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Commentary Article

Choosing a target for targeted radionuclide therapy using biomarkers to personalize treatment

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Dr. William C. Eckelman is currently the editor-in-chief of Nuclear Medicine and Biology and consultant in Targeted Probe Design and Validation. After graduating from Washington University with a Ph.D. in Chemistry, he initiated several key bench to bedside studies using both SPECT and PET radiotracers. At Brookhaven National Laboratory he and Jim Richards developed 'instant kits' which became the basis for all subsequent Tc-99m radiopharmaceutical kits now used routinely in the clinic; at George Washington University he and Dick Reba developed a muscarinic antagonist, I-123 IQNB, which in 1983 led to the first SPECT neuroreceptor image in humans. As Vice President of Diagnostics R&D in the Squibb Institute for Medical Research, he led the team that developed the strontium-82/rubidium-82 generator, Tc-99m labeled teboroxime, and ProHance, a gadolinium labeled MRI contrast agent. In addition, as Chief of the PET Department, Clinical Center/NIH, he led the research group in developing several receptor binding radiotracers including a muscarinic receptor agonist, F-18 FP-TZTP that showed promise as a biomarker for potential Alzheimer's disease in APOE4+ normal subjects. In addition, the PET Department cyclotron group developed techniques to prepare non-traditional PET radionuclides such as Tc-94m, Br-76, Ga-66, Y-86, and I-124. He achieved the rank of Professor on the faculty of the Radiology Departments at George Washington University and UCSD and served as Chair of the Scientific Advisory Committee at Molecular Insight Pharmaceuticals. These efforts have led to over 400 research papers, chapters and books.

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Commentary

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Although targeted radionuclide therapy is perhaps the greatest opportunity to affect patient care, it has not been the major focus of Nuclear Medicine research in spite of the unparalleled early success of [^{131}I]iodide in treating thyroid abnormalities [1,2]. But this seems to be changing slowly [3]. Diagnostic Nuclear Medicine, on the other hand, is still primarily dependent on those radiopharmaceuticals (radiolabeled with Tc-99m) that monitor high capacity sites, e.g., for myocardial and cerebral blood flow, glomerular filtration, phagocytosis, hepatocyte clearance, and bone adsorption for staging disease or the effect of treatment [4].

The pharmaceutical industry has a similar situation in that drugs were based primarily on phenotypic screening in the pre-genomic era and molecular targeted screening in the post-genomic era. Phenotypic screening uses a model of the disease and using high throughput screening, chooses the most effective drug in that model as the lead candidate. The molecular target does not need to be known and in fact, multiple targets and pathways may be involved. The molecular targeting approach uses a single target chosen from studies of variant genes [5]. An example of phenotypic screening in radiopharmaceuticals would be evaluating various Tc-99m chelates as ideal tracers of glomerular filtration in animal models of renal function. An example of a targeted molecular probe is radiolabeled *meta*-iodobenzyl guanidine (MIBG).

Based on the various approaches to linking genetic variants to specific diseases, it is clear that the number of genetic variants for complex diseases is most often large. For example, genome-wide association meta-analyses has confirmed the involvement of multiple variants in prostate cancer, breast cancer, diabetes and schizophrenia [6]. To further complicate the choice of a single target, there are several post-genomic alterations in the process of developing a potential drug target. The various 'omics' such as transcriptomics, epigenetics, miRNA, proteomics, phosphoproteomics and metabolomics introduce further variants to the molecule target for external imaging [7].

As a result, next-generation genomic sequencers, not nuclear imaging *in vivo*, are best for identifying and monitoring variant genes involved in a particular complex disease in order to personalize medicine for the sampled tumor [8]. Choosing a target for a drug or an imaging agent is a more difficult challenge for targeted imaging. Nuclear Imaging, especially, is limited to one or two targets given patient tolerance and absorbed radiation dose.

Why targeted radionuclide therapy for oncology?

At present, there may be applications for certain infections [9], but not in either neurology, psychiatry or cardiology. One advantage of radionuclide therapy over targeted chemotherapy is the target does not have to be a key step in the biochemical pathway of the tumor. Rather, the criteria are a highly

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expressed target that is persistent throughout the course of the disease and preferably accessible on the cell surface.

In order to detect small abnormalities, a high target density and a very high affinity constant are also important because counting statistics, spatial resolution blurring, and target-to-background ratios influence the detection of small volumes by external imaging. The input function of the unmetabolized parent radioligand to the tumor and to the critical normal organ is also a factor in high target to non-target ratios.

The first targeted radiotracers for receptors, enzymes, and transporters studied in humans were of high affinity and the targets were of high density; therefore the early biodistribution was heavily weighted by distribution. For *N*-[^{11}C]methylspiperone, the density of the D_2 receptor in the caudate putamen is high and the affinity is sub-nanomolar so the rule of thumb from *in vitro* radioimmunoassay, namely B_{max}/K_i, is consistent with the high target to non-target ratio at the risk of flow and delivery dependence at early times after injection [10]. Likewise, quinuclidinyl RS 4-[^{123}I]benzylate benefitted from high density targets among the muscarinic acetylcholine subtypes distributed throughout the gray matter and a sub-nanomolar affinity [11].

However, measuring changes in receptor density as a function of disease or treatment at early imaging times required a 'more reversible' ligand with a slightly lower affinity. So the field has been concentrating on 'reversible' ligands to achieve that goal. Now, radionuclide therapy dictates a return to a high affinity ligand targeting a stable high density surface protein.

Prostate specific membrane antigen (PSMA) as a target and radiolabeled glutamate-urea amino acid heterodimeric inhibitors of PSMA best meet these requirements at present. PSMA is upregulated during the various stages of prostate cancer [12] and has demonstrated its value as a target for both nuclear imaging and radionuclide therapy [13]. Given that it is ideal the goal to detect tumors smaller than the physical resolution of the instrument, the highest target to non-target ratio is important for both radiolabeled antagonists and inhibitors.

The affinity constants for the PSMA inhibitors are in the low nanomolar range [14,15]. There are very few studies of the PSMA density in prostate cancer. However, one such study of the receptor density reports a range of 292-4254 ng/mg protein in 5 prostate cancer samples [16]. Using 100,000 Daltons as the mass of PSMA gives 292-4254 fmol/mg protein. To obtain the protein density per mL (g) wet weight tissue, the protein density will be diluted by a factor of 6-12% protein/tissue wet weight [17,18]. Using 10%, this will dilute the protein density to 29.2-425.4 fmol/mg tissue or 29.2-425.4 nM.

This approximation indicates that the B_{max}/K_i for PSMA inhibitors has the potential of high target to non-target ratios. Another method reports that 0.5 ng (range 0.2 to 0.8 ng) is the average total protein content in two cancer cell lines. If one mg protein is recovered from 2 million cancer cells, then 292-

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4254 fmol/2 million cells will result in 146-2127 zmol (10^{-21}) per cell. If that number is multiplied by Avogadro's number, the final PSMA per cell would be 87,600-1,276,200 [19]. Both of these calculations are based on several assumptions, but the B_{max}/K_i and sites per cell are on the high end of previous radiotracer target densities and should yield high ratios *in vivo*.

Two studies are in agreement with these metrics as important in enabling detection of small abnormalities. In one patient the Ga-68 PSMA inhibitor uptake was detected in a tumor, but no uptake was detected with fluoroethylcholine [20]. Another study of 37 patients versus fluoromethylcholine yielded similar results, namely that a Ga-68 labeled inhibitor of prostate membrane antigen (PSMA) gave tumor to gluteal musculature ratios on average of 28.3 with a broad range from 2.9 up to 224, higher than fluoromethylcholine [21]. Choline, acetate, amino acids, fludeoxyglucose and fluorothymidine are among the small molecules that have low SUV values separating normal subjects and patients with prostate cancer and may be a factor in these analyses.

The first criterion for choosing a disease and a target for cancer as proposed by Divgi [3] is 'an unmet therapeutic need in a disease with a dismal prognosis'. The approved radionuclide therapies by the FDA and the EMA include iodine-131 iodide for differentiated thyroid cancer, strontium-89 chloride (Metastron) for bone pain, samarium-153 lexidronam (Quadramet) for bone pain, yttrium-90 ibritumomab tiuxetan (Zevalin®) for NHL, iodine-131 tositumomab (Bexxar) for NHL, radium-223 dichloride (Xofigo) for bone metastases, Lu-177-DOTA-octreotate (Lutathera) for NE tumors. It is interesting that [^{131}I]iodide and [^{223}Ra]radium dichloride are most often used in the clinic. It may be that the unmet need at the time of FDA approval is a key metric for clinical impact.

Given the many genetic factors involved in complicated disease and the time and radiation dose limitations on multiple studies, the most efficacious path to clinical impact of an imaging study is to develop targeted radiolabeled biomarkers/diagnostics for the pharmaceutical industry to use in pre-approval studies, primarily for target occupancy evaluation.

Another approach is to develop biomarker for treatment monitoring of a downstream effect of drug treatment using radioligands (for example, radiolabeled antibodies directed to HER2 to monitor treatment using HSP90 inhibitors [22,23]). A third possibility targets general control points, which are not a specific protein expression product of one disease, but a more general property of many diseases. Examples are radioligands correlated with glucose metabolism (fludeoxyglucose) and proliferation (fluorothymidine), angiogenesis, inflammation, apoptosis, hypoxia, pH change, and tyrosine kinases.

Over the years, the field of radiopharmaceutical sciences has made major technical advances with the Tc-99m generator, instant one-step kits, chelate chemistry given the many oxidation states of Tc, biomedical cyclotrons with sufficient energy and beam current to produce large amounts of radionuclide, cyclotron targets that could withstand the operating conditions, synthetic approaches to incorporating short-lived radionuclides into radiopharmaceuticals and approaches to developing solid

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target technology for longer-lived metallic radionuclides for radiolabeling peptides and antibodies. These were facilitated by the incorporation of the rigor of radiochemistry, organic chemistry, inorganic chemistry, pharmacy and the approaches to validating targeted ligands *in vitro* in use in radioimmunoassay.

A paradigm shift from predominately technical advances requires radioligands designed to have a significant impact on the present standard of care [24]. This shift is especially challenging in diagnosis or staging in neurology, psychiatry, and cardiology and not without challenges in oncology and infectious diseases. A validated single scan approach for diagnostics is critical to the success of Nuclear Medicine [25]. Choosing a high affinity target for radionuclide therapy that is highly expressed through the disease stages is likewise a challenge.

In this paradigm shift, investigators need a clear understanding of whether the goal is monitoring the change in target as a function of disease and treatment or if the goal is to detect as many abnormalities as possible as a function of disease or treatment. With the goal in mind, choosing a target for radionuclide therapy using biomarkers/diagnostics to personalize treatment will increase the impact of this field of targeted Nuclear Medicine.

Conflict of interest

The author has no conflicts of interest.

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Key Article References: 3, 4, 5, 13, 14, 15, 20, 21, 24 & 25

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