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Pharmacokinetics of Protein and Peptide Conjugates

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Abstract

Protein and peptide conjugates have become an important component of therapeutic and diagnostic medicine. These conjugates are primarily designed to improve pharmacokinetics (PK) of those therapeutic or imaging agents, which do not possess optimal disposition characteristics. In this review we have summarized preclinical and clinical PK of diverse protein and peptide conjugates, and have showcased how different conjugation approaches are used to obtain the desired PK. We have classified the conjugates into peptide conjugates, non-targeted protein conjugates, and targeted protein conjugates, and have highlighted diagnostic and therapeutic applications of these conjugates. In general, peptide conjugates demonstrate very short half-life and rapid renal elimination, and they are mainly designed to achieve high contrast ratio for imaging agents or to deliver therapeutic agents at sites not reachable by bulky or non-targeted proteins. Conjugates made from non-targeted proteins like albumin are designed to increase the half-life of rapidly eliminating therapeutic or imaging agents, and improve their delivery to tissues like solid tumors and inflamed joints. Targeted protein conjugates are mainly developed from antibodies, antibody derivatives, or endogenous proteins, and they are designed to improve the contrast ratio of imaging agents or therapeutic index of therapeutic agents, by enhancing their delivery to the site-of-action.

Keywords

Pharmacokinetics; peptide-drug conjugate; antibody-drug conjugate; PEGylation; Radioimmunoconjugate; immunotoxin; albumin conjugates

1. INTRODUCTION

Small molecules are an essential component of therapeutic and diagnostic applications. However, due to their small size, they often suffer from poor pharmacokinetic (PK) and

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pharmacodynamic (PD) properties. For example, small molecules typically demonstrate short half-life and widespread distribution, which makes it difficult to target them at the site-of-action for long durations of time. In addition, due to the small size of the pharmacophore they often demonstrate nonspecific binding, which results in narrow therapeutic index. As such, any strategy that leads to improved PK-PD properties of small molecules can significantly enhance their therapeutic and diagnostic potential. One such increasingly popular strategy is conjugation of small molecules with proteins and peptides. The resulting protein and peptide conjugates typically demonstrate longer half-life and specific distribution to the site-of-action, both of which are desired characteristics for the development of a successful therapeutic or diagnostic agent.

In this review article we have summarized PK characteristics of protein and peptide conjugates. We have categorized the conjugates into three different categories. The first category is peptide-drug conjugates, which are relatively smaller in size and do not lead to dramatic increase in the half-life. However, these conjugates enhance selective distribution of the drug at the site-of-action, and play an important role in diagnostics (where an extended half-life is less critical for the success) and treatment application. The second group is conjugates of non-targeted proteins, such as PEGylated proteins and albumin-drug conjugates. These conjugates generally lead to an improvement in the half-life of a drug or a protein, and also lead to alteration in their distribution. While some non-targeted protein-drug conjugates may exhibit higher distribution to certain tissues (e.g. tumor), in general they do not possess selective distribution ability like the targeted conjugates. The third category is targeted protein-drug conjugates, which are mostly derived from monoclonal antibodies (mAbs) and antibody fragments. These conjugates have high affinity for their target, which allows them to specifically localize at the site-of-action. As such, they have become an important component of therapeutic and diagnostic applications.

2. PEPTIDE CONJUGATES

2.1 Diagnostic Application

Preclinical—Radiolabeled peptides have been extensively investigated as possible diagnostic agents. One prominent example is Bombesin, a peptide derived from the skin of the Bombina frog, which has shown potential for identifying tumors that overexpress gastrin-releasing peptide receptors (GRPR) [1]. In fact, certain Bombesin analogues have been shown to be useful for the detection of both preclinical and clinical tumors when labelled with Technetium-99m (^{99m}Tc), which is a popular radiolabel for tumor detection due to its comparable half-life (~6h) to many tumor targeting drugs [2, 3]. Bombesin analogues have short half-life of 0.5–1.5 h, and their distribution is significantly affected by total peptide charge, the length of the peptide, molecular weight, and the presence of non-natural amino acids. Liolios et al. have thoroughly evaluated the effect of conjugation linker on the PK of ^{99m}Tc labelled Bombesin analogues, and have demonstrated that charge of the linker can play an important role in the ability of these conjugates to diagnose the tumor. Conjugates with positively charged linker were observed to exhibit advantageous PK profiles, demonstrated by enhanced blood clearance, higher tumor targeting ability, reduced upper abdominal radioactivity, and increased tumor/normal tissue contrast ratios [4].

Besides Bombesin, many other peptides have been used for diagnostic applications in oncology. For example, He et al. have developed a synthetic peptide CSNIDARAC, which preferentially binds to lung cancer cells [5]. Using FITC-conjugated CSNIDARAC and H460 mouse xenograft model, the authors demonstrated that the targeted peptide was able to eliminate from the blood quickly and within 2h it was mainly present just in the tumor. In addition, He et al. observed that most of the peptide accumulated in the kidney in very short time, suggesting renal excretion as the primary pathway for elimination for these peptides from the body. Dijkgraaf et al. have developed a dimeric RGD peptide to image integrin $\alpha_v\beta_3$ overexpressing tumors via PET scan [6]. The authors labelled the peptide with NODAGA chelator and $Al^{18}F$, ^{68}Ga , or ^{111}In . Using SK-RC-52 tumor bearing mice the authors were able to demonstrate that all three conjugates cleared from the blood rapidly, and only 0.03 %ID/g peptide was found in the blood 2h after the injection. Similar to the findings of a previously discussed study, a dimeric RGD peptide was found to eliminate via renal excretion and accumulated in the kidneys. It was found that the choice of radiolabel had an effect on the tumor distribution of these conjugates, as 2h after the injection $Al^{18}F$ labelled peptide was present in the tumor at 3.44 %ID/g, whereas ^{68}Ga labelled peptide was present at 6.26 %ID/g, and ^{111}In labelled peptide was present at 4.99 %ID/g. Kawano et al. have tried to exploit specific binding of CTT peptides to MMP-2 antigen, for imaging MMP-2 overexpressing metastatic tumors [7]. They conjugated CTT to the surface of a naturally occurring heat shock protein nanocage via genetic modification, and evaluated near-infrared fluorophore conjugated nanocages in HT1080 (MMP-2 expressing) and HT29 (negative control) tumor bearing mice. Kawano et al. found that maximum uptake of the conjugate in the tumor was observed around 3h, for both target expressing and non-expressing tumors. In addition, the presence of target on the tumor cells led to only 2 times higher exposure of the conjugate in the tumor. It was also found that the conjugates nonspecifically accumulated in the liver, spleen, and kidney (most probably due to renal elimination) to a considerable level. As such, these observations suggest that conjugation of peptide with high molecular weight entities can dramatically alter their PK profile, and may even diminish their ability to specifically diagnose tumor.

Peptides like ApoPep-1, which is an apoptosis targeting peptide, have been used as *in vivo* imaging agents for diagnosing both tumors and Parkinson's disease. Wang et al. have investigated ^{124}I labelled ApoPep-1 in tumor bearing mice, and have showed that the tumor uptake of the conjugate increased by 1.6 times following doxorubicin treatment, suggesting radiolabeled ApoPep-1 could be utilized as an imaging agent for *in vivo* apoptosis [8]. Using a separate biodistribution study in the rats, the authors also found that peptide was able to clear from the circulation very rapidly (within 4h) and renal excretion was the main pathway for the elimination of the peptide. Lee et al. have investigated the same peptide for detecting dying neurons and apoptosis in the brain caused by Parkinson's or other neurodegenerative diseases [9]. Using Cy7.5 and FITC labeled ApoPep-1, and MPTP induced Parkinson's disease mouse model, the authors demonstrated that the fluorophore conjugated peptide was able to enter the brain within 2h and the fluorescent/optical signal in the brain showed a strong correlation with loss of dopaminergic neurons in the brain.

Clinical—When it comes to clinical diagnostics, somatostatin analogues (e.g. octreotide) are routinely used to image neuroendocrine tumors, following conjugation with radiolabels like ^{111}In and $^{99\text{m}}\text{Tc}$. These conjugates are eliminated from the body via excretion through the kidneys and metabolism by the liver. Their uptake into other organs depends on which subtype of the somatostatin receptor (SSTR) the peptides target, and expression profile of SSTR in individual organs [10]. For example, the lymphatic organs mostly contain the SSTR2 subtype, and bind certain analogues with higher affinity than others [11]. Once administered, these conjugates eliminate and distribute rapidly in the body, and exhibit an elimination half-life of about 90 minutes (volume of distribution $\sim 18\text{--}30\text{L}$, total clearance $\sim 160\text{ ml/min}$). Although, these PK parameters may vary for a specific analogue and radiolabel [12]. Sensitivity of the diagnostic tests based on radiolabeled somatostatin analogues can also vary based on the tumor type, and can range from 50 to 95% [13]. More recently, ^{68}Ga has gained momentum as a promising radiolabel for conjugation with somatostatin analogues, as it can be more effectively used with PET/CT imaging. PK of these conjugates has been investigated in the clinic, and distribution and other characteristics were found to be similar to other somatostatin analogues [14].

When it comes to other peptide conjugates, only a few have been successful in the clinic as diagnostic agents. One Bombesin analogue that has advanced to the clinic is RP527, which is radiolabeled to $^{99\text{m}}\text{Tc}$ and engineered to target GRPR positive tumors. Clinically, this conjugate demonstrated similar PK profile as other imaging peptides. Elimination from the blood was very fast, and only 20% of the injected dose remained in the blood after 20 min [15]. Most of the conjugate predominantly cleared by the kidneys and to a lesser extent by the gastrointestinal tract. Mean excretion in the urine at 48 h after the injection was $\sim 58\%$ of the injected activity. The conjugate also demonstrated rapid hepatobiliary excretion, which diminished its ability to image abdominal tumors due to the presence of extensive radioactivity in the bowel. RGD peptide conjugates also have had some success as diagnostic agents in the clinic [16]. ^{18}F Galacto-RGD is one of these peptides, which has been used for imaging angiogenesis. Similar to other peptides, this conjugate demonstrated rapid blood clearance, as only $\sim 25\%$ of the injected dose remained in the circulation after 30 min and $<20\%$ remained after 60 min. The conjugate mainly cleared through renal elimination, and accumulated in the kidneys and bladder. The PK profile of this conjugate played a pivotal role in its clinical implementation as a diagnostic agent, since it was suggested that image acquisition should be done 40–60 min post-injection to minimize the background effect. In addition, to detect the lesions adjacent to urogenital tract and bladder, it was recommended that patients should be instructed to urinate to reduce the degree of tracer uptake in the bladder [17]. Another RGD conjugate ^{18}F FPPRGD₂, which is a PEGylated form of dimeric RGD peptide, has also been used in the clinic for diagnosing tumors [18]. This conjugate was also primarily cleared through the kidneys, and demonstrated rapid clearance, with only $\sim 25\%$ of the dose remaining in the circulation at 30 minutes. The conjugate was observed to mainly distribute to the bladder, spleen, and large intestine.

2.2 Treatment Application

Preclinical—Peptide conjugates have also been used for the treatment of various diseases. One of the peptides discussed earlier for the imaging of lung tumors, CSNIDARAC, has also been used to target doxorubicin loaded liposomes to the tumor [5]. CSNIDARAC conjugated doxorubicin-loaded liposomes were able to selectively home into the tumor, and were found to inhibit tumor growth more efficiently than untargeted liposomes or free doxorubicin. However, it is important to note that since the peptide was conjugated to liposomes, the PK of the conjugate would have been very different than the PK of the peptide itself or the PK of the peptide conjugated to a fluorophore or radiolabel. Another example is Gonadorelin, a luteinizing hormone-releasing hormone receptor (LHRHR) targeting peptide, which has been used to target a liposomal formulation of mitoxantrone to the tumor [19]. Gonadorelin conjugated liposomes were mainly confined to the blood circulation, and had a half-life of ~15h. They were mainly accumulated in the kidney, liver, and tumor, and maximal tumor accumulation was observed ~4h post injection. While liposomes conjugated with Gonadorelin exhibited enhanced inhibition of MCF-7 breast cancer xenografts, no significant difference was observed between the PK of mitoxantrone, when administered in targeted vs. non-targeted liposomes. This suggests that the accumulation of drugs in the tumor was mainly due to nonspecific enhanced permeability and retention (EPR) effect.

A proline-rich antibacterial peptide Bac7, which has been used to protect mice against *Salmonella typhimurium* infection, is another example. While this peptide is efficacious it has very rapid clearance due to renal elimination. Benincasa et al. have shown that conjugation of Bac7 with PEG can be an efficient way to overcome this issue [20]. Using a murine model they demonstrated that PEGylated peptide had much slower renal clearance and wider distribution than the plain peptide. They also observed that PEGylated Bac7 was present in the system 24 hours post administration, and unlike unconjugated peptide the conjugate was able to sustain exposure in liver and peritoneal cavity. In addition, the PEGylated peptide was able to maintain the ability to kill bacteria by penetrating into the cells, suggesting Bac7 has the capacity to internalize a sizeable cargo even after conjugation.

Due to the smaller size, peptides have also been used for improving the permeability of drugs across the blood-brain barrier (BBB) to cure CNS disorders. One example is cell-penetrating peptide synB3, which has been conjugated to endomorphin-1 (EM1) to improve its analgesic activity in the brain [21]. Liu et al. have shown that the nature of the conjugation linker plays a crucial role in synB3 mediated EM1 delivery, and out of the 3 linkers tested the disulfide bond was found to be the most efficient for delivery across the BBB. Conjugation with synB3 also improved the half-life of EM1 in serum from 8.4 to 14.2 min and in brain from 19.6 to 28.4 min. The conjugate was able to enter the brain within 10 min, and the highest levels reached at 20 min. In addition, after 20 min the amount of conjugate in the brain appeared to be almost constant over 1h. Another example is peptide K16ApoE, which has been used to increase brain distribution of cisplatin and methotrexate by 34 and 58 fold, by transiently increasing BBB permeability [22]. However, this peptide is not covalently conjugated with the drugs and just premixed before the injection.

Clinical—GRN1005, which is a conjugate of LRP-1 targeting peptide angiopep-2 and paclitaxel, has been evaluated in the clinic for the treatment of brain cancer [23, 24]. This conjugate employs receptor mediated transcytosis process via the peptide to enhance brain exposure of the anticancer drug. GRN1005 has a half-life of ~3.6 h, and the PK of the conjugate was found to be dose proportional across all patients. Compared to the free paclitaxel, the conjugate resulted in 15-fold increase in total plasma exposure of the drug. CT-2103 is another peptide conjugate, where paclitaxel is attached to poly-L-glutamic acid to improve the PK of the drug [25]. The conjugate had a half-life of >100h in non-small cell lung cancer patients with a low volume of distribution, which allowed the drug molecule to stay in the circulation for an extended period of time [26]. Due to its relatively larger size and longer half-life, the conjugate was able to accumulate in the tumor by taking advantage of the EPR effect.

Other clinically evaluated therapeutic peptide conjugates include EP-100, which consist of an LHRH ligand joined with a cationic α -helical lytic peptide (CLIP-71). This conjugate is designed to deliver the lytic peptide into the cancer cells via specific binding to cell-surface LHRH receptors. In patients with LHRH receptor positive solid tumors, the conjugate showed variable clearance, which generally decreased with an increase in the dose. The volume of distribution also appeared to decrease with an increase in the dose, and at the higher dose it was found to be ~60 mL/kg. The conjugate was eliminated from the system rapidly, with a half-life of 7–16 min [27]. Romiplostim is another peptide conjugate, which belongs to the category of peptibody. This conjugate is designed to treat immune thrombocytopenia, by fusing thrombopoietin receptor targeting peptides with Fc fragment of IgG, to increase the half-life of the peptides [28]. In the clinic, the conjugate exhibited nonlinear PK and signs of target-mediated drug disposition (TMDD). Volume of distribution and clearance of the conjugate decreased with an increase in the dose [29]. For the low dose of 0.3 μ g/kg the half-life was ~1.5h, which increased to ~14h for the high dose of 10 μ g/kg.

Mipsagargin is a novel prodrug peptide conjugate, designed to treat patients with advanced solid tumors. This conjugate is developed by fusing novel thapsigargin-based drug with an inert masking peptide, which is activated by PSMA-mediated cleavage to release an active moiety that inhibits SERCA pump protein to induce cell death. In a phase I trial, the conjugate demonstrated bi-exponential PK profile, with an elimination half-life of ~21 h [30]. The clearance of the conjugate was very low (199 mL/h/m², ~1% of hepatic blood flow) and the volume of distribution was similar to blood volume (4995 mL/m²), suggesting the conjugate was largely confined to the plasma space and did not undergo extensive tissue distribution.

Clinically approved drug Peginesatide is also an example of therapeutic peptide conjugate, where the peptide is conjugated to PEG for improving its PK properties. Peginesatide is an erythropoietin-stimulating agent (ESA), which comprise of two identical erythropoietin mimetic peptides that are conjugated to PEG. As expected, PEGylation leads to an increase in the half-life of the peptide. Table-1 provides a summary of the PK parameters for this conjugate.

3. NON-TARGETED PROTEIN CONJUGATES

3.1 Diagnostic Application

Preclinical—Albumin has been extensively investigated as a non-targeted carrier protein for imaging of tumors [31]. It has a prolonged half-life of ~19 days in human, and can passively distribute to the tumor microenvironment via the EPR effect [32]. Once inside the tumor, albumin can be actively taken-up by the tumor cells via endocytosis, although the exact receptor responsible for this transportation is still not known. Due to these properties albumin can selectively reside at the tumor border where rapidly proliferating tumor cells exist, which makes it an optimal carrier for tumor diagnosis and imaging [33]. Albumin has also been used as an imaging agent for Rheumatoid arthritis (RA). RA is a chronic inflammatory disease marked by joint damage, upregulated metabolism of synovial cells, and increased permeability of the blood vessels in the inflamed joints. The increased fenestration in the blood vessels allow extravasation of albumin at the inflammation site, and synovial cells take up the albumin to fulfil the high demand for nitrogen and energy. Consequently, albumin has been preclinically shown to preferentially accumulate at paws affected by RA, and serves as an attractive carrier to target inflamed joints [34]. While theoretically one can conjugate any number of imaging probes to albumin, the PK of albumin may be affected by the amount of drug/probe conjugated to it. Neumann *et al.* have discussed that on average 1.4 molecules of drug per 1 molecule of albumin provides a physiologically stable conjugate that retains the longer half-life of normal albumin, whereas conjugation of higher number of molecules per albumin lead to higher systemic clearance [35].

Using rhodamine isothiocyanate conjugated bovine serum albumin (BSA) Maeda *et al.* have shown that 24h after the injection fluorescent albumin conjugate specifically accumulated in the S-180 tumor, whereas the free rhodamine was distributed throughout the mouse body [36]. Yang *et al.* have conjugated BSA with gadolinium oxide/copper sulfide to form a Photoacoustic/Magnetic resonance imaging agent, and using SK-OV-3 tumor bearing mice showed that while the conjugate eliminated from the body in 24h the tumor still retained 8 %ID/g of conjugate at that time [37]. Becker *et al.* have conjugated indotricarbocyanine (ITCC) with albumin for *in vivo* optical imaging of HT-29 tumors in mice [38]. They found that maximum tumor contrast of ~1.5 for HSA-ITCC was reached at 6 h after the injection.

Dijke *et al.* have shown the utility of albumin-gadopentetate dimeglumine (Gd-DTPA) conjugate for imaging RA in rabbits using MR imaging [39]. They showed that the albumin conjugate had a half-life of ~3h, and showed a strong signal in the plasma for up to 60 minutes, whereas the free Gd-DTPA decreased by 70% in 5 minutes. They concluded that due to the persistence of the conjugate in the blood, MRI imaging was able to correctly estimate capillary permeability and blood volume in arthritic knee. In another study, Hansch *et al.* injected Cy5.5 into antigen-induced arthritis mouse model for near-infrared range fluorescence (NIRF) imaging, and observed that between 2–72 h the arthritic knee joints showed significantly higher fluorescence compared with contralateral joints [40]. They found that majority of Cy5.5 dye in the serum was bound to albumin and used it as the delivery carrier for depositing at the RA site.

Clinical—5-aminofluorescein (AFL) has been widely used as a fluorescent imaging probe for conjugation with albumin. Kermer *et al.* [41] and Ding *et al.* [42] have demonstrated the application of albumin-AFL conjugate for fluorescent-guided surgical resection of malignant brain tumors. The conjugate is typically administered intravenously 1–4 days prior to the surgery, and able to cross the disrupted BBB to selectively reside in the tumor. The conjugate has a smaller volume of distribution ~6 L and slower clearance ~17 mL/h, which results in a prolonged half-life of ~13 days. Based on PK modeling the time for achieving peak concentrations of the conjugate in the peripheral compartment (e.g. brain) was predicted to be ~81 h, which matches well with 1–4 day time window used in the clinic to achieve the maximum contrast ratio in the tumor. Albumin has also been used in ^{99m}Tc labeled aggregated form (i.e. ^{99m}Tc -nanocolloid and ^{99m}Tc -Albures) to image tumors in the clinic. However, due to the aggregation these molecules are quickly trapped within capillaries and lymph vessels, and the systemic half-life of these aggregates is very short (~11 h) compared to normal albumin. ^{99m}Tc labeled albumin aggregates have also been used as an imaging agent for RA [43].

3.2 Treatment Application

Preclinical—PEGylation is a very common conjugation strategy for the development of non-targeted therapeutic proteins. PEGylated proteins have longer half-life and lower volume of distribution, which make them more desirable compared to their non-PEGylated counterparts. In addition, following subcutaneous administration PEGylated proteins are absorbed very slowly, and sometimes maximum serum concentrations are not reached until a week after the dosing [44], which in turn results in an extended half-life of the conjugate due to the flip-flop kinetics. PEGylated proteins may also display specific uptake characteristics in certain organs, but this is typically governed by the protein itself and not the PEG. In general, PEGylation tend to create more non-specific distribution of the conjugate as PEG itself do not have particularly high affinity for any organ. One such example is PEGylated recombinant human interleukin-6 (rhIL-6). rhIL-6 is a cytokine that exhibits a pleiotropic action, rapid renal clearance, and a broad distribution [45, 46]. The elimination half-life of unconjugated rhIL-6 in rats is ~55 min, which was increased to ~20h (approximately 20 times higher) following PEGylation. PEGylated rhIL-6 also demonstrated mono-exponential decline in the PK following subcutaneous administration, and the PK was found to be linear in the rats [47]. Tissue distribution study showed that the conjugate was mainly confined to the blood, and conjugation was able to successfully reduce broader distribution of rhIL-6 to the liver. Kidney was found to be the major organ responsible for the elimination for PEG-rhIL-6, and ~25% of the injected dose was recovered in the urine following 192 h. Another similar example is PEGylated endostatin (M₂ES), which is a proteolytic fragment of collagen XVIII that is used as an angiogenesis inhibitor. Elimination half-life of endostatin in rats is reported to be ~3.9 h, which was increased to ~60 h (approximately 15 times higher) following PEGylation [48]. M₂ES was found to rapidly and widely distribute to various organs (e.g. kidney, adrenal gland, lung, spleen, bladder and liver), and eliminate primarily via renal excretion. Around 70% of injected dose was recovered in the urine by 432 h.

Albumin conjugates have also been used as therapeutic agents. Due to its higher molecular weight and longer half-life, albumin serves as an ideal carrier for increasing the half-life and improving the PK of rapidly eliminating therapeutic agents. In addition, as mentioned above, it also has a tendency to accumulate in tumor and inflamed joints, which makes it an ideal carrier for delivering drugs to these tissues. A few groups have developed an interesting 'in situ' conjugation technology, where chemically activated drug molecules are administered in the body to make albumin conjugates in vivo. Using diverse drug molecules like doxorubicin and siRNA [49] they have demonstrated that this strategy not only leads to increased half-life of drug molecules, but it also leads to improved efficacy of the drugs. Lau et al. have shown that when an siRNA targeting IGF-1 receptor was administered in the rats it had a half-life of ~5 min, but when the same siRNA was administered in an activated form that can conjugate to albumin in vivo, it resulted in 15 fold increase in the half-life (~75 min) and ~9 fold increase in the exposure of siRNA.

Albumin can also be conjugated to other protein drugs to improve their PK. Byeon et al. have shown that conjugation of the anti-inflammatory protein TRAIL with albumin not only leads longer half-life of TRAIL, but also accumulation of the conjugate in the hind paws of collagen-induced arthritis mice, which makes the conjugate a better modality for the treatment of RA [50]. The TRAIL protein alone had a half-life of 0.23 h in the mouse, which was increase by more than 25 fold to 6.2 h following albumin conjugation. In addition, the clearance of TRAIL was reduced by 23 fold, and exposure was increased by 23 fold following albumin conjugation. The volume of distribution for TRAIL remained the same however. Zhang at al. have demonstrated similar benefits of albumin conjugation using anti-diabetic drug exendin-4 [51]. They observed that exendin-4 had a half-life of 0.58 h in the rhesus monkeys, which was increased by >90 fold to 53.4 h following albumin fusion. In addition, the clearance of exendin-4 was reduced by >500 fold, volume of distribution was reduced by >12 fold, and the exposure was increased by >500,000 fold.

Clinical—Many PEGylated proteins have advanced to the clinic as therapeutics. Majority of these conjugates are designed to increase the half-life of endogenous proteins. A few prominent examples include: methoxy PEG-epoetin beta (Mircera), pegylated interferon alfa-2a (Pegasys), pegylated interferon alfa-2b (PegIntron), PEGylated analog of human growth hormone (Somavert), PEGylated recombinant human granulocyte colony-stimulating factor (Pegfilgrastim), PEGylated recombinant human coagulation factor VIII (Antihemophilic Factor), PEGylated recombinant mammalian uricase (Pegloticase), pegylated phenylalanine ammonialyase (Pegvaliase), PEGylated L-asparaginase (Pegaspargase), and PEGylated bovine adenosine deaminase (Pegademase bovine). Table-1 summarizes the PK characteristics of these conjugates. In general, these conjugates are administered subcutaneously in the clinic, and have diverse half-life and bioavailability. PEGylated proteins typically demonstrate reduced renal clearance, and have reduced volume of distribution that is close to the blood volume. While PEGylation leads to a significant increase in the half-life of a protein, the extent of this enhancement depends on the contribution of TMDD towards the elimination of the conjugate. For example, compared to 2–4h half-life of filgrastim, its PEGylated version (Pegfilgrastim) shows ~10 time increase in the half-life (~40h, range 15–80h). However, this enhancement still provides a half-life of

only couple of days vs. couple of weeks for some other PEGylated proteins (e.g. Certolizumab pegol). In addition, Pegfilgrastim demonstrate a nonlinear PK, where the clearance of the conjugate decreases more than 7 fold when the dose is increased from 30 to 300 µg/kg. This PK behavior mainly stems from a significant contribution of saturable G-CSF receptors in the elimination of PEGylated filgrastim.

Conjugates of albumin, which is one of the most widely used non-targeted endogenous protein, have also advanced to the clinic as therapeutics. These conjugates are mostly designed to increase the half-life of the molecules attached to the albumin, and enhance their distribution to certain tissues (e.g. tumor). Perhaps the most notable example of albumin-drug conjugate is Abraxane® (nab-paclitaxel), which is an 'albumin-bound' nanoparticle formulation of paclitaxel, currently approved for the treatment of metastatic breast cancer, advanced non-small cell lung cancer, and metastatic pancreatic cancer. However, this conjugation does not really increase the half-life of paclitaxel, as both nab-paclitaxel and solvent-based paclitaxel (sb-paclitaxel) shows the half-life of ~20h. Instead, nab-paclitaxel leads to an increase in the unbound fraction of paclitaxel in the plasma from 2.3% (for sb-paclitaxel) to 6.3%. This in turn results in ~3 fold increase in the unbound plasma exposure of paclitaxel, which is associated with augmented antitumor efficacy of nab-paclitaxel compared to sb-paclitaxel. MTX-HAS, which is a covalent conjugate of methotrexate and albumin, is another example of albumin conjugate that is designed to increase the half-life and tumor accumulation of a chemotherapeutic agent. Vis et al. have demonstrated that albumin conjugation increases the half-life of methotrexate from 3–10 h to 9.3–11.4 days in cancer patients [52]. Aldoxorubicin is another clinically evaluated product, which relies on 'in situ' conjugation with albumin to increase the half-life of doxorubicin from 11.5–16 to 20.1–21.1 h. Aldoxorubicin also increases the exposure of doxorubicin by 175–180 fold in patients with advanced solid tumors.

Albuferon (Albinterferon), a fusion on interferon alpha (IFN-α) with human albumin, is an example of albumin conjugate that is developed to increase the half-life of a protein (i.e. IFN-α). IFNα-2b has a short half-life of 2–3 h in humans, which would require daily injections of the protein to treat hepatitis C. However, conjugation with albumin prolongs the half-life of IFN-α to ~6 days, allowing for less frequent administration (every 2–4 weeks) to achieve the efficacy [53]. Antidiabetic drug Albiglutide provides a similar example, where GLP-1 dimer is fused with human albumin to increase its half-life from couple of hours to 6–7 days [54].

4. TARGETED PROTEIN CONJUGATES

4.1 Diagnostic Application

Preclinical—Since the first antibody-based imaging agent OncoScint (a mouse IgG1) was approved by the FDA in 1992, antibodies have been extensively studied as a delivery vehicle for imaging agents. The advantage of using antibodies as the targeted imaging agents is the fact that the PK of antibodies is well understood, and relatively similar between most of the molecules. They demonstrate a long circulation half-life (1–3 weeks) due to FcRn mediated recycling and large molecular size (150 kDa), which prevents glomerular filtration to a great extent. However, this long half-life can become a disadvantage in tumor imaging. When

antibodies are used as tumor imaging agents, high plasma concentration could result in high background noise, which leads to decreased sensitivity. Consequently, most of the time there is 5–7-day delay between antibody administration and imaging. To overcome this limitation, antibody fragments and derivatives with shorter half-life have been developed as tumor imaging agents. These include antibody derivatives like F(ab)₂ (~100 kDa), scFv-Fc (~100 kDa), minibody (~80 kDa), diabody (~50 kDa), scFv (~25 kDa) etc. [55–72]. These engineered antibody fragments retain the binding properties of the full-size antibody, and clear from the systemic circulation rapidly, since there is an inverse relationship between the size of a protein and systemic clearance [73]. This PK property allows them to achieve higher tumor:plasma contrast ratio compared to the full length antibodies.

When it comes to imaging modality, almost any kind of imaging probe (radioisotopes, fluorophores, contrast agents, paramagnetic particles etc.) can be linked to antibodies. These imaging probes are usually covalently linked to the antibody at very low molar ratios to prevent interference with the antigen binding and PK. Imaging modalities commonly employed for molecular imaging of tumor include: positron emission tomography (PET), single-photon emission computed tomography (SPECT), magnetic resonance (MR) imaging, optical imaging (fluorescence and bioluminescence), and photoacoustic (PA) imaging. For each of this technique, the decision on which probe to use depends on the PK of the antibody or antibody fragment. For example, when it comes to radionuclides, the selected radionuclide should have comparable half-life with the targeting agent for optimal resolution and quantitative precision. When intact antibodies are used for PET imaging, ¹²⁴I (half-life ~100 h) and ⁸⁹Zr (half-life ~78 h) are suitable radionuclides since their decay half-life matches the time needed for IgGs to achieve optimal tumor-to-background ratio [74]. If smaller antibody fragments with short plasma half-life are used for PET imaging, ⁶⁸Ga (half-life ~1.1 h) and ¹⁸F (half-life ~1.8 h) may be more suitable.

Apart from the size of the protein, many other factors also play an important role in achieving maximum signal:background (or contrast) ratio for targeted imaging agents. One of the most important factors is the time of the imaging. Tsumura et al. have experimentally investigated this issue using fluorescently labelled anti-tissue factor antibody and its Fab fragment in a mouse xenograft model [75]. They have shown that despite a huge difference in systemic and tumor PK of these molecules, tumor:background ratio for them was the same, but at different time points of 24h and 12h. Thus, if one truly wants to understand the effect of size on maximum contrast ratio, it is important to explore multiple time points as the optimal time may vary for each molecule. The other important factors are expression level of the target, internalization rate of the target, size of the tumor, and physicochemical properties of the targeted agent (e.g. charge and glycosylation). Each of these factors can affect tumor PK and hence the contrast ratio of the imaging agent. Since each of these factors can simultaneously affect tumor PK of the imaging agent, it is hard to select an optimal imaging agent *a priori* without the help of a quantitative framework like a PK model. Wittrup et al. have developed one such model and suggested that there is a “U” shape relationship between maximum tumor uptake and protein size, where proteins ~50 kDa demonstrate the lowest uptake efficiency [76]. We have used a similar model and predicted that proteins ~20 kDa may be ideal for achieving higher tumor:plasma exposure ratio and better imaging sensitivity (data on file).

Half-life extension strategies also have been applied for diagnostic agents, with the hope that reduced clearance and enhanced exposure of a small protein conjugates might lead to the discovery of a better tumor diagnostic agent. For example, Kwon et al. Have designed a ^{64}Cu -labeled bispecific radioimmunoconjugates (bsRIC) composed of trastuzumab Fab fragment, EGF, and PEG₂₄ spacer, for preclinical imaging of various antigen expressing tumors via PET imaging [77]. As expected, they found that PEGylation led to 2.5–7 times increase in the systemic exposure of the conjugate, which ultimately resulted in 2.5–7 fold increase in the %ID/g concentration of the conjugate in the tumor at 48h post dosing. However, it is important to note that at 48h the ratio of tumor:plasma concentration was not significantly better for the PEGylated protein. In addition, since PEGylation led to equivalent reduction in the volume of distribution and clearance of the conjugate, the half-life of the conjugate was not significantly better than the unconjugated protein. Yazaki *et al.* have used albumin to increase the half-life and imaging potential of anti-CEA scFv [68]. Using LS-174T xenografts they showed that the half-life of albumin conjugated scFv was ~15 h, which was ~7 fold higher than the half-life of a typical scFv (~0.5–2 h). Using both PET and SPECT imaging generated with the help of different radiolabels they showed that the longer plasma half-life of the albumin conjugate also resulted in higher tumor uptake of the scFv. The tumor uptake of albumin-scFv conjugate was ~22.7–37.2% ID/g compared to 4.9% ID/g for the anti-CEA scFv, which resulted in a higher tumor:blood ratio of 18:1. Kenanova *et al.* have demonstrated that Fc fusion can also be used as a strategy to increase the half-life of imaging agents [78]. Using a series of fusion proteins generated by attaching anti-CEA scFv with Fc fragments possessing different binding affinities for FcRn, they demonstrated that one can design scFv-Fc fusion proteins with the same size but a desired halflife. The half-life of their fusion proteins ranged from 8h to 289h in mice. Interestingly, in the LS174T xenograft bearing mice they found that the mutant (H310A/H435Q) with the fastest clearance (8h half-life) was the best one for imaging, as at the last time point (~3 days) it was able to produce the highest tumor:background ratio of ~12:1.

Clinical—There are more than dozen antibody-based imaging agents approved by the regulatory agencies [79]. Table-2 summarizes available PK information for these conjugates. All except one have been approved for imaging cancer. Fanolesomab has been approved for the imaging of appendicitis. As mentioned in the preclinical section above, the half-life of these conjugates is a very important parameter for their effectiveness. In order to better exploit target mediated retention of these molecules at the site-of-diagnosis, systemic clearance of these molecules is usually faster so that a very high contrast ratio can be achieved for imaging at the later time points. As shown in Table-2, the half-life of all the conjugates is 5 days or shorter.

4.2 Treatment Application

Preclinical—Antibodies have become extremely popular as targeted therapeutics, especially for oncology disorders. They are often conjugated to cytotoxic molecules and radioactive materials to make antibody-drug conjugates (ADCs) and radio-immuno conjugates (RIC), which can specifically deliver lethal substances to the cancer cells. This conjugation strategy helps improve the therapeutics index of the conjugated molecules, by enhancing their exposure at the site-of-action and reducing their nonspecific exposure at the

site-of-toxicity. Characterization of the PK of these conjugates is very complex, as there are multiple analytes present in vivo, exposure of which at different anatomical site can lead to different pharmacology (i.e. efficacy or toxicity). In general, conjugated antibody is known as the main analyte, and the reported PK parameters of ADC/RIC usually correspond to this analyte. However, it is important to note that sometimes the PK parameters corresponding to these conjugates may refer to other analytes like conjugated drug, unconjugated drug, and total antibody. The PK of conjugated antibody typically depends on the inherent PK of the antibody and the stability of the linker. In general, similar to antibody, the PK of ADC/RIC is marked by slower clearance rates, lower volume of distribution, and longer half-lives [80]. Since most of the time the molecule conjugated to the antibody has a faster clearance, the PK of unconjugated molecule after ADC/RIC administration follows a formation rate limited kinetic, which is marked by a longer half-life equal to that of the conjugate. Of note, due to TMDD the conjugate may also demonstrate nonlinear distribution and clearance, and a half-life that is much shorter than a typical antibody [81, 82]. In addition, the number of drug molecules attached per antibody (also known as drug:antibody ratio or DAR) can also affect the PK of the conjugate, as it has been reported that high drug loading leads to faster elimination of the conjugate.

Since several factors like inherent PK of the antibody, cross-reactivity of the antibody with the preclinical species, target expression levels and turnover rate, severity of the disease, nature of the conjugation linker, the site and extent of drug conjugation, and the inherent PK of the released drug can affect the PK of ADC, PK parameters vary greatly between different ADCs. Table-3 provides a summary of the PK parameters observed for some of the preclinically investigated ADCs, which confirms that the half-life, clearance, and volume of distribution can vary hugely between the ADCs. Nonetheless, there are still a few generalizations one can make. Usually the systemic exposure of unconjugated drug is more than an order of magnitude less than the exposure of ADC. At relatively higher doses, when TMDD is saturated, the volume of distribution for ADCs is close to blood volume and clearance is closer to that of a normal antibody. Whole body disposition studies reveal that the exposure of unconjugated drug in normal tissues can be higher than their systemic exposure, due to partitioning and retention of these small molecules inside the tissues. Tumor disposition studies reveal that the $C_{\max}$ of the ADC and unconjugated drug in the tumor is delayed compared to plasma, and the exposure of unconjugated drug is several fold higher in the tumor compared to plasma and other tissues. Since it is difficult to comprehend and predict this complex PK behavior of ADCs, we and others have developed systems based PK/PD models, which can facilitate quantitative predictions of the PK of different ADC analytes by accounting for system and drug specific parameters [83–86].

ADCs can also show peculiar PK profiles preclinically. For example, anti-5T4 ADC, which has been investigated in mice, rats and monkeys, has shown nonlinear PK in non-cross reactive mice whereas the PK was linear in rats and monkey [87]. While it is difficult to justify this observation, it may stem from Fc-gamma receptor mediated elimination of humanized antibodies in some mouse species. IMMU-132, which is a clinically advanced anti-Trop-2 ADC, is another interesting example where the ADC exhibited plasma half-life of only ~11 h in mice [88]. Such a short half-life was mainly due to the instability of linker-payload and not the short half-life of the antibody. This example suggests that poor PK of

the ADC in preclinical species may not necessarily reflect a clinical failure of the conjugate. HIRMAb-IDS fusion protein, which is a fusion of anti-human insulin receptor antibody and iduronate 2-sulfatase enzyme, is another interesting example of targeted protein conjugate that is designed to increase brain penetration of a protein, which is not able to cross BBB by itself [89]. While the fusion protein had a short half-life of only couple of hours in the monkey, it was deemed to be sufficient to deliver enough enzyme in the brain to cure Hunter syndrome. As the ADC technology is evolving there is also an increase in the number of 'next-generation' ADCs that have better PK profile. One example of this class is 'site-specific ADCs' like ThioMab, which are generated using engineered antibodies to increase homogeneity of ADC formulation and the half-life of ADC. Using antiSTEAP1 ADCs Boswell et al. have shown that while the conventional ADC had a clearance of ~33.6 mL/day/kg, ThioMab based ADC had a clearance of 12.5 mL/day/kg, which was close to unconjugated antibody clearance of ~10 mL/day/kg [90].

RICs remain an important component of targeted protein conjugates, and have recently gained more attention due to their synergy with immune-oncology agents. Their PK also remains variable across molecules (Table-3), but in general they have relatively shorter half-life. The PK of RICs is significantly affected by the type of radiolabel used, the way the label is attached, and the radiolabeling procedure. Batra et al. have provided an interesting example of this phenomena using RICs made from anti-EGFR antibody nimotuzumab [91]. They have shown that different conjugates of nimotuzumab made using ^{177}Lu had always a shorter half-life (7–18 h) in rats compared to the conjugates made using ^{131}I (30 h). In addition, ^{177}Lu labelled RICs exhibited high retention and radioactivity in the liver, which may be the source of toxicity. Along the similar lines, Thompson et al. have investigated if there is a correlation between the efficacy of RICs and the half-life of radioisotopes, using a melanin-binding antibody 6D2 (IgM) in a mouse model of melanoma [92]. They observed that RICs that had similar half-life of the conjugate and the radioisotope had better therapeutic index than RICs that did not have matching half-lives of the two components.

Immunotoxins are also an old and important component of targeted protein conjugates. These conjugates help target toxic molecules to the site-of-action, and may even improve systemic PK of the toxins. Zhou et al. have presented a typical example of such conjugate, in the form of HSGZ, which is generated by fusing anti-Fn14 dimeric single-chain antibody with recombinant gelonin toxin [93]. In a cross-reactive mouse tumor model the conjugate had a half-life of 7.3 h, achieved 4.7–5.1 %ID/g concentration in the tumor, and had a tumor-to-muscle ratio of 5.6–9.0. While the immunotoxin was efficacious, it deposited in unusually high concentrations (10–30 %ID/g) in the liver, kidney, and spleen. This unusual tissue deposition may stem from TMDD, active uptake by the RES system, or immune reaction to gelonin. IL-13PE is another kind of targeted toxin conjugate, where a cytokine (i.e. IL-13) is fused with pseudomonas aeruginosa exotoxin A to target IL-13 receptor $\alpha 2$ (IL-13R $\alpha 2$) expressing gliomas [94]. The conjugate was intracranially administered via convection-enhanced delivery (CED) in mouse, and it was observed that the conjugate attained as high as 4.2 %ID/g concentration in the tumor compared to 0.1–1 %ID/g in all other tissues. Even after 7 days IL-13PE was present in the brain at 1.1 %ID/g concentration.

PEGylated conjugates of antibody and antibody fragments also fall into the category of targeted protein conjugates. As mentioned before, PEGylation can significantly improve the half-life and alter the PK of targeted proteins. As early as two decades ago Lee et al. have shown that PEGylation can increase the half-life of scFv by more than 18 fold (from ~0.7 h to ~13 h) in mice [95]. In addition, it is now well known that the size and number of PEGs conjugated per protein can be altered to attain desired PK of the conjugate [96].

Clinical—More than 70 ADCs have advanced to clinical trials, vast majority of which contain an IgG1 isotype and cleavable linker. Cytotoxic drug molecules conjugated to antibodies mostly include microtubule disrupting agents (e.g. DM1, DM4, MMAE, MMAF, SN-38) and DNA damaging agents (e.g. calicheamicins and PBD). Table-4 summarizes the PK characteristics of some of the clinically investigated ADCs. As shown in the table and mentioned before, due to the complex nature of ADC molecules and contribution of many factors towards their disposition, PK profile of ADCs is hard to generalize and vary greatly between the molecules. Nonetheless, Antoine Deslandes has tried to compare clinical PK of different ADCs [97]. It was observed that in general the plasma AUC of ADCs increased more than dose proportionally at higher doses. In addition, a decrease in clearance with increasing doses was observed for ADCs like AVE9633, BT062, MLN2704, and T-DM1. These observations suggest nonlinear PK of ADC, possibly driven by TMDD. However, other ADCs like huC242-DM1, IMGN901, and PSMA-ADC, showed no dose-dependent change in the clearance. As shown in Table-4, the half-life of ADCs vary between 0.9–14 days, and volume of distribution is mostly close to the blood volume of 50100 mL/kg. It is also reported that in general the exposure of unconjugated drug in plasma is more than an order of magnitude (e.g. 200-fold) less than the plasma exposure of ADC.

There are two clinically approved RICs, ¹³¹I-tositumomab (Bexxar®) and ⁹⁰Y-ibritumomab tiuxetan (Zevalin®), both of which target CD20. In addition, more than dozen RICs have been evaluated in the clinic [98]. Median biological half-life of Zevalin is ~48 h, and it is mainly cleared via the renal system. In the clinic, ~7% of the administered Zevalin was found to eliminate in the urine in the first 7 days. Zevalin is able to selectively distribute to the tumor, especially after pretargeting with the cold antibody, and capable of achieving tumor-to-normal organ ratio of 7. The median half-life for Bexxar is ~67 h (28–115 h), and it also eliminates via the renal system. In the clinic, ~67% of the administered Bexxar was found to eliminate in the urine in the first 5 days.

More than half-a-dozen immunotoxins have also been evaluated in the clinic, but denileukin diftitox (Ontak®) remains the only one approved by the regulatory agencies [99]. It is a fusion of interleukin-2 and diphtheria toxin designed to treat T-cell lymphoma. Denileukin diftitox shows a linear PK, with terminal half-life of ~70–80 minutes. Mean clearance of this immunotoxin is ~0.62.0 mL/min/kg, and the volume of distribution is close to the blood volume (60–90 mL/kg). Interestingly, the clearance of this conjugate increases 2–8 fold following multiple dosing, which may be due to the development of anti-therapeutic antibodies against the toxin. Anti-CD22 immunotoxin moxetumomab pasudotox is another clinically evaluated immunotoxin, generated by covalently fusing anti-CD22 antibody with a fragment of Pseudomonas exotoxin-A (PE38) [100]. This immunotoxin also has a short half-life of ~1h in the clinic, and demonstrate very variable PK between the patients. LMB-2

(anti-Tac(Fv)-PE38) is another similar example of an immunotoxin, which demonstrates terminal half-life of only ~135 minutes [101]. As such, immunotoxins seems to have very short half-lives in the clinic, which may be required to avoid overt toxicity and achieve target specificity.

PEGylated protein like Certolizumab pegol, which is a conjugate of anti-TNF α Fab fragment and ~40 kDa PEG, is also an example of clinically approved targeted protein conjugate. As shown in Table-1, it has a half-life of 2 weeks, and volume of distribution close to the blood volume of 6–8 L. Considering Fab fragments have 12–20 h half-life in human, clinical PK of this conjugate clearly show that PEGylation can lead to a dramatic increase in the half-life of targeted proteins

5. SUMMARY

Each protein and peptide has a unique PK profile, which can be exploited to deliver conjugated molecules at the site-of-action, or can be improved with the help of conjugation. In this article we have reviewed relevant examples that highlight these applications of conjugation for diagnostic and therapeutic purposes. Peptides are very useful to accomplish rapid and specific distribution of a diagnostic or therapeutic agent at the site-of-action, while minimizing the systemic exposure. They can also help conjugated molecule cross physical barrier like BBB. However, they also rapidly eliminate from the renal system and may have very short half-life, which can be improved with the help of PEGylation or conjugation to other non-targeted proteins like Fc fragment or albumin. Albumin do not have any known target, but it can improve the half-life of conjugated diagnostic/therapeutic molecules, and also facilitate accumulation of these molecules at certain anatomical locations (e.g. solid tumor and inflamed joints) because of its longer half-life and larger molecule weight. However, this kind of targeting based on physicochemical or PK property of a protein is not as specific as the one achieved with the help of targeted proteins like antibodies. Consequently, antibodies and their derivatives have become primary proteins for the development of targeted protein conjugates. Since antibodies have long half-life and modular structure, they allow for the developed of diverse derivatives with desired PK and targeting characteristics. Molecules with smaller size and faster elimination are preferred as diagnostic agents, and molecules with longer half-life and enhanced exposure at the site-of-action are preferred as therapeutic agents. Natural ligands like cytokine, which have affinity for specific receptors, can also facilitate the development of targeted protein conjugates. However, since they usually have very short half-life, their use is limited compared to antibody based conjugates. In sum, protein and peptide conjugates possess diverse PK profiles, a thorough understanding of which is required for successful development of a diagnostic or therapeutic agent. In order to reduce translational failure of these conjugates it may be worth designing them *a priori* based on the desired PK profile.

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Table 1.

PK characteristics of approved PEGylated proteins and peptides.

Conjugate Name	Half-life of unconjugated protein (hr)	Half-life of the Conjugate (hr)	CL (mL/hr/kg)	Vss (mL/kg)
Certolizumab pegol [102]		336	0.1535-0.239667	100–133
Peginesatide [103]	9	25	0.5	43.3
Pegaptanib [104]		240	2.33 [105]	48.3 [105]
methoxy PEG-epoetin beta [106]	9 [107]	199	0.47	61
PEGylated interferon alfa-2a [108]	5	160	1.5667	
PEGylated interferon alfa-2b [109]	1.7 [110]	40	22	
PEGylated analog of human growth hormone ^l [111]	2.3	33	9–27	116
PEGylated recombinant human GCSF [112]	4.7 [113]	15–80	63.6 [114]	
PEGylated recombinant human coagulation factor VIII [115]	10.4	14.3	2.76	
PEGylated recombinant mammalian uricase [116]		154–331		
PEGylated phenylalanine ammonia-lyase [117]	21 [118]	46–120		
PEGylated L-asparaginase [119, 120]		72–120	0.105	
PEGylated bovine adenosine deaminase [121]		72–144		

Note: Parameters were normalized to body weight assuming a human body weight of 60 kg and body surface area of 1.7 m².

Table 2:

PK characteristics of approved targeted protein diagnostics.

Generic Name (Trade Name)	Structure (Radiolabel)	Year of Approval	α Half-life (hr)	β Half-Life (hr)	CL (mL/hr/kg)	VD, β (mL/kg)
Satumomab pendetide * (OncoScint)	B72.3 mouse IgG1 (In-111)	1992	6.6 [122]	64.2 [122]	0.716 [122]	66.7 [123]
Arcitumomab (CEA-Scan) [124]	IMMU-4 mouse IgG1 Fab (Tc-99m)	1996	1	12 [124]	~16.667	133 (Vss)
Octreotide (OctreoScan) [125]	Octreotide (In111)	1994	~1	~1.6	~25	300–500
Imciromab pentetate * (Myoscint)	R11D11 mouse IgG2a Fab (In111)	1996	1.5	20.2		
Nofetumomab merpentan (Verluma)	NR-LU-10 mouse IgG2b Fab (Tc99m)	1996	1.5	10.5		
Capromab pendetide (ProstaScint) [126]	7E11-CS.3 mouse IgG1 (In111)	1996		67	0.7	667
Ibritumomab tiuxetan (Zevalin)	2B8 mouse IgG1 (Y-90)	2002		46 [127]		
Tositumomab (Bexxar) [128]	B1 mouse IgG2a (I-131)	2003	2.7	64.1	1.167	75
Fanolesomab * (NeutroSpec former LeuTech)	RB5 mouse IgM (Tc-99m)	2004	0.3	8		
Bectumomab (LymphoScan)	LL2, mouse IgG2a Fab' (Tc99m)	Not in USA				
Votumumab * (HumaSPECT)	88BV59, human IgG3 (Tc-99m)	Not in USA				
Igovomab * (Indimacis-125)	OC125, mouse IgG1 F(ab')2 (In111)	Not in USA				
Sulesomab (LeukoScan)	IMMU-MN3 mouse IgG1 Fab' (Tc-99m)	Not in USA	1 [129]	20 [129]		

* Removed from market.

Note: Parameters were normalized to body weight assuming a human body weight of 60 kg and body surface area of 1.7 m².

Table 3.

Some of the preclinically investigated ADCs/RICs and their PK characteristics

Name	Target	Linker	Dose	Cl (mL/hr/ kg)	Half-life (hr)	Vss (mL/kg)	Animal
ADCT-402 [130]	CD19	VA	0.6 mg/kg		336		Cynomolgus Monkey
Anti-CD22 [131]	CD22	VC	3 mg/kg	0.39		80.0	Cynomolgus Monkey
Anti-CD79b [131]	CD79b	VC	3 mg/kg	0.592		43.1	Cynomolgus Monkey
BMS-936561 [132]	CD70	Maleimide-citrullin valine	22.5 mg/kg	2.42	163	270	Cynomolgus Monkey
7v-Cys-may [133]	CD70	SMCC	30 nmol/kg	0.371	235		Mice
cAC10+MMAE [134]	CD30	VC	40 mg/kg	0.192	314		Mice
h1F6+PBD [135]	CD70	MCVC	1.0 mg/kg		192		Mice
J2898A-SMCC-[³ H]DM1 [136]	EGFR	SMCC	10 mg/kg	1.16		366	Mice
M9346A-sulfo-SPDB[³ H]DM4 [136]	FRα	SPDB	10 mg/kg	1.33		234	Mice
SYD983 [137]	HER2	VC	1 mg/kg		27		Mice
IMMU-132 [88]	TROP2	carbonate	0.2 mg	0.0022	11		Nude Mice
PF-05231023 [138]	FGF21	Maleimide-azetidine	1 mg/kg	2.11	97	111	Rats
IDS+Mab [89]	HIR	N/A	10 mg/kg	31.26+/-2.22	2	89.6+/-4.6	Rhesus Monkey
166Ho/6D2 [92]	melanin	DPTA	100ug 6D2		6.5		Nude Mice
Nimotuzumab+131I [91]	EGFR	DPTA+ p-SCNBn			30		Wistar rats

Abbreviations: valine-alanine (VA); valine-citrulline (VC); succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate (SMCC); maleimidocaproyl (MC); N-succinimidyl 4-(2-pyridyldithio)butanoate (SPDB); diethylene triamine pentaacetic acid (DTPA)

Table 4.

Some of the clinically investigated ADCs and their available PK characteristics

Name	Target	Linker	Dose (mg/kg)	Cl (mL/hr/kg)	Half-life (hr)	Vss (mL/kg)
ABT-414 [139]	EGFR	maleimi do caproyl	1.0		211	
AGS-16C3F [140]	ENPP3	maleimi do caproyl	1.8		168–192	
AGS67E [141]	CD37	MC-VCPABC	1.2		38–54	
AVE9633 [142]	CD33	SPDB	6.43	0.525	94	68.1
BAY 94–9343 [143]	Mesoth elin	SPDB	5.5		120	
Besponsa/ CMC544 [144]	CD22	hydrazo ne	0.068	39	56	2033.33
BT062 [145]	CD138	SPDB	3.95	1	51	
CDX-011 [146]	GPMB	vc	1.88	1.7	28.3	48.7
Denintuzumab Mafodotin [147]	CD19	maleimi do caproyl	0.5–6		336	
DMOT4039A [148]	Mesoth elin	vc	2.4	1.13	50–89	
HuC242-DM1 [97]	CanAg	SPP	5.81	1.01	49	42.7
IMG901 [97]	CD56	SPP	2.77	2.2	0.89 days	56.3
Kadcyla/ Trastuzumab Emtansine [149]	HER2	SMCC	3.6	0.35	21.36	30
MEDI-547 [150]	EphA2	maleimi do caproyl	0.08			
Mirvetuximab soravtansine [151]	FR alpha	SPDB	1.0		79	
MLN2704 [152]	PSMA	thioester	8.48	1.1	60	
PF06263507 [153]	5T4	maleimi do caproyl	1.42	0.9	91	91.667
Polatuzumab vedotin [154]	CD79b	MC-VCPABC	2.4	0.85	128	78.1
PSMA-ADC [155]	PSMA	vc	2.2	1.37	40	62.6
Rovalpituzumab tesirine [156]	DLL3	dipeptid e	0.4	0.17	302	74
SAR3419 [157]	CD19	SPDB	3.95	1	166	53.6

Abbreviations: valine-citrulline (VC); p-aminocarbamate (PABC); succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate (SMCC); maleimidocaproyl (MC); N-succinimidyl 4-(2-pyridyldithio)butanoate (SPDB); N-succinimidyl-4-(2-pyridyldithio)pentanoate (SPP)

Note: Parameters were normalized to body weight assuming a human body weight of 60 kg and body surface area of 1.7 m².