

Invited Perspective

Visualizing T-cell activation: PET imaging of CXCL9 as a window into the tumor immune response

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Abstract: T-cell activation within the tumor microenvironment is a key determinant of response to immunotherapy, yet current biomarkers fail to capture its spatial and temporal dynamics. Traditional assays such as PD-L1 immunohistochemistry, tumor mutational analysis, and circulating cytokine profiling offer static or systemic snapshots that inadequately reflect localized immune engagement. Positron emission tomography (PET) provides a unique opportunity to visualize these processes *in vivo*. Among emerging tracers, CXCL9-targeted imaging stands out as a promising approach to quantify IFN γ -driven T-cell activation and recruitment. Jacobson *et al.* report the development of [¹⁸F]F-h2A12, a high-affinity nanobody PET tracer specific for CXCL9. Preclinical studies demonstrate robust uptake in CXCL9-expressing tumors, close correlation with intratumoral immune activation, and clear distinction from blood-based biomarkers. Compared with existing immune PET tracers that target cytotoxic enzymes, soluble cytokines, or surface activation markers, CXCL9 imaging offers an advantageous balance of specificity, localization, and functional relevance. By visualizing the chemokine gradients that govern T-cell trafficking, CXCL9 PET could serve as an early, noninvasive biomarker of immunotherapy response and a powerful tool for guiding adaptive treatment strategies.

Keywords: PET, CXCL9, tumor, microenvironment

Introduction

The transformative impact of immunotherapy in oncology is undeniable, yet variability in patient response remains a major challenge under active investigation. While the mechanisms driving durable benefit are complex, the composition of the tumor microenvironment (TME) and robust T-cell activation have consistently emerged as critical determinants of therapeutic success. Patients who achieve sufficient activation of cytotoxic T lymphocytes within the TME often experience meaningful and lasting responses to immune checkpoint inhibitors and T-cell engagers, whereas those with poor or absent T-cell activation typically fail to benefit [1, 2].

Traditional biomarkers provide only a partial view of T-cell engagement and the tumor microenvironment. PD-L1 immunohistochemistry, tumor mutational analysis, and circulating tumor DNA capture the static potential for immune recognition but cannot resolve the dynamic kinetics of T-cell activation as treatment unfolds. Circulating cytokines and chemokines provide systemic readouts, yet they are limited by compartmental dilution, short half-lives, and a lack of spatial resolution within tumors [3]. Biopsies offer direct tissue assessment but are constrained by sampling issues, intratumoral variability, and the practical and ethical limitations of repeated acquisition. The aforementioned approaches often fail to capture the temporal and spatial heterogeneity of immune responses, and invasive sampling may miss focal regions of immune activity altogether. Collectively, these limitations

highlight the need for a noninvasive, real-time tool, such as positron emission tomography (PET) combined with a variety of radio-conjugation methods that can directly measure both the extent and the localization of T-cell activation *in vivo* [4-6].

PET imaging holds particular promise for meeting this need. Several tracers have been developed to target immune cell presence, activation markers, or effector molecules. Yet most approaches either interrogate surface receptors, which may not strictly correlate with functional activation, or downstream cytotoxic events, which occur only after T cells have already infiltrated tumors [7, 8]. By contrast, imaging the induction of chemokines downstream of interferon- γ (IFN γ) offers a way to capture the functional activation state of T cells at the moment when they begin to orchestrate antitumor responses. Among these chemokines, CXCL9 is especially compelling, as it is tightly linked to IFN γ signaling, robustly induced during effective immune activation, and critical for recruiting effector T cells into tumors. Jacobson and colleagues' recent development of a fluorine-18 labeled nanobody PET tracer targeting CXCL9 represents an important advance in this pursuit [9].

Literature Highlight: [¹⁸F]F-h2A12

In their study, Jacobson and colleagues generated a llama-derived nanobody with nanomolar affinity for human CXCL9 and subsequently radiolabeled with fluorine-18. The resulting tracer, [¹⁸F]F-h2A12, demonstrated excel-

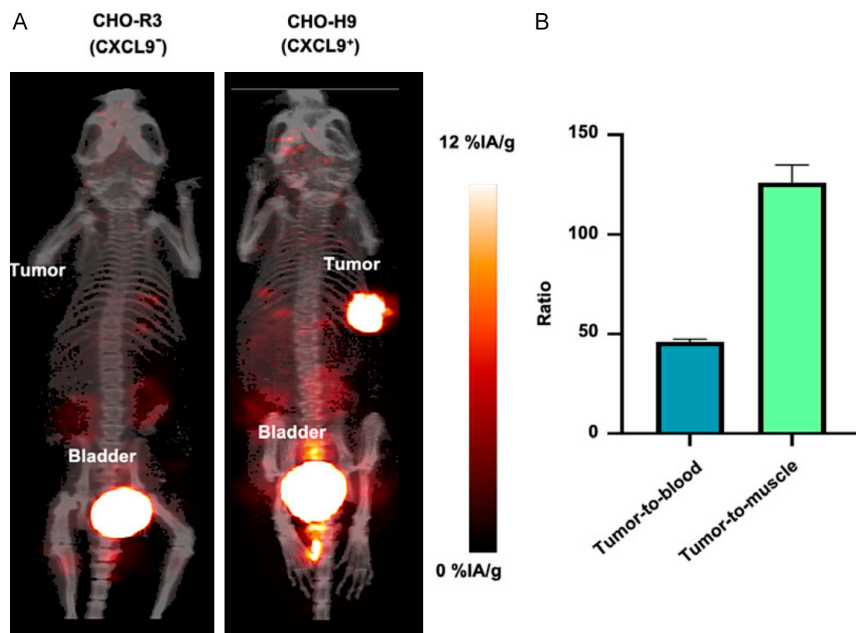


Figure 1. PET/CT imaging of [^{18}F]F-h2A12 in subcutaneous xenograft models. A. Representative projection images of mice bearing CHO-H9 (CXCL9⁺) and CHO-R3 (CXCL9⁻) tumors acquired 2 hours after injection of 3.7 MBq [^{18}F]F-h2A12 demonstrate strong, specific uptake in CXCL9-expressing tumors. B. Quantification of tracer distribution showing high tumor-to-blood and tumor-to-muscle ratios in CHO-H9 xenografts, consistent with high target specificity and favorable pharmacokinetics. Figure reproduced and adapted from Jacobson et al., *J Nucl Med*, 2025.

Comparing PET Imaging Strategies for Immune Activation

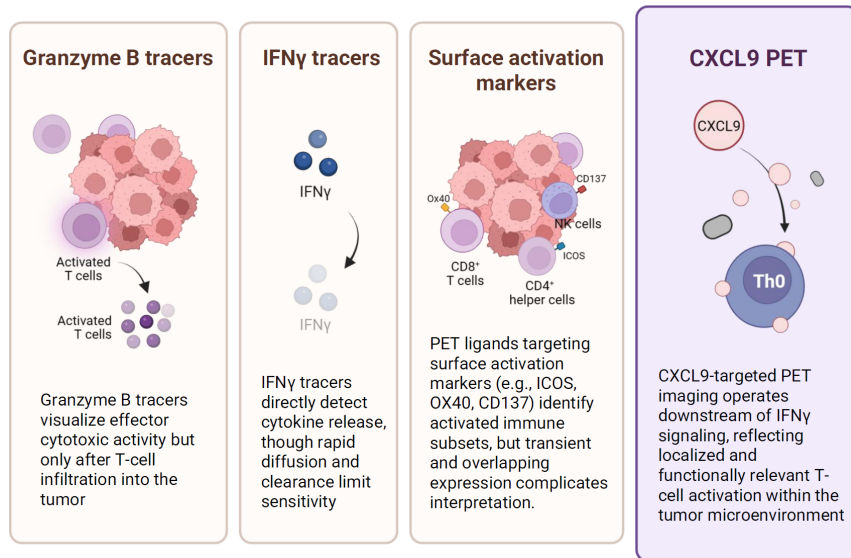


Figure 2. Comparing PET strategies for immune activation: granzyme B (cytotoxicity), IFN γ (cytokine release), surface activation markers (activated subsets), and CXCL9 (localized, downstream IFN γ activity in the TME).

lent specificity, with no appreciable cross-reactivity to closely related chemokines. In xenograft models, uptake in CXCL9-expressing tumors was dramatically higher than in controls, yielding tumor-to-muscle ratios well over 100 within hours (**Figure 1**). In a humanized mouse model of T-cell engager therapy, tracer uptake peaked around

day seven, coinciding with increased intratumoral CXCL9 and IFN γ , and then declined as the immune response contracted. Importantly, serum levels of CXCL9 did not correlate with tracer uptake, underscoring the limitations of blood-based biomarkers and highlighting the unique strength of imaging to capture localized immune activation [9].

To appreciate the distinct value of CXCL9 imaging, it is helpful to compare it with prior immune PET approaches that target T-cells (**Figure 2**). Granzyme B tracers such as [^{18}F]AIF-mNOTA-GZP have been shown to stratify responders and non-responders to checkpoint blockade in murine models by directly visualizing effector cytotoxic activity. These agents are highly specific but only become positive after T cells have infiltrated tumors and engaged targets [10, 11]. PET tracers targeting IFN γ itself have also been developed, but soluble cytokines often diffuse or clear quickly, which limits sensitivity and increases background signal [12]. Probes targeting surface activation markers such as CD69, ICOS, OX-40, or CD137 can detect activated immune subsets, but interpretation may be complicated by receptor internalization, transient kinetics, or expression on regulatory populations [7]. CXCL9 imaging instead occupies a middle ground. It is downstream of IFN γ and reflects a functional immune response, but unlike soluble cytokines it binds to extracellular matrix and cell surfaces, creating focal retention ideal for PET detection. This combination of biological specificity and imaging practicality may make CXCL9 PET uniquely suited to capture the dynamic immune processes that underlie treatment response.

Despite its promise, translation to clinical practice will require addressing several important challenges. Quantification and thresholding will be necessary to determine what constitutes a clinically meaningful level of CXCL9 uptake across tumor types and treatment regimens. Signal attribution may not always be straightforward, as CXCL9 can be secreted

by multiple cell types including macrophages, dendritic cells, endothelial cells, and tumor cells. The kinetics of CXCL9 induction will likely vary across cancer types and therapeutic modalities, meaning the optimal imaging window observed in a T-cell engager model may not generalize to other settings. Safety, dosimetry, and immunogenic-

ity in humans remain to be determined, and while nanobody-based tracers are attractive for their rapid clearance and low background, regulatory-grade production and validation will require considerable effort. Finally, the spatial heterogeneity of immune responses within tumors could hinder assessment, particularly in patients with mixed radiotracer-avid and non-avid lesions.

These hurdles should not obscure the many opportunities that CXCL9 PET presents. If validated in humans, this approach could serve as an early biomarker of immunotherapy response, distinguishing responders from non-responders before structural or metabolic imaging reveals differences. It could be deployed alongside other tracers to build a comprehensive profile of immune dynamics, with CXCL9 imaging indicating early chemokine induction and granzyme B imaging, for example, confirming downstream cytotoxic activity. Such strategies could guide adaptive therapy, where patients failing to mount an early CXCL9 response might be redirected toward combination regimens that augment IFN γ -CXCL9 signaling. Beyond oncology, CXCL9 PET could find utility in transplant rejection, autoimmune disease, and infectious disease, where aberrant T-cell activation drives pathology.

The broader context of radiopharmaceutical development suggests that translation of CXCL9 imaging may occur faster than previously possible. Recent discussions by the FDA and commentaries in the nuclear medicine community have emphasized more streamlined approaches to first-in-human dosimetry and regulatory evaluation, particularly for fluorine-18 labeled nanobody-based tracers [13]. If these frameworks are fully realized, clinical testing of [^{18}F]-h2A12 could proceed with fewer delays, accelerating its path from preclinical discovery to bedside application. At the same time, harmonization of imaging protocols, reproducibility standards, and integration with transcriptomic or circulating biomarkers will be essential for defining how CXCL9 PET fits into the broader biomarker landscape for immunotherapy.

Conclusion

Imaging CXCL9 represents a conceptual and practical advance in immune PET. By capturing the downstream effects of IFN γ signaling and the chemokine gradients that direct T-cell infiltration, CXCL9-targeted PET offers a powerful tool for visualizing immune activation. The preclinical data presented by Jacobson and colleagues set the stage for clinical translation, but the true test will be whether this approach can reliably predict and monitor therapeutic responses in patients. If successful, CXCL9 PET may emerge not only as a window into immune activation but also as a clinical instrument for personalizing immunotherapy and extending the benefits of precision medicine across oncology and beyond.

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Disclosure of conflict of interest

None.

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