Peptide Drug Conjugates in Cancer Therapy

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Drug conjugates have become a significant focus of research in the field of targeted medicine for cancer treatments. Peptide-drug conjugates (PDCs), a subset of drug conjugates, are composed of carrier peptides ranging from 5 to 30 amino acid residues, toxic payloads, and linkers that connect the payload to the peptide. PDCs are further broken down into cell-penetrating peptides (CPPs) and cell-targeting peptides (CTPs), each having their own differences in the delivery of cytotoxic payloads. Generally, PDCs as compared to other drug conjugates—like antibody-drug conjugates (ADCs)—have advantages in tumor penetration, ease of synthesis and cost, and reduced off-target effects. Further, as compared to traditional cancer treatments (e.g., chemotherapy and radiation), PDCs have higher specificity for the target cancer with generally less toxic side effects in smaller doses.

peptide-drug conjugates bioconjugates cancer

1. Introduction

Cancer is a significant cause of death worldwide, with breast and lung cancer having the highest prevalence among women and lung and prostate cancer having the highest prevalence among men [1]. Other leading cancers, according to the world health organization (WHO), include cancers of the colon and rectum, stomach, liver, cervix, and esophagus. Cancer disproportionally affects minority communities within the United States [2][3]. Additionally, the WHO estimates that cancer accounts for 8.97 million deaths worldwide, making cancer the second highest cause of mortality worldwide behind cardiovascular disease [1]. The financial implication of cancer is also severe, with an estimated personal healthcare spending of an estimated 155.5 billion dollars in 2013 in the United States [4].

Historically, the first-line therapy for cancer was surgical excision of a primary tumor [5]. During the 20th century, radiation and chemotherapeutic such as aminopterin, doxorubicin, and cisplatin became available. However, traditional chemotherapy and radiation broadly target rapidly dividing cells, including non-cancerous cells such as hair follicles or enterocytes. Although the standard of care, these methods indiscriminately target cancerous and non-cancerous rapidly proliferating cells, which accounts for the side effects associated with classical chemotherapeutic or radiation treatment, such as hair loss and gastrointestinal upset. Despite advances in oncopharmacology such as immunotherapy, off-target cytotoxicity remains a chief concern, and efforts to mitigate these effects by increasing the targeting specificity of new chemotherapeutic agents.

2. Peptide Drug Conjugates

Although ADCs are clinically established for cancer therapy, PDCs are gaining recognition as a new cancer treatment method by increasing targeted drug delivery with improved efficacy and reduced side effects. PDCs utilize a smaller molecular composition than other marketed anticancer drugs (such as ADCs), contributing to PDC biochemical stability, cell membrane penetration, and overall efficacy [6]. PDCs can be modified to optimize binding affinities and physicochemical properties to ensure proper binding and cleavage [7]. PDCs are classified as cell-penetrating peptides (CPPs) or cell-targeting peptides (CTPs).

2.1. Peptides for Specific Organ Targeting

Directed targeting of specific organs has been considered a crucial step in limiting side effects associated with traditional anticancer therapy. Use of peptides to direct organ specific targeting has emerged as a distinct possibility. Currently there are two main ways to target a peptide 1) rely on natural protein sequences such as vascular endothelial growth factor (VEGF) [8] or somatostatin [9]. Alternatively libraries of peptides can be tested via phage display technique [10]. However, these techniques often yield peptides that can target tumor microenvironments but are poorly directed to specific organs. Likewise certain organs are more easily targeted than others for example N-acetylgalactosamine (GalNAc) can be used to easily target the lungs in adenocarcinoma [11][12]. However, some organs prove more difficult to effectively target when a strong physical barrier is in place as is the case with pancreatic cancers characterized by strong desmoplasia creating a mechanical barrier around the tumor cells [13] or cancers of the brain that necessitate crossing the blood–brain barrier (BBB). That said, developments are underway to utilize phage-derived shuttle peptides which can select against BBB endocytic machinery and used in engineering novel PDCs for brain cancers [14].

2.2. Cell-Penetrating Peptides

The cell membrane provides a physiological barrier that limits the transportation of various molecules, such as macromolecules, proteins, and nucleic acids, across the plasma membrane. However, the cell membrane can also limit drug penetration. Therefore, it is imperative to develop drugs that can cross the cell membrane of cancer cells to induce destruction.

Cell-penetrating peptides (CPPs) can transport drug payloads through cell membranes using specific amino acid sequences ranging from 5 to 30 residues. CPPs provide an effective method for transporting cell-impermeable compounds or drugs to reach their intracellular targets [15]. Various mechanisms of action have been proposed regarding how CPPs penetrate the cell. Two generally accepted mechanisms are (1) direct penetration of the plasma membrane and (2) endocytosis. Direct penetration occurs when positively charged CPPs interact with negatively charged membrane components, destabilizing the membrane and forming a pore [15][16]. Moreover, clathrin-mediated endocytosis and macropinocytosis have been observed to take up CPPs [17]. However, more research needs to be done to elucidate the exact mechanism of cellular entry [18].

Due to the ability of CPPs to enter most cells they come into contact with, their therapeutic effects are limited to intra-tumoral injection. However, some treatments have been developed to target lymphatic metastasis via

intravenous injection using CPPs modified with nanoparticles; in a study by Hu et al., modifying nanoparticles with CPPs suppressed tumor growth rate by 1.4-fold and showed a 63.3% inhibition rate of lymph metastasis in lung cancer [19]. Other advancements have been for specific tumor targeting by activatable cell-penetrating peptides and transducible agents. Coupling shielding polyanions create activatable CPPs (ACPPs) to the peptide with target-specific cleavable linkers [17]. For example, in a study by Cheng et al., the shielding group of 2,3-dimethyl maleic anhydride (DMA) was used to inhibit the CPP at physiological pH. However, at a tumor extracellular pH of 6.8, DMA is hydrolyzed to activate the CPP to sequester the drug inside cancer cells [20]. Transducible agents delivered via intraperitoneal injection use functional domains to modulate the type of tissues CPPs are active against to increase tumor specificity. One notable example is the creation of oxygen-dependent degradation (ODD) domains by fusing hypoxia-inducible factor-1α to β-galactosidase, which helps it target hypoxic tumor cells. This domain is combined with the HIV-TAT protein to reduce tumor growth without causing toxic side effects expected from delivering active caspase-3 [21].

2.3. Cell-Targeting Peptides

Cell-targeting peptides (CTPs) range from 3–14 amino acids long and utilize receptors that are overexpressed on cancer cell surfaces to target the delivery of the drug [15]. Depending on the targeted receptor, CTPs can cause a localized build-up of the drug around the tumor or induce endocytosis upon CTP binding [22][23]. CTPs exhibit similar characteristics to monoclonal antibodies (mAbs) by binding with high affinity to their respective receptor. However, unlike mAbs, CTPs can penetrate tumors better due to their small size [7].

A limitation of CTPs includes the dependency on the expression of a specific receptor to have an effect. Techniques such as phage display can determine a peptide sequence that will specifically bind cancer cells and mitigate the shortcoming mentioned earlier [22]. In a study by Rasmussen et al., phage display was utilized to identify peptide sequences with a 1000-fold or higher binding efficiency and selectivity specific to human colorectal cancer cells [24]. Further, cyclization and multimerization can increase an affinity for the selected receptors. Cyclization forces the peptide into a constrained ring conformation, increasing the resistance to proteases and degradation. Multimerization joins two or more monomers together to improve local concentrations of CTPs, resulting in higher probabilities of peptide-receptor interactions [22]. CTPs can be combined with a CPP to translocate cargo molecules into cancer cells more efficiently. Bolhassani et al. found that delivery of a DNA alkylating agent, chlorambucil, with CREKA (CTP) conjugated to *p*VEC (CPP) (*p*VEC amino acid sequence LLIILRRRIRKQAHAHSK) was more suitable for transportation and led to significantly higher cancer killing than chlorambucil alone [25].

3. Lu-177 Current Clinical Application and Trials

3.1. FDA Approved PDC: Lu-177 DOTA-TATE

The first FDA approved PDC was Lu-177 DOTA-TATE (Lutatera®). Lu-177 DOTA-TATE is a radiolabeled somatostatin analog that was FDA approved in 2018 as a first-in-class drug for the treatment of somatostatin

receptor-positive gastroenteropancreatic neuroendocrine tumors (GEP-NETs) and is administered intravenously (I.V.) to patients [26]. NETs are a type of tumor that originate in endocrine tissues throughout the body. Lu-177 DOTA-TATE binds to malignant cells overexpressing somatostatin receptor type 2. Once Lu-177 DOTA-TATE binds its respective target, Lu-177 DOTA-TATE accumulates within tumor cells and delivers cytotoxic radiation to kill the cells [27]. The 3D structures of the somatostatin receptor in complex with somatostatin and octreotide, a synthetic long-acting cyclic octapeptide somatostatin analog, were recently determined by cryo-electron microscopy [28].

3.2. Examples of PDC Clinical Trials Utilziing Lu-177

Following the success of ^{177}Lu-DOTA-TATE for the treatment of adults with somatostatin receptor–positive GEP-NET and FDA approval, here focuses on other relevant examples of actively recruiting clinical trials that utilize Lu-177 for treatment of various cancers (**Table 1**).

Table 1. Current clinical applications and trials of Lutetium-177 (^{177}Lu).

Intervention	ClinicalTrials.gov Identifier	Phase	Indication	Target
^{177}Lu-PNT2002 versus abiraterone or enzalutamide	NCT04647526	3	Metastatic Castration-resistant Prostate Cancer (mCRPC)	PSMA
^{177}Lu-PSMA-I&T versus Hormone Therapy	NCT05204927	3	mCRPC	PSMA
^{177}Lu-Ludotadipep	NCT05579184	2	mCRPC	PSMA
^{177}Lu-PSMA-617	NCT05114746	2	mCRPC	PSMA
^{177}Lu-PSMA (+/−) Ipilimumab and Nivolumab	NCT05150236	2	mCRPC	PSMA
^{177}Lu-PSMA and enzalutamide (nonsteroidal antiandrogen)	NCT04419402	2	mCRPC	PSMA
^{177}Lu-PSMA (DGUL) and Ga-68-NGUL	NCT05547061	1/2	mCRPC	PSMA
^{177}Lu-PSMA-I&T	NCT05383079	1/2	mCRPC	PSMA
Cabazitaxel in combination with ^{177}Lu-PSMA-617	NCT05340374	1/2	mCRPC	PSMA
Abemaciclib and 177Lu-PSMA-617	NCT05113537	1/2	mCRPC	PSMA
^{177}Lu-rhPSMA-10.1	NCT05413850	1/2	mCRPC	PSMA

Intervention	ClinicalTrials.gov Identifier	Phase	Indication	Target
^{177}Lu-EB-PSMA-617	NCT03780075	1	mCRPC	PSMA
^{177}Lu-PSMA-EB-01 (+/−) radioligand therapy (RLT)	NCT05613738	1	mCRPC	PSMA
^{177}Lu-PSMA + olaparib (PARP inhibitor)	NCT03874884	1	mCRPC	PSMA
^{177}Lu-EB-PSMA (55 mCi)	NCT04996602	1	mCRPC	PSMA
^{177}Lu-Ludotadipep	NCT05458544	1	mCRPC	PSMA
^{177}Lu-DOTA-TLX591	NCT04786847	1	mCRPC	PSMA
Radiometabolic Therapy (RMT) with ^{177}Lu PSMA 617	NCT03454750	2	Castration Resistant Prostate Cancer (CRPC)	PSMA
^{177}Lu-PSMA-617	NCT04443062		Oligo-metastatic Hormone Sensitive Prostate Cancer (mHSP)	PSMA
Standard of Care (SOC) (+/−) ^{177}Lu-PSMA-617	NCT04720157		mHSPC	PSMA
Docetaxel +/− ^{177}Lu-PSMA	NCT04343885	2	metastatic hormone-naive prostate cancer (mHNPC)	PSMA
^{177}Lu-TLX591	NCT05146973	2	PSMA-expressing prostate cancer	PSMA
225Ac-J591 and ^{177}Lu-PSMA-I&T	NCT04886986	1/2	Prostate cancer	PSMA
^{177}Lu-PSMA	NCT05230251	2	Prostate cancer	PSMA
^{177}Lu PSMA 617	NCT04663997	2	Prostate cancer	PSMA
^{177}-Lu-PSMA given before stereotactic body radiotherapy (SBRT)	NCT04597411	2	Prostate cancer	PSMA
^{177}Lu-PSMA-617	NCT05613842	2	Hormone-sensitive disease (cohort A) castrate-resistant Disease (Cohort B)	PSMA
^{177}Lu-PSMA radioligand therapy	NCT05162573	1	node-positive prostate cancer	PSMA

Intervention	ClinicalTrials.gov Identifier	Phase	Indication	Target
^{177}Lu-PP-F11N	NCT02088645	1	Advanced medullary thyroid carcinoma GEP-NET	cholecystokinin-2 receptors
^{177}Lu-AB-3PRGD2	NCT05013086	1	Non-Small Cell Lung Cancer (NSCLC)	Integrin αvβ3
^{177}Lu-DOTA-TATE in combination with carboplatin, etoposide, and tislelizumab	NCT05142696	1	Extensive Stage Small Cell Lung Cancer (ES-SCLC)	STTR
GD2-SADA:177Lu-DOTA complex	NCT05130255	1	GD2 expressing solid tumors (Small Cell Lung Cancer, Sarcoma and Malignant Melanoma)	GD2
Standard of Care (+/−) ^{177}Lu-DOTA-TATE	NCT05109728	1	Glioblastoma	STTR
Intracavitary radioimmunotherapy (iRIT) with a newly developed radioimmunoconjugate ^{177}Lu labeled 6A10-Fab-fragments	NCT05533242	1	Glioblastoma	carbonic anhydrase XII
Combination of ^{177}Lu-girentuximab and nivolumab	NCT05239533	2	Advanced clear cell renal cell carcinoma/ccRCC	Carbonic Anhydrase IX
68Ga-PSMA PET-CT with ^{177}Lu-EB-PSMA-617	NCT05170555	NA	Renal Cell Carcinoma	PSMA
^{177}Lu-PNT6555	NCT05432193	1	Fibroblast Activation Protein (FAP) overexpressing tumors (Colorectal Cancer; Esophageal Cancer; Melanoma; Soft Tissue Sarcoma	FAP
[68Ga]Ga DOTA-5G and ^{177}Lu DOTA-ABM-5G theranostic	NCT04665947	1	Locally advanced or metastatic pancreatic adenocarcinoma (PDAC)	-
^{177}Lu-octreotate versus sunitinib	NCT02230176	2	Progressive pancreatic, inoperable, somatostatin receptor positive, well differentiated pancreatic neuroendocrine tumors (WDpNET).	STTR

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Intervention	ClinicalTrials.gov Identifier	Phase	Indication	Target
^{177}Lu-DOTATATE versus capecitabine and temozolomide	NCT05247905	2	Metastatic Pancreatic Neuroendocrine Tumor and Unresectable Pancreatic Neuroendocrine Carcinoma	STTR
^{177}Lu-DOTATATE hepatic intraarterial infusion	NCT04544098	1	Neuroendocrine Tumors Liver-Dominant Metastatic Pancreatic Neuroendocrine Tumors	STTR
^{177}Lu-DOTATOC	NCT04276597	2	Somatostatin receptor-expressing Pulmonary, Pheochromocytoma, Paraganglioma, and Thymus neuroendocrine tumors	STTR

3.3. PDC Limitations

Despite the many advantages of PDCs, there are several limitations to implementing PDC therapy. Due to their low molecular weight, PDCs exhibit poor stability and undergo rapid renal clearance [29][30][31]. This can lead to limited therapeutic utility, particularly in solid tumors. Researchers have explored both chemical and physical modifications to overcome this challenge. An example of a modification technique is using gold nanoparticles (AuNPs) conjugated with PDCs to increase their overall stability. In a study by Kalimuthu et al., PEG-coated-AuNPs were tested to determine if they could provide a suitable platform for loading PDCs [31]. Their research showed that PDCs conjugated with the PEG-coated-AuNPs were still active after a 72 h pre-incubation period. In contrast, the free PDCs had no cytotoxic activity after a 24 h pre-incubation period [31]. Another technique that has been widely used as a method of improving the enzymatic and chemical stability of peptides is the use of cyclization techniques [30]. For example, peptide stapling, a technique that allows peptides to be locked into a desired confirmation, has been used to enhance a peptide's binding affinity to its target [30].

Another essential factor to consider concerning renal clearance is the overall net charge of the peptide sequence [30]. Increasing the negative charge of the peptide sequence to delay the glomerular filtration by the kidneys has been proposed as a method to lengthen the half-life of peptides [32]. Other ways to delay renal clearance of peptides include increasing the size and hydrodynamic diameter of the peptide and increasing plasma protein binding to prevent the conjugate from being filtered out through the kidneys [32]. One strategy is to conjugate polyethylene glycol (PEG) to PDCs [33]. The inherent qualities of PEG make it an ideal candidate for modification; it is inexpensive, hydrophilic, biocompatible, and non-immunogenic [34]. PDCs possess low immunogenicity compared to ADCs and other more giant molecules, such as proteins. However, they can still benefit from structural modifications to reduce the probability of eliciting an adverse immune reaction. Besides slowing down renal clearance, pegylation can result in less peptide immunogenicity, though PEG has its own immunogenicity

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issues [35][36]. Chemical modification of peptides, then, is yet another avenue of investigation for beneficial modification of PDCs [30][37][38].

Alternatives to pegylation are also emerging as a way to further modify the biochemical and pharmacokinetic aspects of PDCs, and to reduce the inherent immunogenicity complications of PEG [39]. One such alternative to PEG is polysarcosine (PSar) which has low toxicity and unlike PEG, is biodegradable [40]. Furthermore, the unique chemistry of PSar may mitigate the phenomenon of accelerated blood clearance often observed in pegylated therapeutics [41]. Another alternative to PEG is XTEN a class of unstructured hydrophilic and biodegradable protein polymers that have been demonstrated to increase the half-life of bioactive peptides [42] as well as increasing peptide solubility [43]. A final class of alternatives to PEG include the use of proline/alanine/serine (PAS) biopolymers which can function in many of the same ways a PEG but also share the pharmacokinetic benefits of PEG alternatives such as XTEN and PSar such as increased circulating half-life [44] and decreased immunogenicity [45].

Even after FDA approval, failure to demonstrate a survival advantage presents another critical limitation. A prime example of this is the drug melphalan flufenamide (melflufen), initially approved in February 2021 for the treatment of refractory multiple myeloma; it was withdrawn from the U.S. market in October 2021 after consistently failing to demonstrate a survival advantage based on results from a phase 3 randomized controlled trial [46]. This case highlights the role of the FDA's regulatory process and accelerated approval pathways, as well as the importance of rigorous clinical trials backed up by robust clinical data before the marketing and approval of a new drug.

PDCs have limited or non-existent oral bioavailability; this limits their administration to intravenous injection and excludes oral administration [30]. Lack of ease of administration presents a tremendous barrier, particularly for individuals who cannot access a clinical setting regularly. Although this is a common challenge for biologics and peptides, further research is needed to optimize their chemical and enzymatic stability. Lamson et al. have recently reported on a novel way of allowing for the oral delivery of bioactive peptides by using anionic nanoparticles [47]. Although using nanoparticles is an attractive strategy to solve the low oral bioavailability and stability problem, and additional testing is needed to evaluate the feasibility of this technique in future clinical trials. The use of acid-stable coatings, gut enzyme inhibitors, and mucus-penetrating peptides have also been proposed as possible strategies to improve the oral availability of PDCs [48]. For example, acid-stable layers can help to slow down the degradation caused by peptidases [48].

Developing approaches to allow for the oral administration of PDCs is essential to make these drugs more accessible, allow for better therapeutic adherence, and to increase their representation in clinical trials. As the number of clinical trials evaluating the use of PDCs grows, further research is needed to optimize their delivery, improve their systemic stability, reduce fast renal clearance, and lengthen their half-life in vivo.

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