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Review Article

TROP2-targeted molecular imaging: a promising tool for precision oncology

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Abstract: Trophoblast cell surface antigen 2 (TROP2) represents an ideal target in cancer diagnosis and therapy, particularly in antibody-drug conjugate (ADC) treatments. Several TROP2-targeted ADCs have been used for the treatment of end-stage metastatic cancers, demonstrating promising therapeutic efficacy. Research has shown that the efficacy of TROP2-ADCs is closely correlated with TROP2 expression levels, highlighting the potential of TROP2 expression as a key factor for patient stratification and selection, which could significantly predict the therapy response and therefore enhance treatment outcomes. Currently, immunohistochemistry (IHC) is the gold standard for detecting TROP2 expression, although it has certain limitations. Non-invasive molecular imaging techniques offer the potential to overcome these limitations, providing valuable guidance for subsequent treatment strategies. The development of immuno-Positron Emission Tomography (immunoPET) technologies, including radiolabeled monoclonal antibodies, nanobodies, peptides and small molecules, have made the non-invasive measurement of TROP2 expression feasible. TROP2-targeted molecular imaging represents a promising frontier for precision oncology, despite existing challenges in clinical translation. This review systematically summarizes the research progress in TROP2-targeted molecular imaging for tumor diagnosis and therapy, while discussing innovative approaches to overcome current technical limitations and accelerate clinical implementation.

Keywords: TROP2 expression, Antibody-drug conjugate (ADC), molecular imaging, immuno-Positron Emission Tomography (immunoPET), patient stratification

Introduction

Trophoblast cell surface antigen 2 (TROP2) is a transmembrane glycoprotein that is typically overexpressed in various epithelial-derived malignant tumor cells, but is expressed at lower levels in normal tissues [1]. Overexpression of *TROP2* is closely associated with poor prognosis, increased metastasis risk, and tumor progression, particularly in multiple solid tumors such as breast cancer, pancreatic cancer, prostate cancer, lung cancer, and cervical cancer [2, 3]. As a result, TROP2 has become an ideal therapeutic target in cancer treatment, especially in antibody-drug conjugate (ADC) therapies [4]. Given the strong correlation between *TROP2* expression levels and the efficacy of TROP2-ADC therapies, the expression status of *TROP2* holds promise as a key biomarker for predicting treatment outcomes [5]. Therefore, the precise quantification of TROP2 expression levels is crucial for patient stratification and selection. Molecular imaging, particularly immuno-Positron Emission Tomography (immunoPET) imaging technique, has provided new perspectives for the detection and measurement of TROP2. ImmunoPET can non-invasively and in real-time assess TROP2 expression, offering guidance for targeted therapies. Studies have shown that TROP2-targeted radiotracers exhibit superior imaging performance in TROP2-positive tumors compared to stand-of-care examinations, with the added benefit of lower radiation doses, demonstrating excellent clinical translation potential. This review summarizes the development trends of immunoPET-guided tumor-targeted therapies, starting from the existing TROP2 molecular probes.

TROP2 as a key molecular biomarker in cancer therapy

Trophoblast cell surface antigen 2 (TROP2), also known as epithelial glycoprotein 1 (EGP1), gastrointestinal antigen 733-1 (GA733-1), membrane component 1 surface marker 1 (M1S1), and tumor-associated calcium signal transducer2 (TACSTD2), is a member of the GA733 gene family [6]. It is a transmembrane glycoprotein encoded by the *TACSTD2* gene and acts as a tumor-associated calcium ion signal transducer. TROP2 is expressed at very low levels, or is almost absent, in normal tissues, but is significantly overexpressed in a variety of epithelial-derived malignant tumors, including breast cancer, pancreatic cancer, prostate cancer, lung cancer, cervical cancer, urothelial carcinoma, and ovarian cancer [1-3]. Its overexpression is closely associated with tumor metastasis risk

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and poor prognosis, making it an ideal target for cancer therapy [4]. Based on the high expression of *TROP2* in various tumors, TROP2-targeted antibodies and antibody-drug conjugates (ADCs) have been successfully developed, with some advancing to clinical trials and others receiving regulatory approval for clinical use [7, 8].

As a target widely expressed in various solid tumors, TROP2 is not only located on the cell surface but also an internalizing cell membrane receptor, allowing effective recognition by antibodies. Therefore, TROP2-targeting therapeutic agents primarily include antibodies and ADCs. ADCs, as a novel tumor-targeted therapeutic strategy, consist of antibodies, cytotoxic drugs, and chemical linkers, and have been shown to be highly effective and precise in anticancer effects [9]. The basic mechanism of ADCs involves antibody-mediated cytotoxicity (such as antibody-dependent cellular cytotoxicity, ADCC), which enables targeted killing of tumor cells. It is worth noting that certain ADCs can mediate nontargeted cytotoxicity via the "bystander effect", which refers to a phenomenon where antigen-positive (Ag+) tumor cell-targeting ADCs induce antitumor activity in adjacent malignant cells [10]. At present, several TROP2-targeted ADCs have entered clinical trials, with some already approved for clinical use. These agents have demonstrated significant therapeutic efficacy in multiple solid tumors, including breast cancer, urothelial carcinoma, and non-small cell lung cancer, offering novel treatment options for patients with advanced malignancies. The two main TROP2-targeted ADCs are Sacituzumab Govitecan (SG, IMMU-132) and Datopotamab Deruxtecan (Dato-DXd, DS-1062a) [11].

SG is formed by the conjugation of a humanized anti-TROP2 antibody (hRS7) with the topoisomerase I inhibitor, 7-ethyl-10-hydroxycamptothecin (SN-38), and specifically targets TROP2, delivering the cytotoxic drug SN-38 directly to tumor cells [12, 13]. SG has been approved by the Food and Drug Administration (FDA) for therapy of metastatic triple-negative breast cancer (mTNBC), hormone receptor-positive breast cancer, and advanced or metastatic urothelial carcinoma. Up to now, SG is the only TROP2-targeting drug approved for use worldwide [14]. SN-38 is the active form of the chemotherapeutic agent irinotecan [15]. However, a significant limitation of SG is its short half-life (11-14 hours) and plasma instability [16]. In contrast, Dato-DXd is an innovative TROP2-targeted ADC that conjugates an anti-TROP2 antibody with a topoisomerase I inhibitor [13], suggesting stronger clinical efficacy and better safety [17]. The drug is currently being evaluated in multiple clinical trials. The latest data from the TROPION-PanTumor01 Phase I trial indicates that Dato-DXd exhibits significant antitumor activity in patients with mTNBC, with an objective response rate (ORR) of 26.0% and a disease control rate (DCR) of 78.8%, with most patients achieving disease stabilization [18]. The TROPION-Lung01 Phase III trial has demonstrated that the U.S. FDA has accepted a Biological Product License Application (BLA) for Dato-DXd. This therapy is intended for patients with locally advanced or metastatic non-squamous non-small cell lung cancer (NSCLC) who have previously undergone systemic therapy. The Prescription Drug User Fee Act (PDUFA) date is set for December 20, 2024, marking the potential approval of the first Trop2-targeted ADC for lung cancer treatment [19]. Compared to SG, Dato-DXd possesses a longer serum half-life (4-5 days) and a more potent payload, potentially reducing off-target toxicity to normal cells [17].

Correlation between TROP2 expression level and efficacy of TROP2-targeted ADCs

Overexpression of *TROP2* has significant prognostic implications across various cancers, prompting ongoing clinical research into TROP2-targeted therapies. Several antibodies, ADCs, and inhibitors targeting TROP2 have progressed into clinical trials, aiming to mitigate the progression of specific cancers associated with TROP2 overexpression [20]. However, identifying patients who are likely to benefit from these treatments remains a challenge. Therefore, precise quantification of TROP2 expression levels, particularly across different tumor types, is essential for the effective selection of patients who may benefit from these therapies.

Studies have demonstrated that the efficacy of ADCs is contingent upon the expression levels of the target antigen in tumor tissues. Furthermore, downregulation of target antigen expression frequently represents a critical mechanism underlying resistance to ADCs [21]. Based on previous clinical studies on human epidermal growth factor receptor2 (HER2)-ADC therapies (T-DM1, T-DXd) in breast cancer, the results indicated that the ORR in patients with high HER2-expressing breast cancer was significantly higher than those with low HER2 expression [22, 23]. These findings suggest that HER2 expression levels may serve as an effective biomarker for predicting the efficacy of HER2-ADC therapies. Consequently, it is hypothesized that TROP2 expression levels may be significantly correlated with the therapeutic efficacy of TROP2-ADC therapies. A retrospective analysis conducted by Bardia et al. assessed the impact of tumor TROP2 expression on the efficacy of SG in the ASCENT study, a Phase III clinical trial comparing standard chemotherapy with SG in triple-negative breast cancer (TNBC) patients [24]. The results indicated that TNBC patients with high TROP2 expression exhibited significantly improved ORR, progression-free survival (PFS), and overall survival (OS) compared to those with low TROP2 expression (ORR: 44% vs. 22%; PFS: 6.9 vs. 2.7 months; OS: 14.2 vs. 9.3 months). These findings highlight the potential of TROP2 expression levels as a crucial predictive biomarker for the efficacy of TROP2-ADC therapies [5].

In addition, several preclinical studies have further validated the positive correlation between TROP2 expression

levels and the efficacy of ADC drugs. A study on malignant pleural mesothelioma (MPM) cell lines demonstrated that TROP2-positive cell lines were more sensitive to SN-38, the active metabolite released by SG [13]. Similarly, an investigation into the inhibitory activity of Dato-DXd on tumor cell growth in vitro across various tumor types revealed that the drug exhibited significant inhibitory effects on cell lines with high *TROP2* expression, while showing minimal activity in those with low *TROP2* expression [25]. In a mouse tumor model study, tumors with elevated *TROP2* expression showed a significantly better response to SG treatment compared to those with low *TROP2* expression. Furthermore, research on tumors with high TROP2 expression indicated that SG effectively inhibited tumor cell DNA repair through TROP2 targeting, leading to increased DNA damage and promoting tumor cell apoptosis [26]. SG exhibited more pronounced antitumor activity and survival benefits in TROP2-high expressing tumors [27], alongside favorable toxicity profiles characterized by reduced intestinal absorption. This profile enhances patient survival rates and represents an important therapeutic advantage [28].

Before administering TROP2-targeted ADC therapies, precise detection of TROP2 expression status is essential for identifying the most suitable patient population. This approach is crucial for patient stratification, enhancing therapeutic efficacy, and minimizing treatment costs. Furthermore, accurate TROP2 detection methods will enable dynamic assessments of treatment efficacy, allowing for the avoidance of ineffective therapies and the reduction of unnecessary side effects. Therefore, precise TROP2 expression testing plays a pivotal role in shaping clinical treatment strategies and offers valuable insights for the future development and clinical application of ADC drugs [29].

Current techniques for evaluation of TROP2 expression

Immunohistochemistry (IHC) remains the predominant method for evaluating TROP2 expression in clinical practice due to its high sensitivity, specificity, rapid turnaround time, cost-effectiveness and ease of implementation in clinical settings. Given that TROP2 is located on the cell membrane, staining is predominantly observed at the cell membrane in both normal and tumor tissues, with occasional weak cytoplasmic staining. One study employed IHC to determine the baseline expression levels of TROP2 in formalin-fixed, paraffin-embedded (FFPE) primary tumor biopsy samples [30]. Furthermore, in the context of quantitative assessment, the Phase III ASCENT clinical trial implemented a histochemical scoring (H-score) system to stratify TROP2 expression status and evaluate therapeutic outcomes in mTNBC patients treated with SG versus physician's choice of single-agent chemotherapy (TPC) [24]. The H-score was calculated as follows: H-score = Σ pi(i+1) = (3 × proportion of strongly stained cells) + (2 × proportion of moderately stained cells) + (1 × proportion of lightly stained cells), with expression levels categorized as low (H-score: 0-100), moderate (H-score: 100-200), or high (H-score: 200-300). TROP2 positivity was defined as H-scores ≥ 100, whereas scores < 100 were classified as TROP2-negative.

Despite its clinical utility, IHC presents significant limitations in the context of precision oncology. Firstly, the method's dependence on invasive single-lesion biopsies fails to adequately capture tumor heterogeneity, which can manifest both spatially - through inter- and intralesional variability - and temporally, as dynamic expression changes occur during treatment. This inherent sampling bias may lead to an underrepresentation of the true distribution of TROP2, particularly in metastatic diseases [31]. Secondly, variability in procedural factors such as tissue fixation, antibody specificity, and subjective scoring can compromise the reproducibility of results across different laboratories. Thirdly, IHC lacks the capability for non-invasive, serial monitoring of TROP2 dynamics, which limits the ability to assess therapeutic responses in real time. These limitations highlight the urgent need for the development of advanced methodologies that facilitate a comprehensive and longitudinal evaluation of TROP2.

Molecular imaging techniques for non-invasive evaluation of TROP2 expression

In the era of molecular targeted therapy and immunotherapy, emerging targeted molecular imaging modalities are overcoming IHC's limitations by enabling in vivo, whole-body quantification of TROP2 expression [32]. The most commonly utilized molecular imaging technologies include nuclear medicine imaging techniques. Among these, ImmunoPET is a groundbreaking molecular imaging technique that seamlessly merges the remarkable targeting precision of monoclonal antibodies (mAbs) with the high sensitivity of positron emission tomography (PET) technology. This innovative combination enables the non-invasive visualization and quantitative evaluation of antigen expression in living organisms [33]. This innovative approach utilizes radiolabeled antibodies or antibody fragments to achieve high-sensitivity detection and longitudinal monitoring of TROP2-positive lesions. And single-photon emission computed tomography (SPECT) offers alternatives with isotopes [34]. Additionally, magnetic resonance imaging (MRI) and its spectroscopic imaging (MRS), along with optical imaging, and infrared optical tomography are also widely employed in the field.

Nuclear medicine modalities overcome the inherent limitations of IHC by offering three transformative advantages: comprehensive heterogeneity assessment through non-invasive, whole-body imaging that simultaneously captures multifocal and metastatic lesions to resolve spatial heterogeneity; dynamic monitoring via serial scans to track temporal changes in TROP2 expression during ther-

apy, thereby guiding adaptive treatment strategies; and quantitative precision enabled by time-activity curves and standardized uptake values (SUVs) for operator-independent, objective quantification. Consequently, the development and application of radioactive isotope-labeled molecular probes targeting TROP2 are anticipated to significantly enhance the diagnosis of TROP2-positive tumors and their potential metastases. This advancement will facilitate precise screening for patients exhibiting high TROP2-targeted therapies, representing a crucial step in evaluating patient suitability for ADC therapy before treatment [35].

Research progress for TROP2-targeted ImmunoPET

ImmunoPET imaging plays a crucial clinical role in selecting TROP2-positive patients. This novel molecular imaging technique is primarily used for patient stratification by detecting the expression of specific biomarkers in the body. ImmunoPET is particularly effective for screening patients who exhibit moderate to high levels of TROP2 expression, as these patients are more likely to benefit from targeted therapies or immunotherapy. Consequently, immunoPET technology holds promise for providing non-invasive and specific diagnoses for TROP2, as well as offering a method for visualizing and detecting TROP2 expression and distribution at the in vivo level.

Research progress in TROP2-targeted imaging agents

A study by van Rij et al. utilized immunoPET and immuno-SPECT imaging technology based on the hRS7 antibody, successfully visualizing TROP2 expression in tumor models [36]. This technology combines the superior targeting characteristics of mAbs with the high sensitivity of PET, offering a molecular imaging method with high specificity, surpassing traditional metabolic imaging techniques. In recent years, several studies have explored radiolabeled molecular probes for immunoPET detection, with the design and application of these probes highlighting the tremendous potential of immunoPET in TROP2-targeted diagnosis. **Table 1** provides a detailed comparative analysis of radiolabeled targeted molecular probes used for different tumor subtypes. This analysis covers several important aspects, including imaging techniques, characteristics of the probes, types of studies performed, and the key findings obtained from these research efforts.

Monoclonal antibodies-based radiotracers for TROP2-targeted PET imaging

With advancements in radiochemistry and conjugation strategies, several radionuclides and monoclonal antibodies have been successfully applied in the development of immunoPET probes. Currently, multiple monoclonal antibodies targeting TROP2 have been developed and are extensively employed in imaging and radioimmunotherapy (RIT) [37]. These radiolabeled probes provide enhanced specificity and accuracy for clinical tumor diagnosis.

In clinical settings, isotopes with longer half-life (defined as the time required for 50% of its atoms to undergo radioactive decay), such as ^{89}Zr (half-life of approximately 3.3 days), are preferred over those with shorter half-life, such as ^{64}Cu (half-life of 12 hours) [38]. The relatively long physical half-life of ^{89}Zr allows for optimal alignment with the pharmacokinetics of antibodies. Additionally, ^{89}Zr's high gamma-ray energy and excellent spatial resolution enable the production of high-resolution, high-quality PET images. Consequently, ^{89}Zr has emerged as one of the most commonly used radionuclides in antibody radiolabeling and PET imaging research [39].

For example, Weiyu Chen et al. Conducted TROP2-targeted PET imaging and biodistribution studies using the [^{89}Zr]Zr-DFO-AF650 probe in three pancreatic cancer xenograft mouse models (BxPC-3, MIA PaCa-2, and AsPC-1) [37]. The study demonstrated that the probe exhibited high specificity for TROP2 in BxPC-3 cells, effectively distinguishing primary tumors and significantly enhancing the visualization of small lesions. Moreover, PET imaging results were highly consistent with biodistribution findings, validating the high sensitivity and accuracy of immunoPET technology in detecting malignant pancreatic tissues and small lesions.

In another study, an ^{89}Zr/^{177}Lu dual-labeled anti-TROP2 antibody (NY003) was developed for immunoPET and SPECT imaging [35]. This antibody was conjugated with DFO and DTPA to facilitate radiolabeling with ^{89}Zr and ^{177}Lu, respectively, and was evaluated in a subcutaneous TROP2-positive TNBC xenograft mouse model. The results from immunoPET, SPECT imaging, and RIT demonstrated that [^{89}Zr]Zr/[^{177}Lu]Lu-labeled anti-TROP2 antibody NY003 not only enabled non-invasive screening of patients who may benefit from SG ADC therapy but also effectively inhibited the growth of TROP2-positive TNBC tumors. The application of [^{177}Lu]Lu-DTPA-NY003 exhibited minimal toxicity to surrounding healthy tissues and organs. However, the study highlighted the need for further optimization of the NY003 structure and improvement of its pharmacokinetic properties to enhance its clinical application potential.

Therefore, Zeng et al. developed a novel TROP2-targeted molecular probe, [^{124}I]I-IMMU-132, to better elucidate TROP2 expression [40]. [^{124}I]I-IMMU-132 is the first radiolabeled molecular probe constructed based on the ADC drug IMMU-132. The [^{124}I]I-labeled IMMU-132 conjugate was administered to the mouse model. Micro-PET/CT imaging revealed specific targeting of the radioconjugate to both TROP2-positive pancreatic cancer cell lines (Capan-1) and TNBC tumors (MDA-MB-468), as illustrated in **Figure 1**. Its cellular uptake in vivo demonstrated a time-dependent increase, indicating robust targeting and binding capabilities.

Table 1. Comparative analysis of radiolabeled targeted molecular probes across oncologic subtypes: imaging modalities, probe characteristics, study type, and main findings

Types of Cancer	Imaging Modality	Imaging Agents/Targeted Drugs	Preclinical/ Translational Study	Main Findings
TNBC	PET, SPECT, RIT	[^{89}Zr]Zr-DFO-NY003/ [^{177}Lu]Lu-DTPA-NY003 [35]	Preclinical study	[^{89}Zr]Zr/[^{177}Lu]Lu-NY003 enables imaging-guided optimization of SG-ADC therapy and suppresses TROP2-positive TNBC tumor growth.
	Confocal microscopic	DOX-ST-NPs [49]	Preclinical study	[TROP2]-ST-NPs selectively target TNBC cells and enable rapid doxorubicin (DOX) release via intracellular disulfide reduction.
	RIT	[^{131}I]I-IMP-R4-hRS7 [50]	Preclinical study	[^{131}I]I-R4-hRS7 demonstrates preclinical potential for targeted radioimmunotherapy.
Prostate cancer	PET	TF12-[^{68}Ga]Ga-IMP288 [51]	Preclinical study	TF12 pretargeted [^{68}Ga]Ga-hapten-peptide immuno-PET achieves high-contrast prostate cancer imaging.
	PET	TF12-[^{111}In]In/[^{177}Lu]Lu-IMP288 [52]	Preclinical study	TF12-mediated [^{68}Ga]Ga-IMP288 uptake enhances TROP2^{+} prostate tumor visualization.
	PET	[^{64}Cu]Cu-NOTA-AF650/ [^{90}Y]Y-DTPA-AF650 [53]	Preclinical study	[^{64}Cu]Cu-NOTA-AF650 detects TROP2^{+} prostate cancer, while [^{90}Y]Y-DTPA-AF650 inhibits tumor growth with minimal toxicity.
	PET, SPECT	[^{89}Zr]Zr/[^{111}In]In-hRS7 [36]	Preclinical study	[^{111}In]In-hRS7/[^{89}Zr]Zr-hRS7 specifically accumulate in PC3 xenografts, enabling tumor imaging via SPECT/PET.
	SPECT, NIRF	[^{111}In]In-TF12-RDC018 [54]	Preclinical study	[^{111}In]In-RDC018 shows TROP2-specific uptake on micro-SPECT/CT; near-infrared fluorescence (NIRF) imaging guides precise tumor resection.
Pancreatic cancer	NIR	Anti-TROP2 mAb-IR700 [55]	Preclinical study	TROP2-targeted photoimmunotherapy (PIT) suppresses pancreatic/cholangiocarcinoma xenograft growth.
	ADCs	TROP2Fab-DOX [56]	Preclinical study	TROP2Fab-DOX inhibits pancreatic cancer proliferation in vitro and tumor growth in vivo.
	PET, SPECT	[^{64}Cu]Cu/[^{177}Lu]Lu-hIMB1636 [57]	Preclinical study	[^{64}Cu]Cu/[^{177}Lu]Lu-hIMB1636 evaluates TROP2 expression and suppresses pancreatic tumors; [^{177}Lu]Lu-hIMB1636 SPECT monitors therapy efficacy.
	PET	[^{124}I]I-IMMU-132 [40]	Translational study	[^{124}I]I-IMMU-132 PET delineates TROP2^{+} tumors.
	PET	[^{89}Zr]Zr-DFO-AF650 [37]	Preclinical study	[^{89}Zr]Zr-DFO-AF650 enables TROP2-targeted pancreatic cancer detection via immuno-PET.
	PET	[^{68}Ga]Ga-NOTA-T4/T5 [29]	Translational study	[^{68}Ga]Ga-NOTA-T4 confirms TROP2 specificity in high-expression models and pancreatic CDX.
	PET	[^{68}Ga]Ga-NOTA-RTD01 [32]	Preclinical study	[^{68}Ga]Ga-NOTA-RTD01 has demonstrated favorable diagnostic performance in preclinical models of pancreatic cancer.
	PET	[^{68}Ga]Ga-NOTA-RTD98 [32]	Preclinical study	[^{68}Ga]Ga-NOTA-RTD98 immuno-PET differentiates TROP2 expression in PDAC with high sensitivity.
Gastric cancer	PET	[^{68}Ga]Ga-NOTA-RTD98 [32]	Preclinical study	[^{68}Ga]Ga-NOTA-RID98 demonstrates diagnostic potential in gastric cancer models.
Lung cancer	PET	[^{18}F]F-AlF-RESCA-T4/RT4 [42]	Translational study	[^{18}F]F-AlF-RESCA-T4/RT4 distinguish pulmonary inflammation from malignancy.
Solid tumors	PET	[^{68}Ga]Ga-NOTA-T4/T5 [29]	Translational study	[^{68}Ga]Ga-NOTA-T4 visualizes TROP2^{+} tumors in patients, enabling noninvasive expression assessment.
	PET	[^{68}Ga]Ga-MY6349 [34]	Translational study	[^{68}Ga]Ga-MY6349 PET/CT noninvasively assesses TR2 expression for personalized therapy.

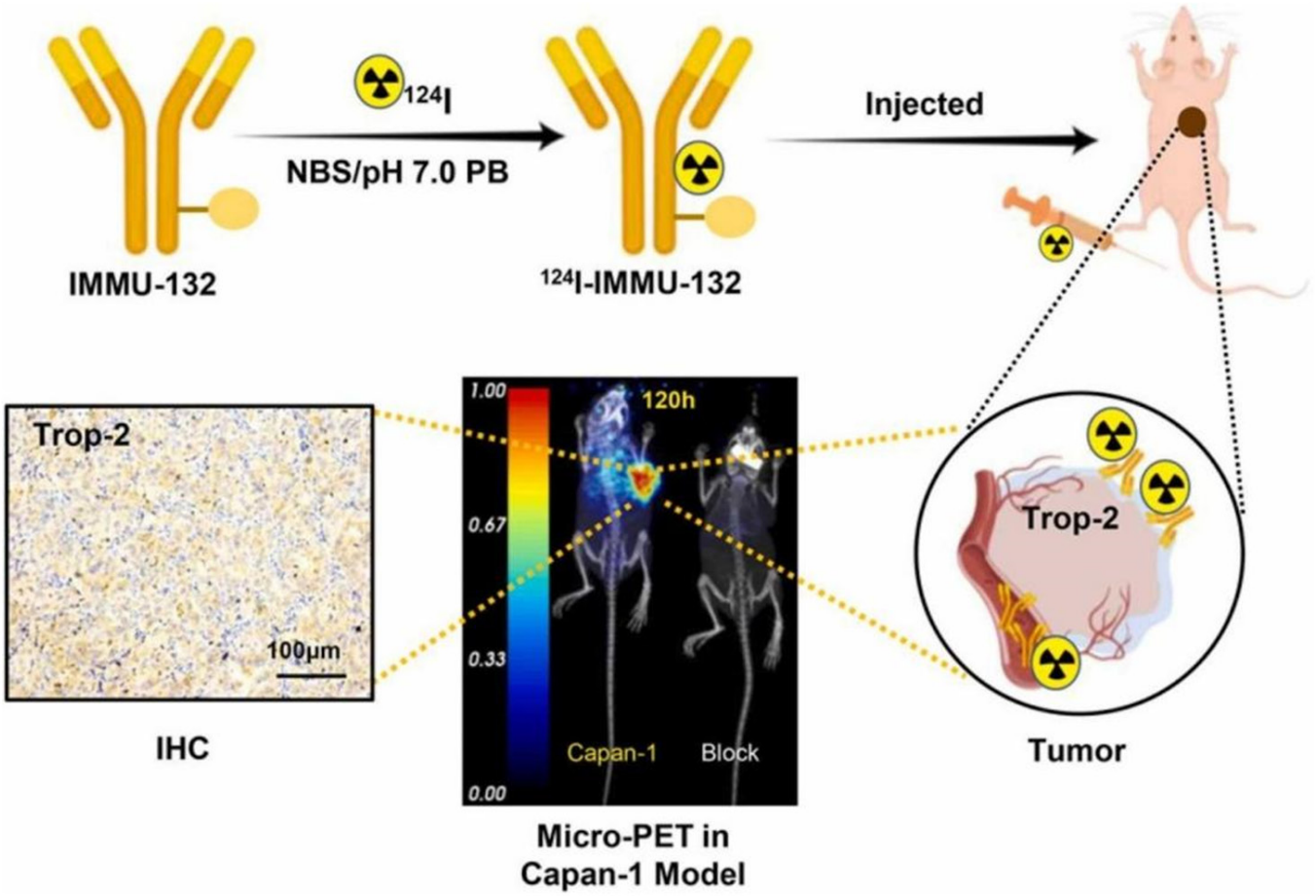

Figure 1. The construction of [^{124}I]I-IMMU-132. An iodine-124 radiolabeled TROP2-targeting molecular probe developed for micro-PET imaging applications. Source: From Ziqing Zeng, Yong Zheng et al. (2024) with modifications. Permission: Permission to reproduce this figure was obtained. Originally published in Biomedicine & Pharmacotherapy (2024; 178: 117151).

The probe's radiochemical properties and binding affinity were confirmed to be stable through experiments on cellular uptake, PET/CT imaging, biodistribution and pharmacokinetics in mouse models. [^{124}I]I-IMMU-132 exhibited specific targeting and tracing abilities for TROP2 protein and TROP2-positive tumors, establishing it as a valuable tool for the clinical diagnosis and therapeutic monitoring of tumors with high TROP2 expression.

One notable advantage of [^{124}I]I-IMMU-132 is its incorporation of ^{124}I, which has a relatively long half-life of approximately 4.2 days. This characteristic makes it well-suited to align with the in vivo metabolic profiles of ADC drugs. The longer half-life facilitates delayed imaging, yielding clearer and more persistent imaging results, thereby enhancing its potential for clinical application. Moreover, [^{124}I]I-IMMU-132 has already progressed to clinical trial stages, underscoring its promise in advancing TROP2-targeted diagnostics and therapies.

Advances in nanobody-based radiotracers for TROP2-targeted PET imaging

In addition to conventional tumor-targeting vectors, recent advancements have led to the development of nanobodies with short circulation times and bispecific antibodies with strong binding affinities, aimed at designing novel immunoPET probes. Nanobodies are single-domain antibody fragments, approximately 13 kDa in size, derived from the heavy-chain-only antibodies found in camelids [41]. Their small size, high stability, and rapid tissue penetration make them ideal for molecular imaging and targeted therapy. They exhibit strong binding affinity to antigens, even in complex tumor microenvironments.

Characterized by their simple molecular structure, high stability, and rapid in vivo metabolism, nanobodies are ideal candidates for radiolabeling with short half-life radionuclides. These properties position nanobodies as promising PET/CT molecular probes with significant clinical potential. Nanobody-based probes not only exhibit low radiation doses and excellent structural stability but also provide robust support for the diagnosis of TROP2-positive tumors.

With ongoing research and optimization, nanobody probes are expected to emerge as pivotal tools for diagnosing and targeting TROP2-positive tumors, offering both precise imaging and therapeutic capabilities [40].

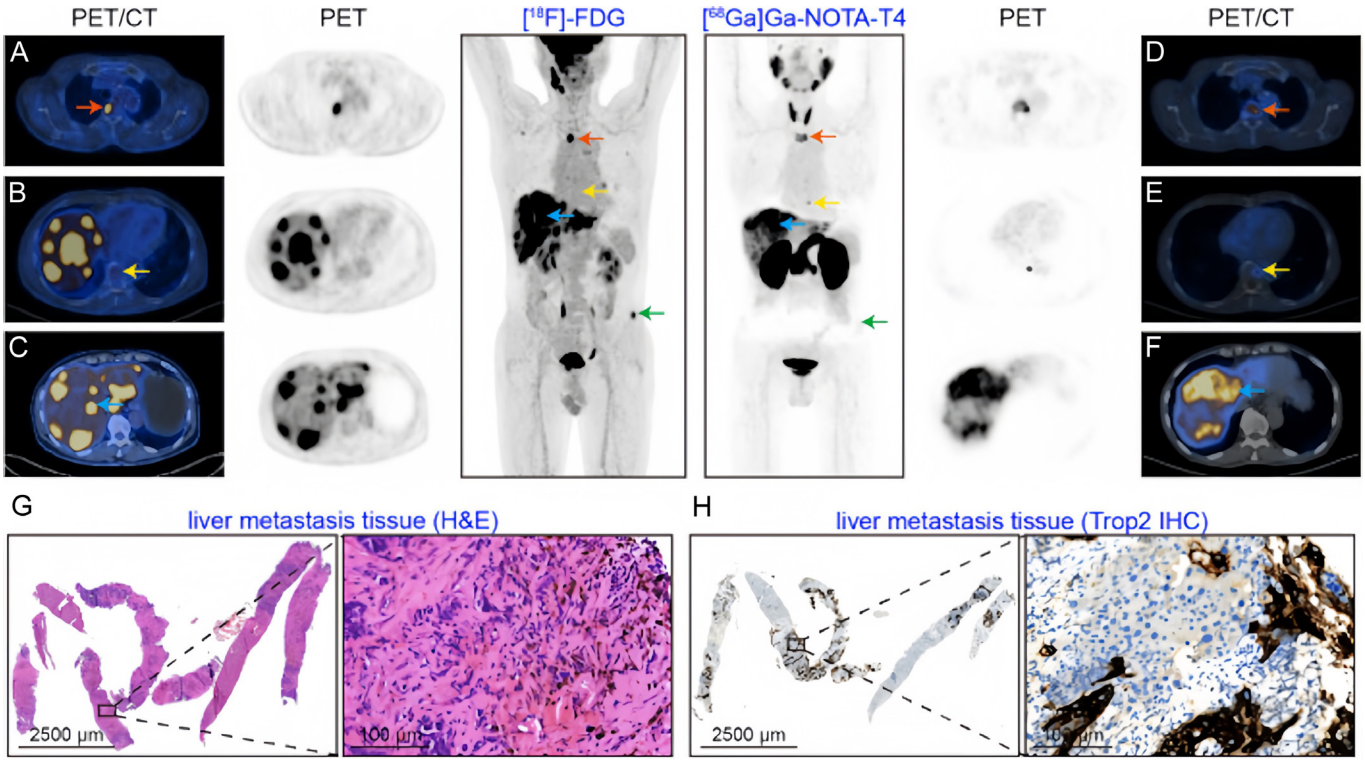

Figure 2. Impact of [^{18}F]FDG and [^{68}Ga]Ga-NOTA-T4 PET/CT imaging on the assessment of TROP2-positive metastatic nasopharyngeal carcinoma. A patient with nasopharyngeal carcinoma and multiple metastases to the liver, bone, and lymph nodes underwent sequential [^{18}F]FDG PET/CT (A-C) and [^{68}Ga]Ga-NOTA-T4 PET/CT (D-F) imaging. [^{68}Ga]Ga-NOTA-T4 immuno-PET/CT identified metastatic lesions in the bone and liver, suggesting positive TROP2 expression. Newly developed bone metastases (yellow arrows) exhibited significant radiotracer uptake. Histological examination of liver biopsy samples included H&E staining (G) and TROP2 IHC staining (H). Source: From Wei Huang, You Zhang et al. (2024) with modifications. Permission: Permission to reproduce this figure was obtained. Originally published in EMBO molecular medicine (2024; 16(5): 1143-1161).

In one study, two nanobodies targeting TROP2 (RTD98 and RTD01) were developed and radiolabeled with gallium-68 (^{68}Ga, half-life: 1.1 hours) as the immunoPET imaging probes [32]. Evaluations in mouse models bearing pancreatic and gastric tumors with varying TROP2 expression levels revealed that [^{68}Ga]Ga-NOTA-RTD98 demonstrated significant diagnostic sensitivity and specificity. This probe effectively delineated subcutaneous tumors, exhibiting a superior tumor-to-muscle (T/M) ratio compared to conventional [^{18}F]FDG or nonspecific probes like [^{68}Ga]Ga-NOTA-RTD161. These findings highlight the potential of [^{68}Ga]Ga-NOTA-RTD98 to effectively highlight differences in TROP2 expression in preclinical tumor models. Furthermore, [^{68}Ga]Ga-NOTA-RTD01, another developed probe, exhibited promising diagnostic performance in pancreatic cancer preclinical models.

However, these probes also present certain limitations. [^{68}Ga]Ga-NOTA-RTD98 exhibits high hepatic background uptake, which may impair imaging clarity for tumors located in the liver region. Meanwhile, [^{68}Ga]Ga-NOTA-RTD01 suffers from a short tumor retention time, resulting in a limited imaging window and a low target-to-background ratio in PET images.

Based on the pharmacokinetic properties of the aforementioned nanobodies, Huang and colleagues further developed two TROP2-targeted nanobodies with improved in vivo pharmacokinetics, [^{68}Ga]Ga-NOTA-T4 and [^{68}Ga]Ga-NOTA-T5 [29]. Comparative studies in preclinical mouse models demonstrated that [^{68}Ga]Ga-NOTA-T4 exhibited superior diagnostic performance compared to [^{68}Ga]Ga-NOTA-T5, establishing it as the preferred probe for subsequent immunoPET imaging research. However, the small cohort sizes in preclinical studies may limit statistical robustness and generalizability, as larger cohorts are needed to account for interindividual variability in tumor uptake. Further investigations revealed that [^{68}Ga]Ga-NOTA-T4 performed exceptionally well in canine models, displaying pharmacokinetic stability that underscores its translational potential. Consequently, [^{68}Ga]Ga-NOTA-T4 has been employed in initial clinical translation studies and successfully visualized TROP2 expression non-invasively in preclinical solid tumor models and a subset of solid tumor patients. In a patient diagnosed with metastatic nasopharyngeal carcinoma exhibiting multiorgan involvement, sequential [^{68}Ga]Ga-NOTA-T4 PET/CT scans demonstrated intense radiotracer uptake in osseous and hepatic metastases expressing TROP2 (**Figure 2**). Despite these promising results, physiological uptake in normal tissues (e.g., pancreas and kidneys) may obscure imaging clarity for renal and pancreatic tumors and raise concerns about off-target effects in therapeutic applications.

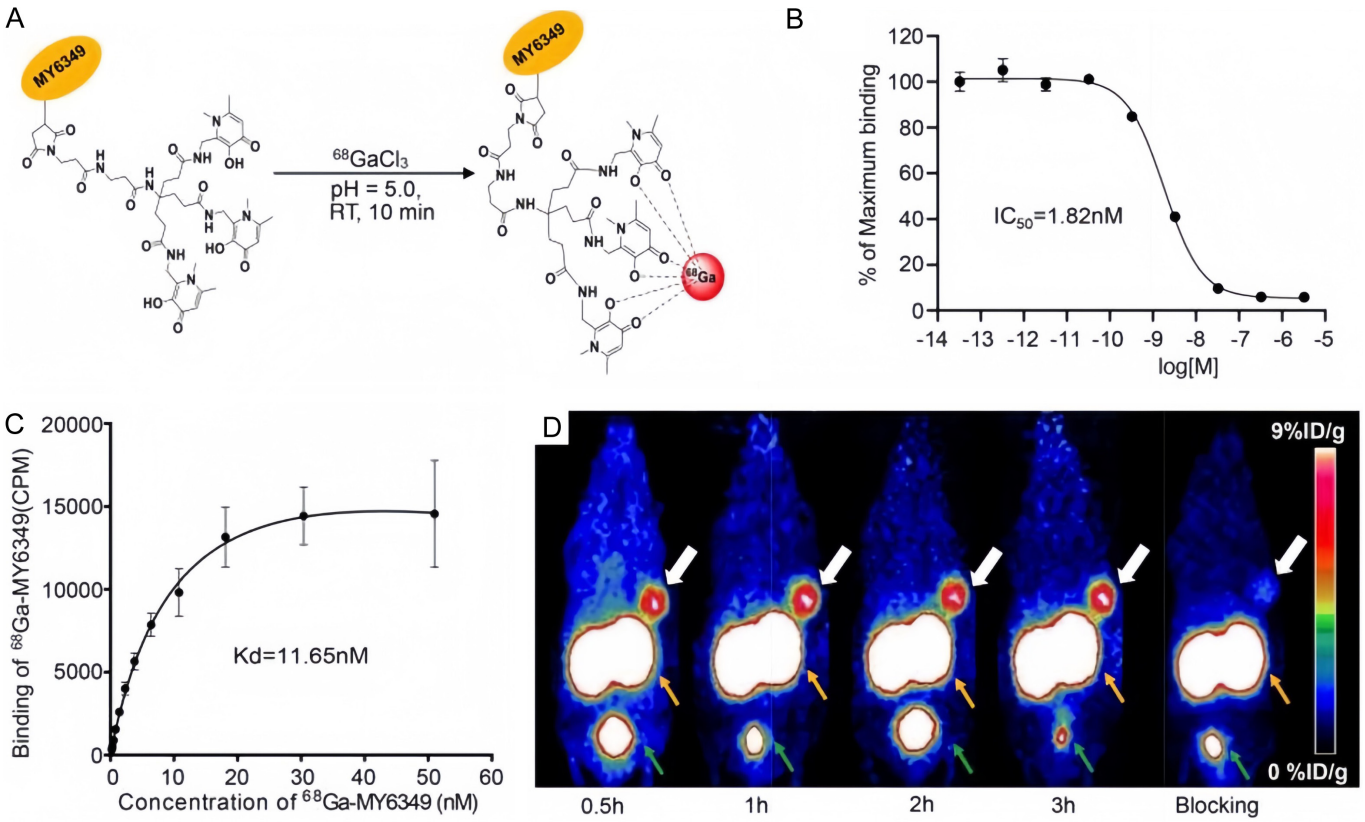

Figure 3. Preparation and preclinical evaluation of [^{68}Ga]Ga-MY6439. A. The mechanism of [^{68}Ga]Ga-MY6349 radiolabeling at room temperature (RT). B, C. The TROP2 binding affinity of [^{68}Ga]Ga-MY6349 was assessed using IC50 and equilibrium dissociation constant analyses. D. Representative PET imaging of [^{68}Ga]Ga-MY6349, including blocking experiments in a BxPC-3 tumor model. Yellow arrows indicate renal uptake, green arrows represent bladder activity, and white arrows mark tumor uptake. Source: From Haojun Chen, Liang Zhao et al. (2024) with modifications. Permission: Permission to reproduce this figure was obtained. Originally published in The Journal of clinical investigation (2024; 135(1): e185408).

IHC staining confirmed that [^{68}Ga]Ga-NOTA-T4 accurately delineated negative, positive, and heterogeneous TROP2 expression in both primary and metastatic tumors in three patients with solid tumors, highlighting its potential in patient stratification and therapeutic monitoring. These studies demonstrated that [^{68}Ga]Ga-NOTA-T4 could provide real-time monitoring and therapeutic efficacy evaluation for TROP2-targeted treatments, offering a critical basis for clinical decision-making.

The novel PET imaging probes [^{18}F]F-AlF-RESCA-T4 and [^{18}F]F-AlF-RESCA-RT4 have been successfully developed and applied, significantly enhancing the efficiency and stability of ^{18}F radiolabeling [42]. In preclinical studies, both probes demonstrated specific uptake in TROP2 positive tumors within the T3M-4 tumor model. Notably, [^{18}F]F-AlF-RESCA-RT4, which lacks the His-tag, exhibited optimized pharmacokinetic properties, including reduced renal radioactivity accumulation without compromising tumor uptake. Nevertheless, interspecies differences in renal reabsorption mechanisms between mice and humans may lead to an underestimation of clinical nephrotoxicity risks. This highlights the urgent need for further dosimetry studies to accurately assess these risks. Furthermore, an initial clinical study involving three patients highlighted the promising application of [^{18}F]F-AlF-RESCA-T4 and [^{18}F]F-AlF-RESCA-RT4 PET/CT imaging in lung cancer diagnosis and treatment. These radiotracers, in particular, demonstrated potential in differentiating inflammatory diseases from malignant tumors, paving the way for their clinical translation. However, the limited clinical sample size (n = 3) and the absence of multicenter validation hinder the ability to draw definitive conclusions about diagnostic accuracy across various tumors. Despite these limitations, the probes exhibit rapid targeting kinetics (optimal imaging window: 45-150 minutes) and demonstrate high tumor-to-background ratios (TBR > 3), which facilitate their potential clinical translation.

In a pioneering clinical translational study, Chen et al. engineered a TROP2-specific nanobody-based radiopharmaceutical, [^{68}Ga]Ga-MY6349, quantitative analysis revealed high-affinity binding to TROP2 receptors (IC50 = 1.82 nM; Kd = 11.65 nM) (**Figure 3A-C**) [34]. In subsequent in vivo evaluations using BxPC-3 tumor xenografts, it was observed that [^{68}Ga]Ga-MY6349 exhibited a time-dependent reduction in blood pool uptake. Notably, [^{68}Ga]Ga-MY6349 demonstrated rapid tumor accumulation within 0.5 hours post-injection, achieving a stable physiological distribution. Furthermore, it maintained a sustained level of tumor uptake for up to 3 hours after injection, as evidenced by PET imaging (**Figure 3D**). Notably,

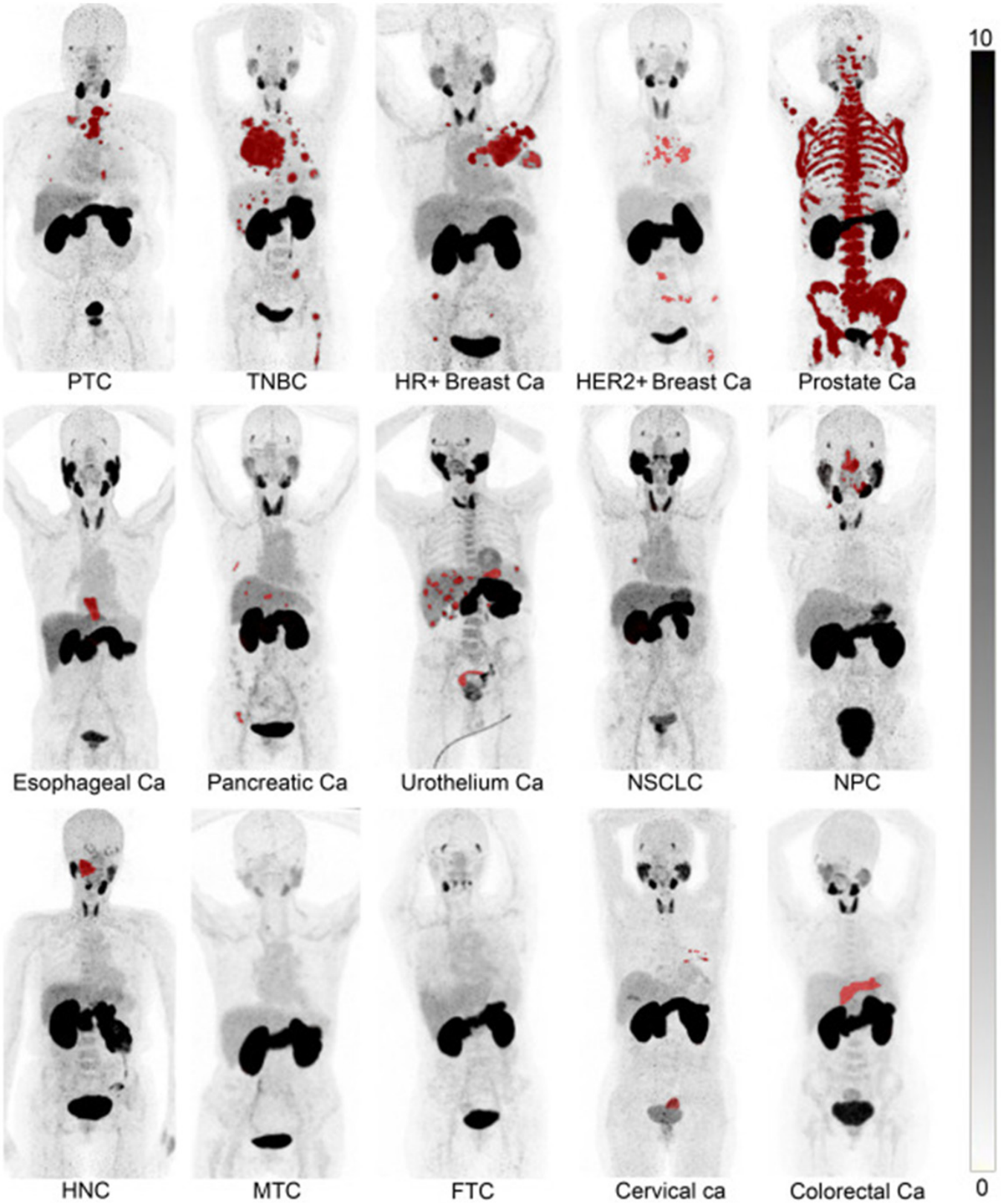

Figure 4. Detection of fifteen histopathologically confirmed solid tumors using [^{68}Ga]Ga-MY6349 PET/CT. Ca, carcinoma; FTC, follicular thyroid carcinoma; HER2, human epidermal growth factor receptor 2; HNC, head and neck cancer; HR, hormone receptor; MTC, medullary thyroid carcinoma; NPC, nasopharyngeal carcinoma; NSCLC, non-small cell lung cancer; PTC, papillary thyroid carcinoma; TNBC, triple-negative breast cancer. Source: From Haojun Chen, Liang Zhao et al. (2024) with modifications. Permission: Permission to reproduce this figure was obtained. Originally published in The Journal of clinical investigation (2024; 135(1): e185408).

while preclinical models have validated the specificity of [^{68}Ga]Ga-MY6349, interspecies differences in TROP2 epitope accessibility between murine xenografts and human tumors may lead to an overestimation of clinical perfor-

mance. Building on these findings, the investigators initiated a translation study (NCT06188468) to explore its clinical application across various tumor types. Furthermore, the limited subgroup analyses, particularly the inclusion of only 12 patients with prostate cancer, may compromise the statistical power necessary for drawing tumor-specific conclusions.

This favorable in vivo distribution pattern provided a flexible time window for PET imaging, ranging from 30 min to 180 min. As time goes on, the rapid systemic clearance of [^{68}Ga]Ga-MY6349 from the blood pool facilitated a TBR exceeding 3 across most malignancies, enabling precise delineation of tumor margins and enhanced diagnostic accuracy in PET/CT assessments. In a comprehensive semiquantitative analysis utilizing PET/CT imaging across 15 types of solid tumors, we observed a markedly increased [^{68}Ga]Ga-MY6349 uptake in TROP2-positive lesions, with an average SUVmax exceeding 10. This elevated uptake was particularly prominent in TNBC, hormone receptor-positive breast cancer, and prostate carcinoma, among others, when compared to normal tissue counterparts (**Figure 4**). Additionally, the metrics of SUVmax and TBR are inherently constrained by the spatial resolution of PET imaging, which could result in an underestimation of subcentimeter lesions, despite an observed improvement in TBR. Even so, this characteristic still indicates its robust diagnostic efficacy.

Furthermore, [^{68}Ga]Ga-MY6349 targeted distinct TROP2 epitopes in combination with TROP2-ADC drugs, providing novel strategies for therapeutic monitoring. This finding highlights its potential as a real-time evaluation tool for TROP2-targeted immunotherapies during treatment. Lastly, the small sample size (n = 2) and the absence of epitope mapping data highlight the need for further validation to elucidate the competitive binding dynamics between the tracer and ADCs.

Additionally, representative clinical imaging analyses demonstrated superior radiotracer uptake of [^{68}Ga]Ga-MY6349 in metastatic lesions across multiple malignancies, particularly in detecting lymph node metastases, osseous metastases, and hepatic metastases. Comparative semiquantitative analysis revealed significantly higher SUVmax and TBR with [^{68}Ga]Ga-MY6349 PET/CT than with [^{18}F]FDG PET/CT (**Figure 5A-C**), enabling the detection of additional metastatic foci and improved diagnostic sensitivity for specific cancer subtypes. Intriguingly, [^{68}Ga]Ga-MY6349 PET/CT demonstrated SUVmax values comparable to those of [^{18}F]FDG PET/CT in TNBC lesions. However, its notably higher TBR enhanced the detection of occult or subcentimeter lesions (**Figure 5D**). These findings highlight the clinical potential of TROP2-targeted molecular probes as next-generation pan-tumor imaging agents with enhanced lesion conspicuity.

Challenges and future directions of TROP2-targeted molecular imaging

Current research on the standardization of TROP2 quantification in PET/CT primarily focuses on three essential areas: the development of high-performance radiotracers, the optimization of quantitative analytical techniques, and the standardization of image acquisition protocols. Although a universally accepted framework for quantitative standardization remains unattainable, preliminary protocols utilizing SUV thresholding, multi-time point scanning, and artificial intelligence (AI)-assisted analytical systems have been documented. The implementation pathway comprises several sequential steps: First, the optimization of the TROP2-targeted radiotracer and administered dosage is crucial to achieve optimal tumor uptake and an appropriate in vivo distribution pattern. Second, PET imaging with multi-time point acquisitions may be employed to assess the pharmacokinetic characteristics of TROP2-targeted probes [40]. Third, optimizing PET acquisition parameters is crucial for ensuring reproducibility in imaging studies. Specifically, the uptake time, which should be standardized to a range of 60 to 120 minutes post-injection, along with the acquisition time, typically set to 2 to 3 minutes per bed, must be carefully calibrated. This standardization will enhance the consistency and reliability of PET imaging results across different studies and applications [34]. Furthermore, emerging AI-driven methodologies significantly improve analytical precision by automating tumor segmentation (region-of-interest, ROI) and employing machine learning models that correlate TROP2 expression levels with the therapeutic efficacy of ADCs. Furthermore, subsequent image reconstruction and correction processes should incorporate iterative algorithms that account for attenuation and scatter corrections.

While the standardization of TROP2 quantification in PET/CT has seen notable progress, significant challenges persist in translating TROP2-targeted immunoPET into clinical practice. First, conventional full-length antibody-based probes suffer from unfavorable pharmacokinetic profiles, including slow blood clearance (prolonging circulation time to > 7 days), high background noise in non-target tissues, and cumulative radiation doses to critical organs such as bone marrow [43]. These limitations are particularly problematic for TROP2 imaging, given its frequent overexpression in epithelial tumors with inherently heterogeneous tracer distribution patterns. Furthermore, the spatiotemporal dynamics of TROP2 expression during treatment pose diagnostic dilemmas: antigen loss may cause false-negative interpretations, while nonspecific uptake in inflammatory lesions or antigen sinking could generate false-positive signals [44]. At the level of production quality control, good manufacturing practice (GMP)-compliant production of ^{89}Zr/^{64}Cu-labeled anti-

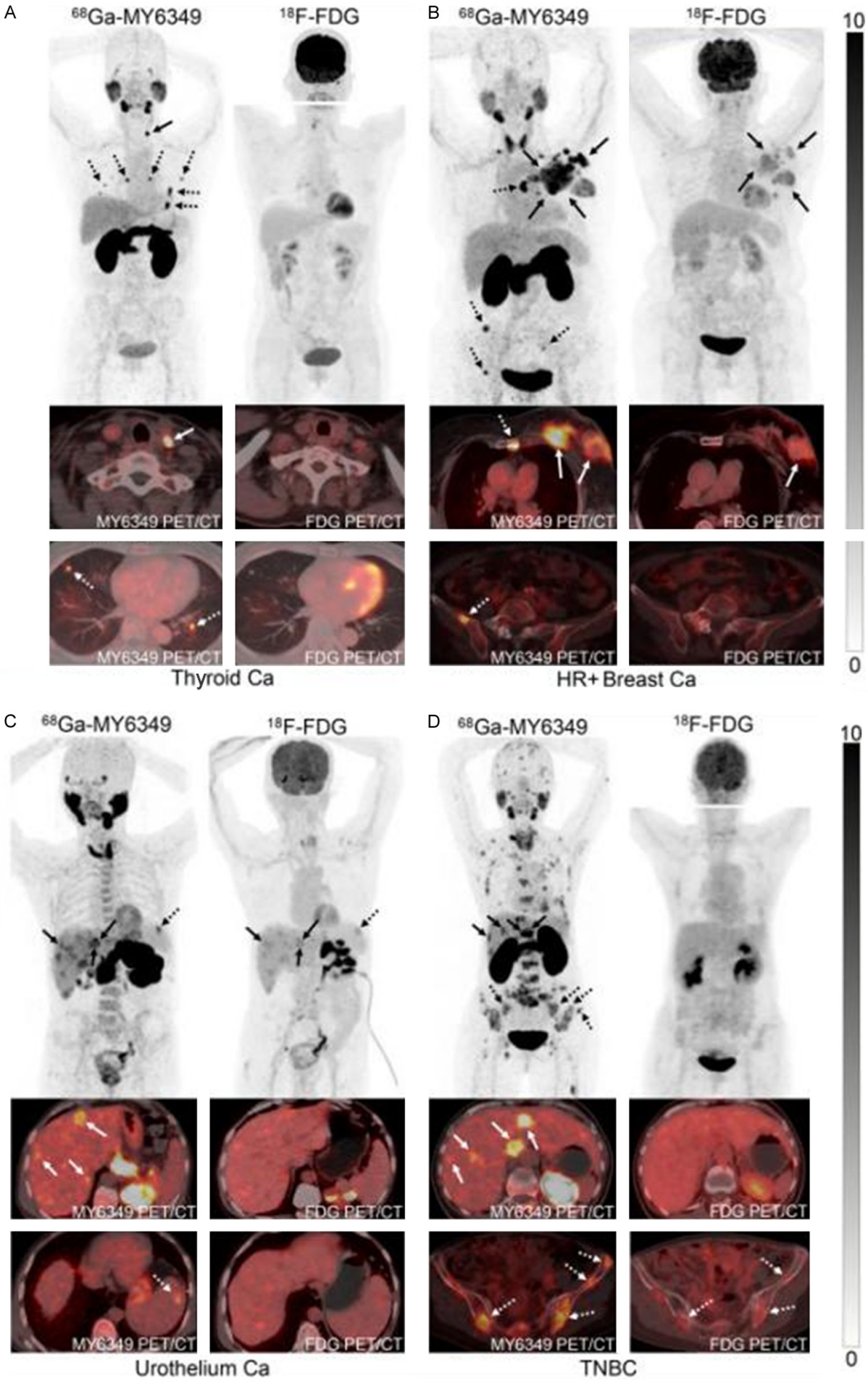
A
68Ga-MY6349
18F-FDG
MY6349 PET/CT
FDG PET/CT
Thyroid Ca
B
68Ga-MY6349
18F-FDG
MY6349 PET/CT
FDG PET/CT
HR+ Breast Ca
10
0
C
68Ga-MY6349
18F-FDG
MY6349 PET/CT
FDG PET/CT
Urothelium Ca
D
68Ga-MY6349
18F-FDG
MY6349 PET/CT
FDG PET/CT
TNBC
10
0

Figure 5. Representative [^{18}F]FDG PET/CT and [^{68}Ga]Ga-MY6349 PET/CT imaging in patients with various malignancies. (A) [^{68}Ga]Ga-MY6349 PET/CT was performed to assess post-surgical tumor recurrence in a 68-year-old female patient with papillary thyroid carcinoma. [^{68}Ga]Ga-MY6349 PET/CT detected significant radiotracer uptake in cervical lymph nodes (solid arrows) and pulmonary metastases (dashed arrows), whereas [^{18}F]FDG PET/CT yielded false-negative findings. (B) [^{68}Ga]Ga-MY6349 PET/CT was utilized for tumor staging in a 74-year-old patient with HR+ breast cancer. Compared to [^{18}F]FDG PET/CT, [^{68}Ga]Ga-MY6349 PET/CT exhibited higher uptake in the primary tumor (solid arrows), lymph nodes, and bone metastases (dashed arrows), whereas [^{18}F]FDG PET/CT displayed false-negative uptake in bone metastases. The HR+ breast cancer imaging in **Figure 4** is referenced in panel (B, C). [^{68}Ga]Ga-MY6349 PET/CT was applied for the initial staging of urothelial carcinoma in a 63-year-old patient. Compared to [^{18}F]FDG PET/CT, [^{68}Ga]Ga-MY6349 PET/CT demonstrated superior radiotracer uptake and enhanced imaging contrast in both primary and metastatic lesions, particularly in hepatic metastases (solid arrows) and splenic metastases (dashed arrows). The urothelial carcinoma imaging in **Figure 4** is referenced in panel (C, D). [^{68}Ga]Ga-MY6349 PET/CT was employed for restaging TNBC in a 65-year-old female patient following treatment. The scan revealed intense tracer uptake in lymph nodes, liver (solid arrows), and extensive bone metastases (dashed arrows). In contrast, [^{18}F]FDG PET/CT exhibited low to moderate uptake across most metastatic sites. Source: From Haojun Chen, Liang Zhao et al. (2024) with modifications. Permission: Permission to reproduce this figure was obtained. Originally published in The Journal of clinical investigation (2024; 135(1): e185408).

TROP2 antibodies requires specialized infrastructure for site-specific conjugation and rigorous batch-to-batch stability testing, substantially increasing manufacturing costs compared to small-molecule radiotracers [45]. Finally, the lack of harmonized regulatory frameworks for antibody-based radiopharmaceuticals prolongs clinical validation timelines, delaying the implementation of multicenter trials essential for establishing TROP2 PET quantification thresholds.

To overcome these translational bottlenecks, we propose a dual-pronged strategy: probe optimization should prioritize engineered antibody fragments with accelerated clearance kinetics (targeting < 72 hours plasma half-life), paired with radionuclides like ^{18}F or ^{68}Ga to balance dosimetry and image resolution. Additionally, theranostic integration could leverage TROP2's dual role as a diagnostic biomarker and therapeutic target by developing isotope-labeled bifunctional probes for companion diagnostics during ADC therapies (theranostic is a medical technology that combines diagnosis and therapy, with the objective of improving treatment efficacy while minimizing side effects. This is achieved by accurately identifying disease characteristics and simultaneously administering targeted therapeutic interventions) [46, 47]. The establishment of a standardization system should focus on three key aspects: first, protocol harmonization through SNMMI/EANM-endorsed acquisition protocols (e.g., optimal 72-96 hours post-injection imaging windows for intact antibodies); second, the creation of a GMP pilot-scale platform for antibody labeling through collaborative efforts between industry, academia, and research institutions to streamline ^{89}Zr-TROP2 antibody production [45]; and third, regulatory acceleration via National Major New Drug Development Project, utilizing existing clinical data from TROP2-targeted therapies to support investigational new drug applications [46].

Despite the progress made in the development of Trop2-targeted radiopharmaceuticals, significant limitations remain in their theranostic application. The majority of current Trop2-targeted radioligands utilize low-molecular-weight nanobodies as targeting vectors, capitalizing on their superior tumor penetration and rapid systemic clearance. However, these pharmacokinetic characteristics inadvertently lead to considerable renal tubular reabsorption, resulting in alarming levels of radionuclide accumulation within the renal parenchyma, ranging from 30 to 50% ID/g. From a theranostic perspective, this situation presents a critical dilemma: balancing target specificity with optimal biodistribution. Excessive retention in the kidneys not only imposes dose-limiting toxicity thresholds but also heightens the risks of radiation-induced nephropathy, which currently stands as the foremost obstacle to clinical implementation. To tackle these challenges, it is imperative to implement additional structural modifications aimed at improving pharmacokinetic profiles and minimizing nonspecific retention. To date, various strategies have been devised to mitigate nanobody tracer uptake in the kidneys. Notably, one effective approach involves the use of enzymatically cleavable linkers, which allow for the selective release of radionuclides upon renal filtration. This mechanism promotes urinary excretion through bladder metabolism while significantly reducing renal radiotoxicity [48]. Additionally, the advancement of peptide-based targeting probes represents a promising alternative strategy for optimizing Trop2-directed radiopharmaceuticals.

Overall, these findings elucidate the substantial potential of TROP2-based molecular imaging for patient selection and response evaluation, while highlighting an unmet need for radiopharmaceutical innovation and clinical validation in Trop2-directed theranostic pipelines.

Summary

In conclusion, molecular imaging technologies, particularly immunoPET, offer a novel approach for the non-invasive evaluation of TROP2 and provide a critical foundation for the precise selection of targeted therapies. With ongoing advancements in molecular imaging, PET-based molecular probes targeting TROP2 are poised to become a valuable diagnostic tool for visualizing TROP2 expression in clinical tumor settings. This is especially relevant in the context of TROP2 ADC-targeted therapies, where TROP2 plays a crucial role in patient screening, early ther-

apeutic assessment, and guiding targeted treatment strategies.

To enhance the future development of TROP2-targeted immunoPET, it is essential to focus on the integration of AI and machine learning (ML) with TROP2 imaging technologies. This integration aims to optimize prediction models for pharmacokinetic characteristics. The creation of dual-targeting probes is designed to tackle tumor heterogeneity through co-localization strategies, while computational simulations can be utilized to improve probe performance. Additionally, establishing interdisciplinary collaborations that combine radiomics and bioengineering is crucial for developing standardized quantitative analytical frameworks. These coordinated efforts will ultimately pave the way for the implementation of precision theranostic applications, which seamlessly integrate diagnostic and therapeutic capabilities.

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Disclosure of conflict of interest

None.

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