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Bioanalytical Assays for Pharmacokinetic and Biodistribution Study of Antibody-Drug Conjugates

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ABSTRACT

Antibody-drug conjugates (ADCs) are produced by the chemical linkage of cytotoxic agents and monoclonal antibodies. The complexity and heterogeneity of ADCs and the low concentration of cytotoxic agent released in vivo poses big challenges to their bioanalysis. Understanding the pharmacokinetic behavior, exposure-safety, and exposure-efficacy relationships of ADCs is needed for their successful development. Accurate analytical methods are required to evaluate intact ADCs, total antibody, released small molecule cytotoxins, and related metabolites. The selection of appropriate bioanalysis methods for comprehensive analysis of ADCs is mainly dependent on the properties of cytotoxic agents, the chemical linker, and the attachment sites. The quality of the information about the whole pharmacokinetic profile of ADCs has been improved due to the

development and improvement of analytical strategies for detection of ADCs, such as ligand-binding assays and mass spectrometry-related techniques. In this article, we will focus on the bioanalytical assays that have been used in the pharmacokinetic study of ADCs and discuss their advantages, current limitations, and potential challenges.

SIGNIFICANCE STATEMENT

This article describes bioanalysis methods which have been used in pharmacokinetic study of ADCs and discusses the advantages, disadvantages and potential challenges of these assays. This review is useful and helpful and will provide insights and reference for bioanalysis and development of ADCs.

Introduction

Antibody-drug conjugates (ADCs) are an innovative and significant kind of complex biopharmaceuticals with highly cytotoxic small molecular compounds (payloads) covalently bound to monoclonal antibodies through a chemical linker (McCombs and Owen, 2015; Parslow et al., 2016; Zhang et al., 2022). The typical chemical structure of an ADC is shown in Fig. 1A. Compared with monoclonal antibodies, the chemical structures of ADCs are more complex and heterogeneous. The objective

of development of ADCs is the specific and targeted delivery of small molecular cytotoxic payload to cancer cells. Meanwhile, they are less exposed in normal tissues, which results in improving the therapeutic index of anti-tumor treatment and minimizing the corresponding toxic side effects (Abdollahpour-Alitappeh et al., 2019; Hafeez et al., 2020; Marei et al., 2022; Tarantino et al., 2022).

Generally, the chemical structure of ADCs has three parts: monoclonal antibody, chemical linker, and small molecular cytotoxic payload (Dal Corso et al., 2017; Bargh et al., 2019; Ashman et al., 2023). ADCs specifically combine with the highly expressed proteins (target antigen) on the surface of cancer cells through monoclonal antibody to generate ADC antigen complex, and then enter the cells through the endocytosis mediated by clathrin; thereafter, the drug carrier enters the tumor cells to release drug and kill tumor cells. The metabolism and action mechanism of ADC is shown in Fig. 1, B and C. The antibody part of ADCs should be able to selectively combine with the antigen molecules that are specifically expressed or overexpressed on the surface of cancer cells (McCombs and Owen, 2015; Yao et al., 2016). The cytotoxicity of the payload of ADC should be very high so that the payload can kill tumor cells at low concentrations reached in the cells after the ADC is

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ABBREVIATIONS: ADC, antibody-drug conjugate; CIQA, cryoimaging quantitative autoradiography; DAR, drug-to-antibody ratio; DM1, mertansine; FMT, fluorescence molecular tomography; LBA, ligand-binding assay; LC-MS/MS, liquid chromatography tandem mass spectrometry; LSC, liquid scintillation counter; MS, mass spectrometry; PET, positron emission tomography; SPE, solid phase extraction; SPECT, single photon emission computed tomography; T-DM1, trastuzumab emtansine.

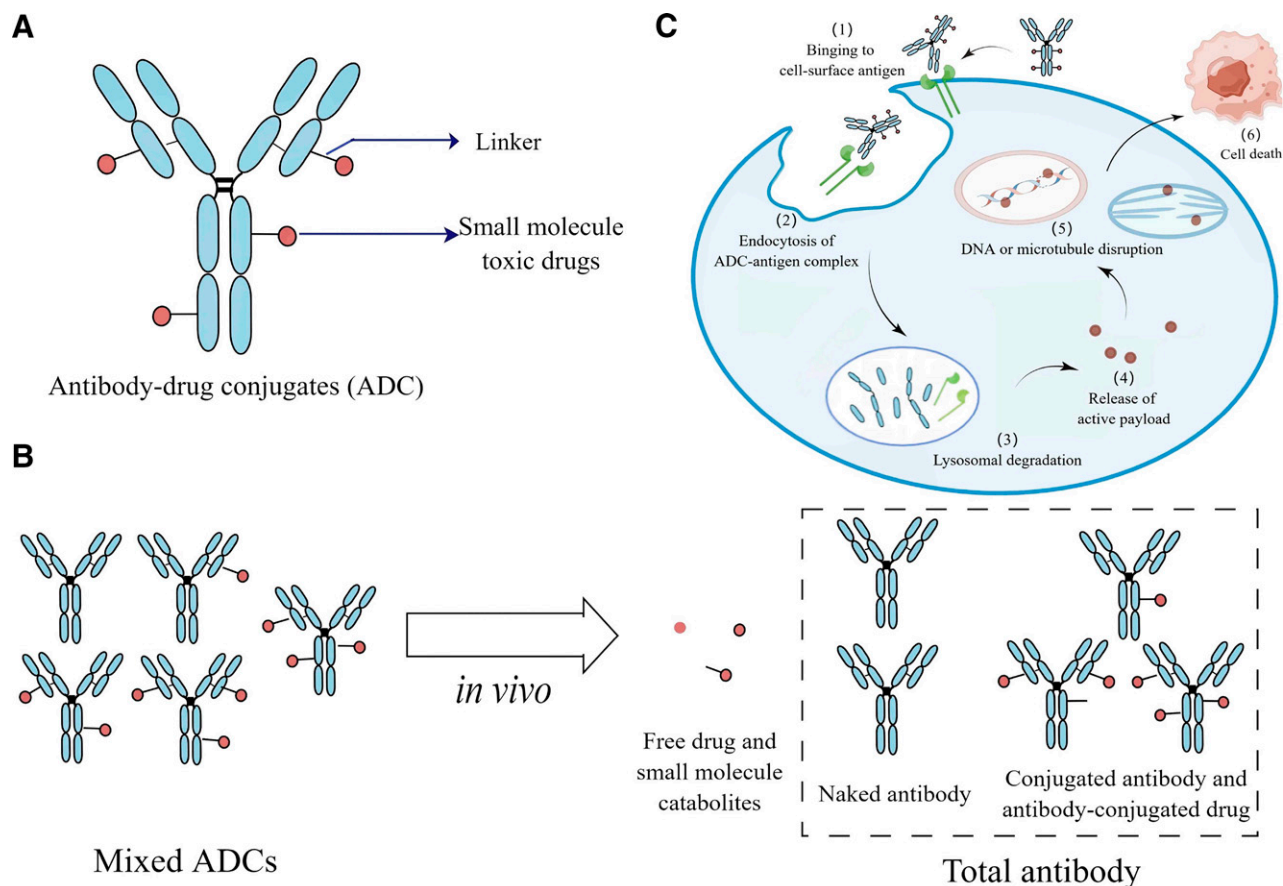


Fig. 1. (A) Typical chemical structure of ADC. (B) Metabolism of ADC in vivo. (C) Action mechanism of ADC.

distributed to solid tumor tissue (McCombs and Owen, 2015). The number of cytotoxic agents connected with the antibody molecule is limited; therefore, the biophysical and pharmacokinetic characteristics of ADCs are not seriously affected contrast to the corresponding monoclonal antibodies. ADCs are heterogeneous mixtures which have different drug-to-antibody ratios (DARs). The DAR distribution of ADCs is dynamically changing in vivo. Furthermore, the linkers of ADCs should be stable enough in systemic circulation to ensure that the payloads remain connected to the antibodies, but once the ADCs distribute into solid tumor tissue and enter the cancer cells, the linkers should be broken easily and completely to release the small molecular cytotoxic payload to kill the cancer cells (Cazzamalli et al., 2017; Dal Corso et al., 2017; Deonarain and Yahioğlu, 2021; Nicolaou et al., 2021; Teicher and Morris, 2022; Ashman et al., 2023).

ADC is a hot track of innovative drugs at present, and its indications are mainly solid tumors (Joubert et al., 2020; Chia, 2022). So far, there are 310 ADC drugs with known targets in the world. Most of these drugs are in the early clinical stage. Only 15 of them have been successfully marketed and approved by the U.S. Food and Drug Administration for cancer treatment in clinical applications (summarized in Table 1), 13 candidate drugs have entered the clinical stage III, and 156 and 137 drugs are in the preclinical stage and clinical stage I. Understanding the pharmacokinetic behavior of ADCs is important for their development, optimization, and clinical application (Han and Zhao, 2014; Kraynov et al., 2016; Zhang et al., 2019). The pharmacokinetic behaviors of ADCs are summarized and shown in Table 2. Accurate analytical methods for pharmacokinetic study of ADCs should monitor different kinds of analytes, such as intact antibody drug conjugates, conjugated small-

molecular toxins, total antibodies, and free small-molecular toxins and their related metabolites (Dere et al., 2013). The selection of appropriate analytical methods for comprehensive analysis of ADCs is mainly associated with the properties of the chemical linker, small molecular cytotoxic agents, and the attachment sites (Wakankar et al., 2011; Källsten et al., 2018; Cahuzac and Devel, 2020; Pretto and FitzGerald, 2021). At present, label-free bioanalytical assays and labeled bioanalytical methods are the two main types of bioanalytical assays for determination of ADCs-derived compounds in plasma or tissues. Label-free bioanalytical assays mainly refer to ligand-binding assays (LBAs), mass spectrometry (MS)-based methods, or the combination of the above assays (Wang et al., 2016; Todoroki et al., 2018; Lechner et al., 2019; Chang and Shah, 2020; Todoroki et al., 2020; Zhang et al., 2023). In most cases of labeled ADCs, a radioactive isotope or a fluorescent reporter should be incorporated within the structure of the payload and/or the monoclonal antibody. The in vivo fate of ADCs can be monitored by noninvasive imaging assays just like positron emission tomography (PET), single photon emission computed tomography (SPECT) and fluorescence molecular tomography (FMT) (Ilovich et al., 2015; Giddabasappa et al., 2016; Brand et al., 2018; Carmon and Azhdarinia, 2018).

The aim of this article is to outline the bioanalytical methods that have been used in pharmacokinetic study of ADCs and discuss their associated merits, current limitations and potential challenges. We believe this review is helpful and will provide insights and reference for bioanalysis and development of ADCs.

TABLE 1
ADCs approved by the Food and Drug Administration

ADC	Target Antigen	Payload	Linker	Average DAR	Indication	FDA Approval
Mylotarg	CD33	Calicheamicins	Cleavable	2-3	Acute myeloid leukemia	2000
Adcetris	CD30	Monomethy lauristatin E (MMAE)	Cleavable	4	Hodgkin's lymphoma	2011
Kadcyla	HER2	Mertansine (DM1)	Noncleavable	3.5	Breast cancer	2013
Besponsa	CD22	Calicheamicins	Cleavable	5-7	B-cell precursor acute lymphoblastic leukemia	2017
Lumoxiti	CD22	Pseudomonas aeruginosa exotoxin (PE38)	Cleavable	/	Hyperleukocytic acute leukemia	2018
Polivy	CD79b	Monomethy lauristatin E (MMAE)	Cleavable	3.5	Non-Hodgkin's lymphoma	2019
Euhertu	HER2	Exatecan derivative (Dxd)	Cleavable	8	Breast cancer, gastric cancer	2019
Padcev	Nectin-4	Monomethy lauristatin E (MMAE)	Cleavable	4	Urothelial carcinoma	2019
Trodelyv	TROP2	SN38 (7-Ethyl-10-hydroxycamptothecin)	Cleavable	8	Triple negative breast cancer	2020
Blenrep	BCMA	Mono Methy lauristatin F (MMAF)	Cleavable	4	Multiple myeloma	2020
Akalux	EGFR	IRDye700DX	Noncleavable	/	Head and neck cancer	2020
Zynlonta	CD19	PBD	Cleavable	2.3	Non-Hodgkin's lymphoma	2021
Disitamab Vedotin For Iicction	HER2	Monomethy lauristatin E (MMAE)	Cleavable	3.5	Gastric cancer	2021
Tivdak	TF	Monomethy lauristatin E (MMAE)	Cleavable	4	Cervical carcinoma	2021
Elahere	FR α	DM4	Noncleavable	3.4	Ovarian cancer	2022

Analysis of ADCs by Ligand-Binding Assays

The LBAs are generally used for detection of biologic large molecular compounds (Thway, 2016). The critical procedure of LBAs is the equilibrium reaction between the ligand and the protein or antibody that is directed against the target analytes. The final time point of the reaction reveals the amounts of the monitored analytes in different biologic samples. The analytes in biologic samples can be captured and detected by most LBAs with antibodies as critical reagents (Jani et al., 2016). ELISAs are the gold standard of LBAs for detection of different analytes in various biologic samples (Fischer et al., 2015). Monoclonal antibodies and polyclonal antibodies are the preferred critical reagents in LBAs. To generate monoclonal antibodies or polyclonal antibodies, rabbits, goats, and sheep are usually used as the host species, which are immunized with therapeutic proteins and adjuvant (Lipman et al., 2005; Thway, 2016).

The chemical structure of ADCs is very complicated. Bioanalysis and pharmacokinetic study of ADCs is a difficult task. The efficacy and toxicity of ADCs are associated with antibody conjugated drug, free drug, and total antibody, which should be monitored simultaneously. For analysis of ADCs, liquid chromatography tandem mass spectrometry (LC-MS/MS) is usually used to detect payload with small molecular weight and LBAs are usually used to detect total antibody or conjugated

antibody whose molecular weight is large. For example, Stephan et al. developed bioanalysis assay based on the ELISA technique for determination of MC-MMAF conjugates and anti-CD22-MCC-DM1 in mouse plasma. For analysis of the total anti-CD22 antibodies, the antibodies were captured by CD22 extracellular domain (ECD) and detected by a goat anti-human IgG Fc horseradish peroxidase-conjugated antibody GxhFc-horseradish peroxidase. Moreover, for analysis of the drug-conjugated anti-CD22 antibody, recombinant CD22 extracellular domain and anti-mertansine (DM1) or anti-MMAF biotinylated antibody were used as the capture reagent and detection reagent, respectively (Stephan et al., 2008). Lewis Phillips and his collaborators developed ELISA-based analytical method for determination of trastuzumab-maytansinoid conjugates (HER2 breast cancer treatment drug) in mouse plasma. For detection of trastuzumab-maytansinoid, HER2 extracellular domain and goat anti-human Fc integrated horseradish peroxidase-conjugated were used as the capture reagent and detection reagent, respectively (Lewis Phillips et al., 2008). Generally speaking, the advantages of LBAs include low cost, high throughput, easy implementation, and high sensitivity for determination of large molecules. The disadvantages of LBAs are summarized as follows (Mou et al., 2018): (1) LBAs could not measure the DAR of ADCs; (2) the sensitivity of LBAs is limited for detecting the metabolites of ADCs; (3) the selectivity

TABLE 2
Pharmacokinetic characteristics of ADCs

	ADCs
Molecular weight (Da)	150K
Administration route	Intravenous administration
Distribution	The apparent distribution volume of ADCs is generally small, close to plasma volume, and limited tissue distribution; The apparent distribution volume of payload is relatively large, and it is easier to enter the tissue; May be the substrate of transporter
Metabolism	Phase I and II metabolism, proteolysis.
Excretion	ADCs and antibodies mainly circulate in the body; The main excretory pathway for payload and its metabolites are bile and renal excretion.
Half-life time	The half-life time of both ADC and free small-molecular toxins are relatively longer.
Pharmacokinetic linearity	Generally, they are linear at high dose, but nonlinear at low dose.
Target analytes	Antibody drug conjugates, total antibodies, conjugated small-molecular toxins, free small-molecular toxins and their related metabolites
Bioanalysis method	Ligand binding assay, LC-MS/MS, CE-MS/MS, etc.
Immunogenicity	Yes
Heterogeneity	Mixture

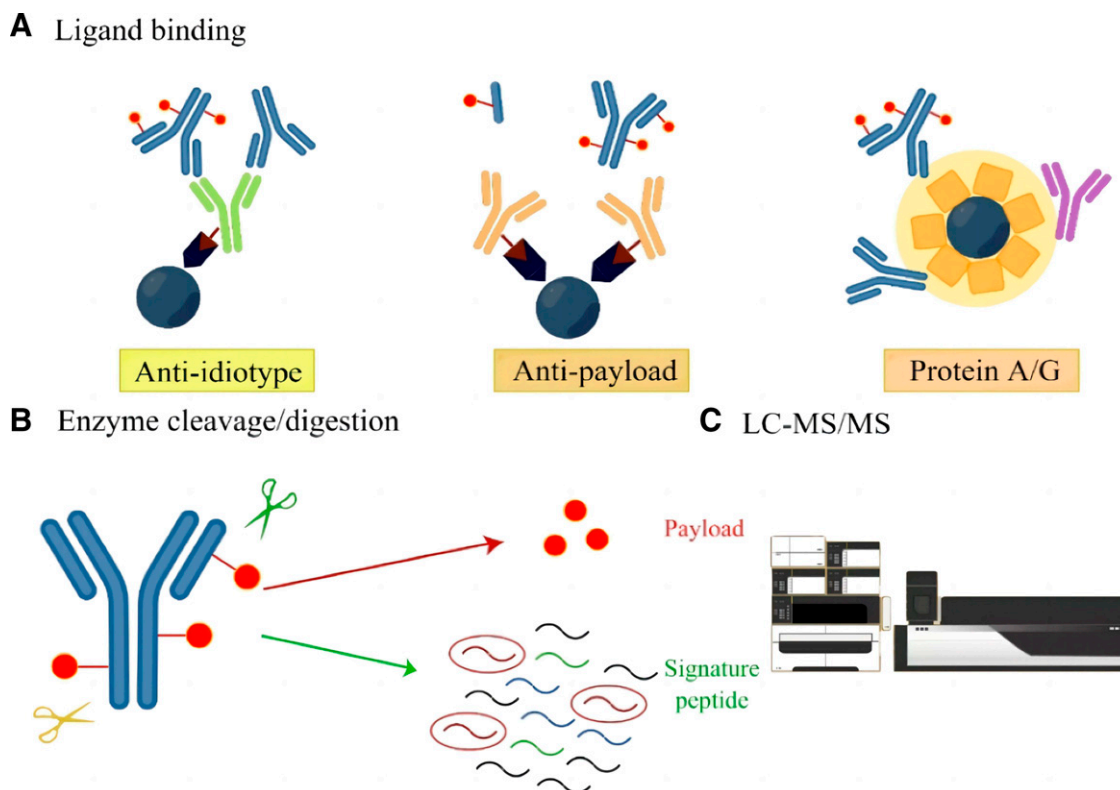


Fig. 2. The typical procedures of hybrid LBA-LC-MS/MS assays: (A) ligand binding, (B) enzyme cleavage/digestion; (C) LC-MS/MS analysis.

of LBAs with antibodies to capture and detect the analytes is not high; (4) LBAs cannot provide the comprehensive information about chemical structure and sequence of ADCs; (5) the antibodies used in LBAs have potential cross-reactivity; (6) the multiplexing capability of LBAs is limited; and (7) the time for development of antibodies of LBAs is long.

Analysis of ADCs by Hybrid LBA-LC-MS/MS Assays

Hybrid LBA-LC-MS/MS assays are complementary alternatives to LBAs for bioanalysis of ADCs. The typical procedures for bioanalysis of conjugated-payload of ADCs by hybrid LBA and LC-MS/MS methods are as follows: first, the conjugated-payload of ADCs are captured by corresponding reagents, such as generic capture reagents, anti-payload, or anti-idiotype capture reagents; second, after elution, the conjugated-payload of ADCs are digested with trypsin or cathepsin-B enzyme; and third, the generated peptides from enzyme digestion of the conjugated-payload of ADCs are detected by LC-MS/MS with MRM transitions (Qu et al., 2017; Wei et al., 2018; Zhu et al., 2020; Panda et al., 2022). To avoid interference from endogenous human IgGs, unique peptides from variable regions are usually selected for detection of ADCs. Anti-human Fc antibodies could be selected as the immuno-affinity capture reagent to capture and purify the targeted antibodies in biologic matrix, which leads to the improved specificity and sensitivity of the hybrid LBA-LC-MS/MS assay (Ouyang et al., 2012; Furlong et al., 2014). Fig. 2 illustrates the typical procedures of hybrid LBA/LC-MS/MS assay (Wang et al., 2016; Zhu et al., 2020). For example, Liu et al. developed a hybrid LBA and LC-MS/MS assay to determine Bristol-Myers Squibb ADC in monkey plasma. The selective capture reagent used in their study was a unique monoclonal antibody. The reagents were biotinylated with EZ-Link Sulfo-NHS-LC-Biotins, and the corresponding analytes in biologic matrix were immunocaptured. Samples were slowly loaded onto the kit. The captured analytes were eluted from

the cartridges using ammonium bicarbonate and then digested with cathepsin B enzyme. After that, the generated peptides of the conjugated payload can be analyzed by LC-MS/MS method (Liu et al., 2015). Moreover, a hybrid LBA and LC-MS/MS assay was developed by Faria et al. to quantify total ADC and total antibody of MEDI4276 in human plasma. In their study, anti-idiotype antibodies were used to capture MEDI4276 followed by enzyme digestion with trypsin. The released payloads were used to measure total ADC and the produced peptide from enzyme digestion of the complementary determining region of the corresponding ADC was selected to determine total antibodies (Faria et al., 2019). Generally, hybrid LBA-LC-MS/MS methods have numerous advantages such as high sensitivity, high selectivity and multiplex capability. Furthermore, the methods can also offer valuable structure information of ADCs, such as DAR and drug load. Moreover, hybrid LBA-LC-MS/MS assays also have some disadvantages, for example, high cost, complicated data interpretation, complicated instrument operation, lower throughput, and low sensitivity for detection of intact ADC.

Analysis of Payload of ADCs by MS Methods

The payloads of ADCs are the small molecule toxins that are not linked to the antibody. The small molecule toxins can be released from the catabolism of ADCs in vivo. LC-MS/MS assay is an excellent technique to detect payloads of ADCs due to its high selectivity and high sensitivity. Solid phase extraction (SPE) and protein precipitation with organic solvents are usually selected as the sample preparation assay to remove proteins in biologic matrix before chromatographic separation. The lower limit of quantification of the LC-MS/MS assay should be much lower because the concentration of the released drug is much low in biologic samples. For instance, Olivier Heudi et al. developed and validated a specific and sensitive LC-MS/MS assay coupled with online SPE technology to determine maytansinoid in human serum. The free

thiol moiety of DM1 was first reduced by the chemical reagent [tris (2-carboxyethyl) phosphine] and then blocked with another agent N-ethylmaleimide. After that, the samples were diluted before the online SPE on a C₁₈ cartridge. The LC-MS/MS assay coupled with on-line SPE was successfully used for the determination of DM1 in human serum (Heudi et al., 2016). Liu et al. developed and validated a convenient LC-MS/MS assay for simultaneous quantification of three catabolites (DM1, MCC-DM1, Lys-MCC-DM1) of the ADC trastuzumab emtansine (T-DM1) in vitro cellular samples. Protein precipitation was selected as the sample processing method in this study. The developed assay showed excellent linearity (1–100 nM) with an r^2 value more than 0.995 (Liu et al., 2017). Generally speaking, the MS method for determination of payloads of ADC has numerous advantages, such as high selectivity, high sensitivity, and multiplex capability. The MS method also has some drawbacks; for example, the MS method may be affected by the non-volatile salts and endogenous compounds in biologic samples (Redman et al., 2016; Qu et al., 2017).

Analysis of ADCs by Labeled Bioanalytical Methods

The pharmacokinetic of ADCs in vivo can be also studied with radio-labeled techniques (Shadid et al., 2017). Radiolabeled ADCs can be used as the typical model for their pharmacokinetic study. The payload or antibody component of ADCs can be labeled with radioisotope. Then the in vivo fate of ADCs can be investigated by monitoring the radioisotopes through the use of imaging techniques, such as PET, SPECT, and FMT, which are sensitive and noninvasive (Chia et al., 2020; Chomet et al., 2020; Pellico et al., 2021). The temporal window for imaging can be dictated by the half-life of the labeled radioisotope. The biodistribution of ADCs could be revealed by the monitoring of the radiometals (Marciscano and Thorek, 2018). For example, Winnik et al. studied the biodistribution of [⁸⁹Zr]Zr-Desferrioxamine*-T-DM1 in Balb/c and NOD/SCID mice by Micro PET/CT image technique (Fig. 3A). The results of their study indicated that uptake (dose/g) of [⁸⁹Zr]Zr-DFO*-T-DM1 was $5.0 \pm 1.8\%$ (Cho et al., 2020). Sijbrandi et al. studied the pharmacokinetic behaviors of ⁸⁹Zr-trastuzumab, ⁸⁹Zr-trastuzumab-Mal-AF and ⁸⁹Zr-trastuzumab-Lx-AF in NCI-N87-bearing mice by PET-CT image technology. The results of their study suggested that the pharmacokinetic and tissue distribution properties of these three ADCs are similar. The concentrations of these three ADCs are much higher in tumor tissues compared with the normal tissues which means that the ADCs have excellent tumor targeting (Sijbrandi et al., 2017).

Lutje et al. used SPECT to investigate the biodistribution of ¹¹¹In-labeled ADCs in BALB/c nude mice (Lutje et al., 2018). The distribution of different ¹¹¹In-labeled ADCs in tumor tissues could be visualized clearly at 3 days after subcutaneous injection. Boswell et al. studied the tumor to heart ratio of ¹¹¹In-labeled anti-tomoregulin monomethyl auristatin E in male C.B-17 SCID beige mice by SPECT-CT imaging technology (Fig. 3B). The tumor-to-heart ratio decreased along with the increment of the dose of ADC (Boswell et al., 2012).

Gupta et al. investigated the distribution and tumor targeting potential of an anti-IL13R α_2 ADC in A375 xenograft-bearing mice by FMT imaging technique (Gupta et al., 2021). FMT revealed an excellent distribution profile for anti-IL13R α_2 ADC. The tested ADC showed a dose associated anti-tumor effect on A375 xenograft-bearing mice. When the dose is 3 mg/kg, the proportion of complete responders is 90%. Giddabasappa et al. studied the biodistribution and tumor targeting of Anti-5T4 ADC in female nu/nu tumor bearing mice by FMT imaging technology (Giddabasappa et al., 2016). The protocol for assessing the distribution and tumor targeting of biologic drugs by FMT imaging technique is shown in Fig. 3C. Their study proved that FMT is a useful technique to reveal the pharmacokinetic behavior of ADCs in vivo.

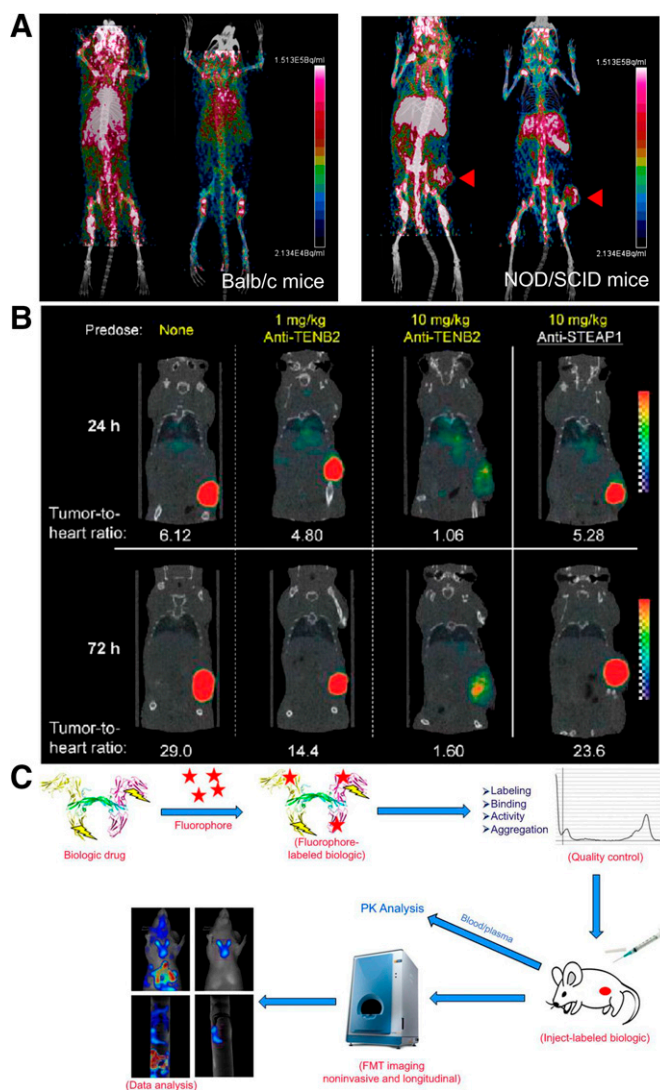


Fig. 3. (A) Biodistribution study of [⁸⁹Zr]Zr-DFO-T-DM1 and [⁸⁹Zr]Zr-DFO-T-DM1 in non-tumor bearing Balb/c mice and NOD/SCID mice with s.c HER2-overexpressing SK-OV-3 human ovarian cancer xenografts (arrow) by microPET/CT. Reprinted with permission from Cho et al., Nuclear Medicine and Biology 2020, 84–85:11–19. Copyright © 2019 Elsevier Inc. All rights reserved. (B) Biodistribution study of ¹¹¹In-anti-TENB2-MMAE (3 mg/kg) at 24 hours or 72 hours after injection in mice by SPECT/CT. Reprinted with permission from Boswell et al., Journal of Nuclear Medicine 2012, 53(9):1454–1461. Copyright © 2018 SNMMI. (C) Schematic representing the stepwise protocol followed in evaluating biodistribution and targeting of biologic drugs using FMT imaging. Ab/ADC (biologic drug) was labeled with VT680 by NHS ester reaction. After labeling, the VT680-conjugated Ab/ADCs were qualitatively and/or quantitatively evaluated for VT680 labeling, stability, binding to the antigen, and cytotoxic activity by in vitro methods. After these quality control evaluations, in vivo biodistribution and tumor targeting were determined longitudinally by FMT imaging and PK analysis of blood and tissues. Reprinted with permission from Giddabasappa et al., Mol Cancer Ther 2016, 15:2530–2540. Copyright © 2016, American Association for Cancer Research.

Dual radiolabeling of different components of ADCs is also a good option which can be used to study the in vivo behavior of ADCs (Alley et al., 2009). For example, Joey A. Muns et al. studied the biodistribution and blood kinetics of a dual radiolabeled trastuzumab-[^{195m}Pt]Lx-DFO-⁸⁹Zr by immuno-PET imaging technique (Muns et al., 2018). As indicated via ^{195m}Pt/⁸⁹Zr dual labeling, the results of their study showed that the tested ADC trastuzumab-[^{195m}Pt] Lx-DFO-⁸⁹Zr is stable in blood and its tumor targeting is good. Ilovich et al. used dual-isotope cryo-imaging quantitative autoradiography (CIQA) imaging technique

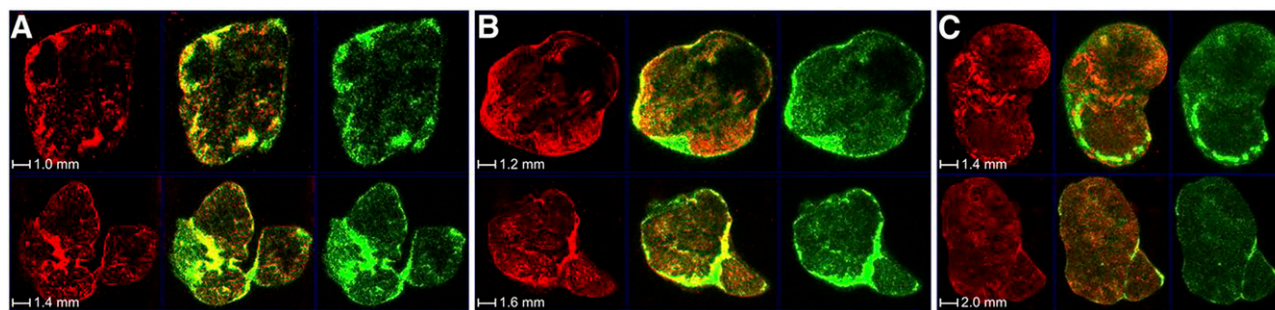


Fig. 4. Representative sections of HEK-293 GCC2 tumors (top) and HEK-293 tumors (bottom) excised at 1 hour (A), 24 hours (B), and 96 hours (C) after tracer injection. Voxels are 0.025, 0.025, and 0.025 mm. H signal is red, ^{111}In signal is green, and both signals coregistered is yellow. Strikingly, image of HEK-293 GCC2 at 24 hours shows initial diffusion of drug away from antibody accumulation site and deeper into tumor. Reprinted with permission from Ilovich et al., *J Nucl Med* 2018, 59:1461–1466. Copyright © 2018 SNMMI.

to investigate the biodistribution and payload delivery of an ADC which has ^{111}In -labeled antibody and ^3H -labeled payload MMAE (Ilovich et al., 2018). In their study, CIQA clearly showed the amount of the released payload in blood and tissues at different time points (Fig. 4). Chari et al. investigated the biodistribution of an ADC SGN-75 in tumor-bearing mice which has [^{14}C] labeled MMAF and [^3H] labeled anti-CD70 antibody (Chari et al., 2014). The accumulation of ^{14}C from MMAF or ^3H from antibody in tumor and various normal tissues could be detected by CIQA. They found that MMAF-derived radioactivity accumulated preferentially in tumor tissues. Cahuzac et al. used dual radiolabeling and ex vivo digital imaging technique to monitor the in vivo fate of an ADC (Fig. 5) which was dual-labeled with ^3H and ^{14}C (Cahuzac et al., 2022). These studies confirmed the feasibility of dual radiolabeling for pharmacokinetic study of ADCs. Moreover, liquid scintillation counter (LSC) is a radioactivity meter that uses a liquid scintillator to accept radiation and convert it into fluorescent photons. It is a useful tool for pharmacokinetics study of ADCs (Kamath and Iyer, 2016). Total radiation and radioactivity can be quantified from biologic samples by LSC. For example, Okeley et al. studied the cellular kinetics and uptake of ^{14}C -labeled SGN-35 by LSC (Okeley et al., 2010). Girish et al. investigated the pharmacokinetic behavior of [^3H]-labeled Trastuzumab Emtansine (T-DM1) in rats by LSC. The total radioactivity in rat plasma, bile, tissues, urine, and feces are determined by the LSC (Shen et al., 2012). Bolleddula et al. studied the catabolism and pharmacokinetic of [^3H] labeled TAK-164 in tumor-bearing mice by LSC (Bolleddula et al., 2020). The results of their study showed that the terminal half-life of the [^3H]-labeled TAK-164 is 41 hours in mice

plasma, and its clearance is 0.75 mL/h per kilogram. However, LSC also has some limitations. For instance, chemical, color, or physical quenching will happen during sample processing which result in reducing the counting efficiency and thus the quenching correction should be performed for LSC detection. Moreover, for LSC, the energy used for separation of α and β particles is depended on many factors. Therefore, in the process of measuring the total α and β radioactivity in LSC, the correct setting of different parameters is very important. In addition, the energy released by the anions and particles in the solution can also affect the detection process of LSC (Stapleton, 2022).

Generally, labeled analytical methods for bioanalysis of ADC have some advantages, such as high sensitivity, visualization, and being non-invasive. However, the selectivity of labeled analytical assays is not high. Labeled analytical assays cannot distinguish the ADCs and their metabolites when they all have the radioactive element. Furthermore, the radioactive labeled element may cause immunoreactivity and change the in vivo behavior of ADCs.

Current Challenges and Future Perspectives

ADCs are a novel and unique kind of anticancer therapeutic. Due to the complexity and heterogeneity of ADCs and their dynamic changes in vivo, the pharmacokinetic profile of ADCs is correspondingly complex, which poses big challenges for the bioanalysis of ADCs. Bioanalysis of ADCs should simultaneously focus on intact ADCs, total antibody, released small molecule cytotoxins, and related metabolites. During these years, analytical methods requiring a prelabeling of ADCs (such as PET and PMT) and label-free analytical methods (such as LBA and MS based assays) have been widely used for pharmacokinetic study of ADCs. The future development of bioanalysis technologies, especially the application of new mass spectrometry-based techniques, can support more accurate and comprehensive analytical tools for monitoring the in vivo fate of ADCs. Improvements of chromatographic technologies can provide high resolution separations for ADCs derived analytes. The combination of different analytical technologies is leading to a better and a more thorough understanding of the pharmacokinetic behavior, structure-exposure-safety/efficacy relationships of ADCs, which is helpful and important for the designing and development of ADCs.

Data Availability

The authors declare that all the data supporting the findings of this study are contained within the paper.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Yin, Xu, Zhao, Gu.

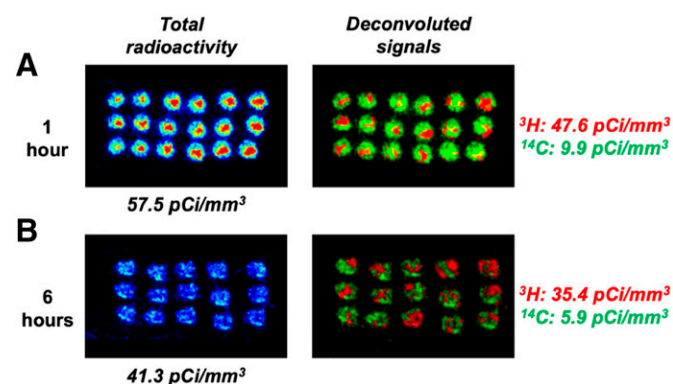


Fig. 5. Ex vivo dual radio-imaging of 4T1 tumor sections enables the quantification of both components of [$^3\text{H}/^{14}\text{C}$]-2 1 hour (A) or 6 hours (B) after administration ($n = 3$ mice per time). Reprinted with permission from Cahuzac et al., *J Med Chem* 2022, 65:6953–6968. Copyright © 2022 American Chemical Society.

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