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Molecular imaging: What is right and what is an illusion?

William E. Klunk*

University of Pittsburgh, Pittsburgh, PA, USA

Abstract Over the past 40 years, brain molecular imaging has evolved from measuring cerebral metabolism with fluorodeoxyglucose, to neuroreceptor imaging, to imaging pathological protein deposits. In the early going, the characteristics of successful molecular imaging radiotracers were defined, and a detailed "Process" was developed for the collection of basic pharmacodynamic and pharmacokinetic data. These data are essential for the interpretation of in vivo imaging data and for defining the strengths, weaknesses, and limitations of new tracers. This perspective discusses the use of this "Process" in the development of the amyloid β positron emission tomography radiotracer, Pittsburgh Compound-B, and discusses some of the current controversies and difficulties in the field of tau positron emission tomography in the context of human data that preceded completion of this radiotracer characterization process—which still remains to be completed. As a field, we must decide which data are valid and which are artifacts and determine that when the artifacts are so overwhelming, the data are merely an illusion.

Keywords: Positron emission tomography; Amyloid; Tau; Radiotracer development; Illusion

Fifty years before AIC-2017 and a decade before the first fluorodeoxyglucose positron emission tomography (PET) studies were performed, the recent Rock & Roll Hall of Fame inductee, Graeme Edge of "The Moody Blues" wrote a poem called "Morning Glory" that became a part of their iconic album "Days of Future Past." Some of Edge's words apply well to the current state of molecular imaging—a field with a brief history and many still active in the field have been around for most of it (and for the music of the late 60s). The poem reflects on how the moon changes our perception of colors, and the pertinent part for this perspective goes something like, "…red is gray and yellow white, but we decide which is right…and which is an illusion" [1]. The field of PET radiotracer development fashioned a "Process" long ago to help us make these decisions [2]. This perspective reflects on the advantages of following that "Process" and the pitfalls of forgetting it.

Fifteen years, before the presentations highlighted in this special issue, the first Pittsburgh Compound-B (PiB) data were presented at AIC-2002 (Stockholm). It was thought-provoking to pull out that old presentation and flip through it in preparation for writing this perspective. Ninety percent of the presentation was preclinical technical data reflecting the culmination of more than a decade of work, describing the "Process" for the development of a novel class of amyloid β (Aβ) radiotracers including (1) criteria for acceptance of a good Aβ PET tracer; (2) structure-activity relationships for the binding of benzothiazole derivatives to postmortem Alzheimer's disease (AD) and control brains; (3) correlations with brain Aβ load measured biochemically; (4) pharmacokinetics in

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*Corresponding author. Tel.: 412-246-6460; Fax: 412-246-6465.
E-mail address: klunkwe@upmc.edu

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primates; (5) two-photon studies in mice; (6) receptor/enzyme pharmacology; and (7) toxicology. A human image was shown only briefly in one slide as an advertisement for what was then aptly called a "Hot Topics" presentation later in the week at the main conference (then called the International Conference on Alzheimer's Disease) by Henry Engler of Uppsala University (where the first human PiB study was performed 5 months earlier on February 14, 2002). It would be 18 months before the first manuscript on PiB imaging in an expanded cohort was published in January 2004 [3]—a manuscript that included more "Process" data such as autoradiographic data from postmortem human brain, time-activity curves from gray and white matter of AD and control subjects, and a lengthy discussion of the potential limitations of the new technology. In turn, these promising preliminary results were soon followed by a fully quantitative pharmacokinetic study using arterial blood data that included metabolite analyses in humans [4] that were complemented by metabolite analyses in rodent and postmortem human brain tissues [5]. These detailed, dynamic human PiB PET data sets clearly showed reversible binding of PiB and pinpointed when equilibrium was reached in the brain. These data were then used as the foundation for characterization of simpler, shorter scanning protocols without need for arterial lines and the use of 20-minute acquisition protocols using standardized uptake value ratios with cerebellar gray matter as the reference region [6]. An understanding was gained of the tradeoffs between the convenience of the shortened studies and the complete data sets from the longer dynamic studies. No large-scale human studies had yet been initiated at this point, but I think we had a good grasp on "which was right and which was an illusion" when it came to interpreting PiB PET data because of this careful developmental "Process." For example, we knew very early that there was substantial, nonspecific white matter retention of PiB that was equivalent in both AD and controls and that had to be excluded from the analyses of gray matter retention. We also knew there was substantial specific PiB retention in the striatum, contrary to what many believed at the time. Within a couple of years, postmortem correlative data began to appear that showed gray matter PiB retention correlated closely with Aβ load measured immunohistochemically and biochemically [7,8]. By late 2008, the FDA adopted postmortem correlation studies as the prescribed pathway to the approval of Aβ PET tracers for clinical use—a pathway that has been successfully traversed by three Aβ tracers: florbetapir (Amyvid), flutemetamol (Vizamyl), and florbetaben (Neuraceq) [9–11]. By 2010, Aβ PET imaging was being included as a secondary outcome in trials of anti-amyloid passive immunotherapy [12], a practice that has now become routine. This use of Aβ imaging has shown a (probably too) weak reduction of Aβ load by at least two immunotherapies that have failed to meet their clinical endpoints [13–15] and has shown some impressive reductions in Aβ load with aggressive immunotherapy that, at the highest dose, appeared to nearly normalize Aβ load and perhaps slow cognitive decline in prodromal and mild AD patients [16].

If you found the preceding paragraph boring at points... well, you should have. That's even without getting get into the unsuccessful, yet instructive, decade before 2002 during which we struggled to develop Aβ radiotracers based on Congo red. It was during that struggle that we developed the acceptance criteria later used to judge subsequent Aβ PET tracers based on Thioflavin-T [17]. The intent of the preceding paragraph was to show that the development of a good molecular imaging radiotracer is a long, often slow, and technically tedious "Process." I do not pretend that the "Process" described above was perfect. It was not. There were steps that were skipped. For example, no one has ever completed an in vivo blocking study in which an Aβ radiotracer is displaced by excess unlabeled compound—mainly due to the high number of Aβ binding sites and the difficulty obtaining approval for the administration of such high doses of unlabeled compound to humans and the lack of a good animal model [18,19]. The most important point of the preceding paragraph is that there was a relatively standard "Process" for the development of what most would agree ultimately turned out to be a successful group of radiotracers. That "Process" and the fundamental rules upon which it is based existed long before the idea of using PET to assess Aβ burden [2]. Although increasingly ignored, the "Process" continues to apply today and will continue to apply in the future, and discarding it will (and has) lead to more false starts and backtracking than might be necessary.

I wonder if the success of the Aβ PET radiotracers may have actually led to the "Process" being pushed aside. Once the field believed we could accurately image Aβ deposition—a belief that did not always come easily in the early days—it found the notion that we could accurately image tau burden much easier to believe. So easy, perhaps, that tau-PET tracers were rushed into relatively large-scale use. Personal experienced proved that reviewers were hungry to see tau PET included in grant proposals even before any detailed information was in the literature. It was not long until problems arose and the "Process" was remembered—at least by some. I can vividly remember one of the most experienced (i.e., "old") and respected PET scientists who was around since the beginning of molecular imaging lamenting about "what happened to the 'Process?'" at a recent Human Amyloid Imaging meeting when commenting on some of the difficulties in developing and employing tau-PET radiotracers. Could it be that too many steps of the "Process" had been skipped? And could this be leading to some of the difficulty the field has experienced in deciding "which is right and which is an illusion" in the tau-PET literature? Let us look at some examples. To be fair, although the majority of these examples focus on [F-18]AV-1451 (AKA: flortaucipir), this is only a reflection of the fact that this was the first and most widely used tau-PET tracer and is not meant to detract from

the fact that this a very functional radiotracer which we and many other groups currently employ to their advantage.

The first issue was highlighted with the lack of dynamic and arterial [F-18]AV-1451 data. This lack of data made the already adopted use of 80- to 100-minute standardized uptake value ratio data questionable when the point of equilibrium had not been clearly reached in subjects with high tau burden. This made it unclear whether the change in [F-18]AV-1451 retention was linearly related to changes in tau load—a relationship that is critical for longitudinal and therapeutic studies. This has now been mostly resolved showing the 80- to 100-minute standardized uptake value ratio data to be acceptable, but not without significant limitations [20–24].

A second issue with [F-18]AV-1451 is that of off-target binding—in the midbrain, basal ganglia, and choroid plexus, as well as to neuromelanin, melanin, and blood components—even in clinically normal elderly whose brains are not expected to harbor tau pathology outside the medial temporal lobe [25]. The off-target binding in the choroid plexus was first hypothesized in the literature in 2016 [26] but is still poorly understood [25,27]. This poor understanding led some early users of [F-18]AV-1451 to incorrectly identify the signal from the choroid plexus as coming from the nearby hippocampus [28]. Retention of [F-18]AV-1451 in the basal ganglia and midbrain complicates interpretation of the in vivo signal in non-AD tauopathies with significant tau deposition in these areas, such as progressive supranuclear palsy [29]. Much confusion still exists over the mismatch between apparent on-target in vivo retention and lack of binding in the same areas in postmortem brains; in some cases, from the same subjects [25,30]. Does the would-be off-target in vivo binding create the illusion of specific [F-18]AV-1451 retention or do the harsh, nonphysiological conditions of the autoradiography (e.g., 100% methanol) create the illusion of no binding? How do we decide which is right? We will have to stay tuned.

Another illusion was recognized at a later stage of evolution of a tau-PET tracer. [F-18]THK-5351 looked to be a promising tau-PET tracer that may have had some advantages over [F-18]AV-1451 [31–35]. While [F-18]THK-5351 does bind to tau deposits, only recently was it appreciated that a substantial portion of its in vivo retention is to monoamine oxidase-B [36]. The significant problem is that this off-target monoamine oxidase-B binding occurs in the cortical gray matter and is inseparable from on-target binding.

Other novel tau-PET tracers are being developed and debuted each year. Two recent additions include Piramal's [F-18]PI-2620 and Merck/Cerveau's [F-18]MK-6240. Both look promising in the early going (or we would not have ever seen them, of course), but little data are yet available on how well (or if) these tracers will traverse the "Process."

Again reflecting on the words of Graeme Edge [1], abandoning the "Process" can leave us wondering in the twilight zone of neuroimaging where the moon, Graeme's "cold-hearted orb," can "remove the colors from our sight" or worse yet insert colors where they should not be—especially when red is the choroid plexus and yellow is monoamine oxidase-B. Following the "Process" is critical because it is only through the careful acquisition and interpretation of data amassed through a fundamental and complete characterization of a new radiotracer that we will be able to correctly "decide which is right and which is an illusion."

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RESEARCH IN CONTEXT

1. Systematic review: The author based this perspective on his experience developing Aβ PET imaging agents. No systematic review was performed.
2. Interpretation: My interpretation of the process employed (or not employed) in the development of tau-PET imaging tracers contributes a cautionary approach to the interpretation of data from radiotracers that have not yet been fully characterized.
3. Future directions: This perspective describes a process for the evaluation of new radiotracers that has been fairly standard for decades, but has not been adequately applied to newer tau-PET tracers. Future work should address these shortcomings in order to better interpret tau-PET data.

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