

## Specific and Non-Specific Uptake in Quantitative $^{89}\text{Zr}$ -Immuno-PET

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### TO THE EDITOR:

In a recent review, Van Dongen et al. illustrated why  $^{89}\text{Zr}$ -immuno-PET has become an important tool for the *in vivo* characterization of novel biological drugs and their targets (1). A technical State of the Art summarized PET quantification of  $^{89}\text{Zr}$ -tracer uptake, stressing that total-tissue uptake results from a target-specific and a non-specific contribution. The latter involves a first, so-called, reversible part related to free tracer in blood and interstitium, quantified by Patlak y-intercept ( $V_i$ ). The second irreversible part is related to  $^{89}\text{Zr}$  residualization after monoclonal-antibody (mAb) uptake and degradation by antigen-negative cells, quantified by Patlak uptake-rate-constant ( $K_i$ ). This description is fully in line with a previous study co-authored by van Dongen, using Patlak analysis in normal tissues (kidney–liver–lung–spleen) without known target expression for four  $^{89}\text{Zr}$ -labeled mAbs, respectively (2). Van Dongen et al. thus suggested that future quantitative  $^{89}\text{Zr}$ -immuno-PET studies should consider multiple-time-point acquisitions to assess non-specific-uptake versus time, with at least three late time-points, and that sophisticated modelling strategies should be developed (1,2).

We believe that this suggestion warrants further comments that might be helpful for anticipating quantitative  $^{89}\text{Zr}$ -immuno-PET studies in tumors, designed for assessing *in-vivo* target engagement. First, the non-specific-irreversible uptake should be quantitatively compared to the total-tumor uptake, in order to actually determine whether it might be significant or negligible (1,2). To justify this proposal, let us consider recent results about

<sup>89</sup>Zr-anti-PD-L1, designed for monitoring *in-vivo* chemotherapy-mediated modulation of tumor-PD-L1 expression (3). After extracting tracer input function and tumor data showing irreversible uptake (using the Web-Plot-Digitizer software in Jung et al.'s Figures 2B-3B, respectively), Patlak analysis provides a total-tumor  $K_i$  of 0.0289 mL·g<sup>-1</sup>·h<sup>-1</sup> ( $R^2=0.9993$ ). For comparison, combining four <sup>89</sup>Zr-labeled mAbs, baseline value of the non-specific  $K_i$  in kidney–liver–lung–spleen was previously found to be: 0.0007–0.0011–0.0002–0.0005 mL·g<sup>-1</sup>·h<sup>-1</sup>, respectively (2). The total-tumor  $K_i$  value of the <sup>89</sup>Zr-anti-PD-L1 random example thus appears to be between 26–145-fold higher than the non-specific  $K_i$  values of normal tissues. Even assuming that the non-specific contribution might vary depending on tumors and patients, unlike for normal tissues across patients, we do suggest this first issue deserves consideration.

Second, we suggest that the principle of a three-time-point method, previously described for quantitative <sup>64</sup>Cu-immuno-PET, might be adapted to <sup>89</sup>Zr-immuno-PET (4). Rather than the three late time-points suggested by van Dongen et al., three time-points are needed at early (after reaching equilibrium), mid and late imaging, for assessing  $K_i$ ,  $V_t$  and a release-rate constant ( $k_R$ ). Indeed, we believe the Patlak assumption of irreversible uptake cannot be justified in an arbitrary tissue, including tumors, as evidenced by <sup>64</sup>Cu-NOTA-RamAb in VEGFR-2-positive HCC4006 tumors:  $K_i = 0.0314$  mL·g<sup>-1</sup>·h<sup>-1</sup>,  $k_R = 0.0387$  h<sup>-1</sup> and  $V_t = 0.2075$  mL·cm<sup>-3</sup> (without RamAb blocking dose)(4). Noteworthy, this method cannot differentiate between specific and non-specific uptake, and the actual meaning of the three kinetic parameters should be specified under each situation. However, it should be emphasized that a kinetic modelling analysis able to differentiate between specific and non-specific uptake may probably increase the number of parameters involved in fitting three-time-point PET-data, which is contrary to Akaike criteria (5). Finally, if non-specific uptake has proven quantitatively negligible compared to specific, or, alternatively, if differentiating between them has proven unrealistic in current clinical practice, we suggest that a single time-point for optimal quantitative <sup>89</sup>Zr-immuno-PET might be probed (under irreversible-trapping condition)(6).

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## REFERENCES

1. Van Dongen GAMS, Beaino W, Windhorst AD, et al. The navigating and de-risking role of  $^{89}\text{Zr}$ -immuno-PET in the development of biopharmaceuticals. *J Nucl Med.* 2020 Dec 4;jnumed.119.239558. doi: 10.2967/jnumed.119.239558. Online ahead of print.
2. Jauw YWS, O' Donoghue JA, Zijlstra JM, et al.  $^{89}\text{Zr}$ -immuno-PET: toward a noninvasive clinical tool to measure target engagement of therapeutic antibodies in vivo. *J Nucl Med.* 2019;60:1825–1832.
3. Jung KH, Park JW, Lee JH, Lee EJ, Moon SH, Cho YS, Lee KH.  $^{89}\text{Zr}$  labelled anti-PD-L1 antibody PET monitors gemcitabine therapy-induced modulation of tumor PD-L1 expression. *J Nucl Med.* 2020 Sep 11;jnumed.120.250720. doi: 10.2967/jnumed.120.250720. Online ahead of print.
4. Laffon E, Marthan R. A three-time-point method for assessing kinetic parameters of  $^{64}\text{Cu}$ -labeled Ramucirumab trapping in VEGFR-2 positive lung tumors. *Phys Med.* 2017;43:1–5.
5. Akaike H. A new look at the statistical model identification. *Automatic Control, IEEE Transactions on.* 1974;19:716–723.
6. Laffon E, Marthan R. Is there a relevant imaging time for optimal quantitative  $^{89}\text{Zr}$ -DFO-daratumumab PET imaging? *Radiology.* Accepted for publication.