluation [2], one might wonder about the margin of advantage of PDCs over their corresponding parent drugs in terms of overall survival and the patient's quality of life. Such an advantage of a drug is evaluated only at phase III of the clinical trial, thus since only 2 PDCs have undergone phase III clinical trials (Opaxio and NKTR-102) and the clinical data is available to-date only for Opaxio, any conclusion with regards to the overall survival and patient's quality of life is derived from Opaxio's performance. Opaxio, in comparison with its conventional paclitaxel-based treatment, showed some safety-related advantages such as alopecia was rare, nausea and vomiting were uncommon and hypersensitivity reactions were rarely observed. A significant survival benefit has also been observed for women receiving Opaxio *vs.* paclitaxel [7]. These promising results have provided the confidence that PDCs can give therapeutic benefits in the field of oncology. The use of PDCs in combination with the application of diagnostic tools to measure protease expression and EPR effect would be a rational approach for the further development and clinical use of PDCs.

4. Conclusions

In this study, we have reported analysis of data from the literature whereby we have documented and examined the expression of enzymes (cathepsins) within various tumour types, the magnitude of the EPR effect in the various tumour types, and the clinical responses observed for PDCs. We have determined that the highest percentages of clinical responses to PDCs were observed for lung cancer, which was also found to express high levels of the cathepsin enzymes. Breast and ovarian cancer also showed high clinical responses to PDCs, which correlated well with high levels of cathepsins observed in these tumour types, and with reports indicating the presence of the EPR effect. This finding is in line with other studies [13,14] and in agreement with that concluded by others (e.g., [13]). Moreover, our study also suggests that careful patient selection, in the form of pre-screening for enzyme content and the EPR effect, would be a rational approach for the further development and clinical application of PDCs. This could ultimately result in a more consistent efficacy of this drug delivery system in the clinical setting.

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Author Contributions

A.K.R. and D.R. jointly carried out the research and wrote the manuscript; H.M.I.O. co-led the research and writing of the manuscript; F.G. conceived the original idea, led the research and writing of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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