

## Invited Perspective

# FIAU: From reporter gene imaging to imaging of bacterial proliferation

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**Abstract:** The radioiodinated thymidine analogue, FIAU, is a tracer that has been developed for reporter gene, for cells that were transfected with herpes simplex virus thymidine kinase, HSV-TK. FIAU is also a specific substrate of bacterial TK due to the homology between viral and bacterial TK. In this issue of AJNMMI (<http://www.ajnmml.us>), Pullambhatla *et al.* reported that the accumulation of  $^{125}\text{I}$ -FIAU in pulmonary infectious foci correlated with the bacterial burden in the lungs.  $^{125}\text{I}$ -FIAU could be used to monitor the efficacy of anti-microbial therapy in mice. Potentially  $^{124}\text{I}$ -FIAU PET could be used to discriminate microbial from sterile inflammation in patients with prosthetic implants.

**Keywords:** Infection, inflammation, reporter gene imaging, positron emission tomography (PET)

In their editorial in the Journal of Nuclear Medicine in 1991 Corstens and van der Meer defined the requirements of the ideal radiotracer for imaging focal sites of infection and inflammation [1]; high uptake in infectious/inflamed foci, rapid background clearance, nontoxic, low cost and preferably discrimination between infection and non-microbial inflammation. Since then a wide series of radiotracers has been proposed for infection/inflammation imaging based on antibodies, cytokines, anti-microbial or chemotactic peptides or antibiotics. Some of these tracers had in vivo characteristics that met most of the criteria mentioned above. However, mainly due to commercial hick-ups none of these agents has been made commercially available for routine clinical use and in most hospitals autologous leukocytes labeled with  $^{111}\text{In}$  or  $^{99\text{m}}\text{Tc}$ ,  $^{67}\text{Ga}$  citrate and more recently  $^{18}\text{F}$ -FDG are commonly used to determine the localization and the extent of infectious disease in patients.

In the current issue of the American Journal of Nuclear Medicine and Molecular Imaging Pullambhatla *et al.* report that focal bacterial infections in the lungs in mice can be visualized with  $^{125}\text{I}$ -labeled 2'-fluoro-2'-deoxy-1 $\beta$ -D-arabinofuranosyl-5-[ $^{125}\text{I}$ ]iodouracil ( $^{125}\text{I}$ -FIAU) [2].

$^{124/5}\text{I}$ -FIAU is a tracer that was developed for imaging of cells transfected with the herpes simplex virus 1 (HSV1) thymidine kinase reporter gene. This enzyme transfers a  $\gamma$  phosphate group from ATP to the 5' hydroxyl group of pyrimidine deoxynucleosides. The lipophilic tracer diffuses into the cell and is trapped in the cell with HSV1-TK activity, because the phosphorylated tracer cannot pass the plasma membrane.

In 2005 the group at Johns Hopkins University hypothesized that the TK gene of bacteria was sufficiently similar to that of the viral TK of HSV1 that FIAU could also be phosphorylated by the endogenous bacterial TK [3]. In their report published in PNAS they provided convincing evidence that indeed FIAU is a substrate of *E. coli* TK. They showed that FIAU inhibited the growth of the wild-type bacteria, but not a TK-deficient strain. In mice models, focal infections induced by five different genera of bacteria were imaged with  $^{125}\text{I}$ -FIAU.

In the present study, Pullambhatla and coworkers show that the tracer can be used to monitor the efficacy of anti-microbial therapy, because the  $^{125}\text{I}$ -FIAU signal intensity is proportional to the bacterial load. It is suggested that imaging

with FIAU could be used to evaluate the efficacy of newly developed antibiotics. The authors carefully assessed the sensitivity of imaging *E. coli* with microSPECT using  $^{125}\text{I}$ -FIAU as a tracer: in the bacterial concentrations  $\geq 10^9$  CFU/ml could be detected. The method is less sensitive than the methods based on the use of fluorescent probes or bioluminescence, but in contrast to these optical imaging modalities, radionuclide imaging allows imaging of deep seated foci. The authors speculate that the sensitivity can be improved by exploiting the enhanced sensitivity of PET using  $^{124}\text{I}$ -FIAU as a tracer. The feasibility of PET imaging of bacterial infections with  $^{124}\text{I}$ -FIAU was demonstrated by the same group, by successfully imaging musculoskeletal infections in seven patients and one healthy subject [4]. In that study all bacterial infections were visualized as early as 2 h after injection of the tracer. Obviously, the sensitivity of  $^{124}\text{I}$ -FIAU PET for imaging bacterial infection in various patients suspected of focal bacterial infections has yet to be assessed.

Most importantly, the present report shows that FIAU imaging can indeed discriminate between microbial infection and sterile inflammation, because tracer accumulation is dependent of the presence of microbial TK activity. Pulmonary inflammation induced by lipopolysaccharide (LPS) did not show enhanced tracer uptake. It has been claimed that  $^{99\text{m}}\text{Tc}$ -labeled ciprofloxacin also specifically imaged bacterial infectious foci, due to the specific interaction of ciprofloxacin with bacterial DNA gyrase and topoisomerase IV [5]. However, several studies have shown that this tracer could also be retained in non-microbial inflammatory sites [6, 7]. Later it was argued that non-bacterial infection could be distinguished from bacterial infection by imaging at two time points, however, in a Phase II trial it was demonstrated that this tracer washed-out from sites of infection as well as from inflammation at the same rate [8].

In clinical practice there is a great need for an imaging agent that could (a) discriminate between microbial infection and sterile inflammation, e.g. to discriminate infection of prosthetic implants from the sterile inflammatory response in aseptic complications and (b) monitor antibiotic treatment by determining successful eradication of bacteria. It would be of great interest to determine the diagnostic accuracy of  $^{124}\text{I}$ -FIAU PET in these patients. It would be a major

step forward when clinical studies could demonstrate that a tracer that was developed for reporter gene imaging could be a valuable tool for evaluating the presence of active bacterial infection in patients.

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