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INTRODUCTION

There have been many applications of the peaceful use of the atom. Probably none have been as effective as the radiopharmaceuticals developed to better understand human biology and to improve patient care through diagnosis or radiotherapy [1]. The latter goal is the focus of this review using parallel approaches in the pharmaceutical industry as a reference point for the development of new radiopharmaceuticals. In this review, we emphasize translational radiopharmaceutical development with the goal of translating radiopharmaceutical to human imaging with the goal of impact patient health. This goal does not preclude the development of imaging agents with purely scientific goals; however, the emphasis of this review is on the compounds most likely to have an impact on patient treatment.

Several Nuclear Medicine societies and journals have changed their names recently by adding Molecular Imaging to their previous titles [2]. But molecular imaging, which is synonymous with targeted imaging, has been a major part of radiopharmaceutical development from the 70s, dictated by the nature of external imaging. External radiotracer imaging is perhaps best described as a method of converting photon emissions (whether emission from positron annihilation or single photon emission) to biological metrics. This can best be achieved when the radioligand interacts with a single receptor or enzymatic protein and is either bound or is metabolically trapped as is [^{18}F]fludeoxyglucose as the 6-phosphate. There are several examples of targeted radioligands that when studied in humans yielded biological information some of which went forward to gain human use approval (Table 1). By the nature of external imaging, monitoring a single biochemical target, the so-called molecular target screening approach, compared to the phenotypic screening approach, which involves several biochemical entities, is an important distinction [3]. The single target is the end goal and does not preclude other biochemical steps, but does not lead to more than one final target. Radioligands that measure myocardial or cerebral flow and clearance by renal or hepatic pathways can be considered targeted molecules but with a high capacity target [4].

THE PHARMACEUTICAL COMPANIES APPROACH TO IDENTIFYING AND DEVELOPING NEW DRUGS

Ironically, in the pregenomic era pharmaceutical companies (pharma) identified new drugs using the phenotypic screening approach and did not emphasize the molecular target screening approach until the genomic era. The difference between the two lies in the approach to identifying the most effective drug (Fig 1). Phenotypic screening uses a model of the disease and, using high throughput screening, chooses the most effective drug in that disease model. In the approach based on phenotype screening, the molecular target does not need to be known and, in fact, multiple targets and pathways may be involved [3].

By distinction, molecular target screening uses a single target chosen from studies of variant genes followed by progressive testing in cells and disease models. Pharma have developed a number of single target drugs, but companies are returning to the phenotypic screening approach given the decrease in new chemical entities being approved for routine clinical care. In the period 1999 to 2008, 75 were first-in-class drugs divided into 50 small molecules and 25 biologics. More were developed using phenotypic screening (28) than by molecular target screening (17) [5]. In addition, some single-targeted drugs that appeared highly effective in pre-clinical studies did not fair well in clinical trials, due to resistance mechanisms that emerged quickly, typically by disease processes that bypassed the specific single target of these highly targeted drugs. For example, in the 1990's and early 2000's, excitement regarding cancer drugs targeted to a single receptor tyrosine kinase (RTK), gave way to a return to cancer drugs with several RTK targets, many of which continue have has significant role in recent cancer care – for example sunitinib [6] and sorafenib [7]. Once called “dirty kinases”, these drug were re-marketed as “multi-kinase drugs” and form an important component of the current compendium of cancer drugs [8]. The complexity of drug targeting

for these “multi-kinase drugs” demands, perhaps even more so than for targeted agents, methods that can provide a robust readout of downstream efficacy representative of therapeutic response or resistance.

THE DEVELOPMENT OF THE TARGETED MOLECULE CONCEPT

Paul Ehrlich coined the expression ‘magic bullet’ at the beginning of the 20 century [9],[10]. The German word ‘Zauberkegel’ appeared earlier in his publications, on the basis of his view of ‘side chains’, the precursor of the concept of receptors, and on the desirable properties of drugs that do not harm the host but do attack the parasitic invader. Ehrlich’s first magic bullet, arsphenamine (Salvarsan), was discovered in 1909 and provided the best cure for syphilis at that time. Obviously, arsphenamine was developed without the benefit of knowing the target or being able to image a target in vivo. In the nomenclature introduced above, this would be considered phenotypic screening (Fig 2). Ehrlich also thought of attaching toxins to antibodies whereby the antibody would carry the deadly freight to the site of the invading parasite, but not to normal tissue. Depending on the specificity of the antibody, this could be characterized as either phenotypic screening or molecular target screening.

THE PHENOTYPIC SCREENING APPROACH IS NOT USED IN RADIOPHARMACEUTICAL SCIENCE.

Phenotypic screening is not an approach that can be used in radiopharmaceutical development. In fact, radiopharmaceuticals have always been used to image and obtain biological information on a single low capacity/low density site. The exceptions are the Tc-99m radiopharmaceuticals used in clinical Nuclear Medicine for myocardial or cerebral blood

flow, glomerular filtration, phagocytosis, and hepatocyte clearance. These are often characterized as higher capacity processes although the mass of the radiotracer injected is still relatively small compared to the mass used in drug treatment [11]. These categories are not discrete, but rather a continuum. For example, high capacity sites such as beta-amyloid (A β) deposits ($B_{\max}$ 1000–2000 nM) requires a K_d of 2 nM to obtain a Binding Potential of ~2 by external imaging [12]. The ratio $B_{\max} / K_d$ is best used to eliminate potential radiolabeled antagonists, enzyme inhibitors, and transporter inhibitors before beginning preclinical studies *in vivo* since it is taken from *in vitro* studies to predict the maximum bound to free ratio. *In vitro* measurements involve very low nonspecific binding (because it can be subtracted) and are conducted under true equilibrium conditions in the absence of confounding factors such as metabolism, blood flow, and permeability. Therefore, these ratios provide best-condition values, which have never been achieved *in vivo*. Although exact ratios that will lead to a successful radioligand that can be imaged are difficult to assign, maximal ratio of >25 would warrant further investigation. The case of radioligands for beta-amyloid deposits is a clear exception to the rule.

A recent example of a drug developed using phenotypic screening is proposed for treatment of behavioral disturbances associated with dementia. Although the chemical structure was not identified by Intra-Cellular Therapies, Inc. in the clinicaltrials.gov protocol, the drug (ITI-007-200) “combines potent serotonin 5-HT_{2A} receptor antagonism, dopamine receptor phosphoprotein modulation (DPPM), glutamatergic modulation and serotonin reuptake inhibition into a single drug candidate. At dopamine D₂ receptors, ITI-007 has been demonstrated to have dual properties and to act as both a post-synaptic antagonist and a pre-synaptic partial agonist. ITI-007 has also been demonstrated to stimulate phosphorylation of glutamatergic NMDA NR2B, or GluN2B, receptors in a mesolimbic specific manner”. Given the multiple interactions of this drug, it is unlikely that patient outcome would be affected by imaging the radiolabeled drug itself; however, imaging drugs in existence of development that image the many targets of the drug could be useful in early testing, especially for

proof-mechanism and perhaps inferring the importance of the differing targets in producing a clinical outcome.

In summary, if the molecular target is not known and/or if multiple targets and pathways are involved (usually a result of phenotypic screening), it is difficult if not impossible to analyze the resulting changes in biology by converting photon emissions to biological metrics using external nuclear imaging for a single target, or limited combination of targets. On the other hand, multiple scans for many different single targets usually is limited by patient tolerance and absorbed radiation dose.

CHOOSING A DRUG FOR AN UNMET CLINICAL NEED

Choosing a drug for a disease that has an unmet need was based on autopsy data, linkage information, and effective natural products in the pregenomic era [13]. In the post genomic era, completion of the human genome sequence identified variant genes that underlie complex disease. Our understanding of quantitative traits based on as few as 3 to 5 genes is minimal. For complex diseases such as diabetes, breast cancer, prostate cancer, and schizophrenia on the order of 40 or more variant genes play a major role [14],[15],[16]. Microarray analysis of a subset of genes is best for identifying variant genes involved in a particular complex disease [17]. The various “omics” such as transcriptomics, epigenetics, miRNA proteomics, phosphoproteomics, and metabolomics introduce further perturbations to the potential protein targeted for external imaging [18]. These considerations support the need for in vivo characterization of disease-related cell surface proteins, a strength of radiotracer imaging. Proteomics is developing to the point of identifying leads for nuclear imaging [19].

WHAT TARGETS ARE MOST OFTEN STUDIED?

There are few analyses in the literature that list the proposed targets by the number of studies. One such listing, although obviously incomplete, is the Molecular Imaging and Contrast Agent Database (MICAD), which identified ~5,000 publications that contained a new probe with accompanying biological data [20]. Before the program was terminated by the National Institutes of Health, 1400 chapters were analyzed identifying a series of targets with the integrin receptors and epidermal growth factor receptor as the most studied (Fig 2). The question for the radiopharmaceutical scientists is whether a potential target is worth pursuing given the number of previous publications, the extent of validation of the target, and the potential effect on patient outcome.

SHIFTING EMPHASIS IN RADIOPHARMACEUTICAL SCIENCES

Should radiopharmaceutical scientists change their emphasis in radiopharmaceutical research and development? Radiopharmaceutical scientists have made tremendous progress over the years in developing the technical aspects required in the development of new targeted radioligands. The development required diverse expertise including radiochemists, inorganic chemists, organic chemists, accelerator and reactor physicists, and materials scientists. The ^{99m}Tc generator and instant, one-step kits for clinical studies were available early on for development of new ^{99m}Tc radiotracers [21]. The development of the biomedical cyclotron, targetry for high beam currents, and automated radiochemistry for short-lived radiotracer synthesis was key to PET radiopharmaceutical development as evidenced by the change in major technical topics from year to year at major meetings such as the International Symposium of Radiopharmaceutical Sciences [22]. But a new paradigm is needed where the

choice of the target becomes paramount and the technical development of the ideal radiotracer is secondary. For example, how many of the targeted radiotracers in Figure 2 have a high probability of affecting clinical care as the primary goal? As the pharmaceutical industry has discovered, it is a low success rate, but never the less the choice of a radioligand that will affect patient care is still an important design criteria. In 1981, an editorial was published that spoke of the low success rate of discovery in pharma (1 in 3000) and the achievements of the group at the University of Michigan who through a long range synthetic chemistry commitment and the combined effort of an interdisciplinary team produced [^{123}I]metaiodobenzylguanidine. Besides the early identification of [^{131}I]iodide, this was one of the first targeted radiotracers developed (Fig 3).

As it is in pharma, unmet clinical need should be the primary goal. This requires identifying the potential effect on patient outcome using the present standard of care (SOC) or emerging treatment paradigms for the disease with physicians treating patients with the disease. In addition to clinical need one needs to consider new radiopharmaceuticals in the context of other current imaging procedures, the existence and use of ex vivo diagnostic tests (blood and urine assays, biopsy) that will lessen the impact of- and need for- a new radiopharmaceutical.

Given the many genetic factors involved in complicated disease and the patient's time commitment and radiation dose limitations that restrict multiple external imaging studies, the choice of a target must be limited, and directed by clinical and biologic considerations. Since the investments of pharmaceutical companies in identifying potential unmet needs in patient care and the target that has potential to impact patient care, radiopharmaceutical diagnostic imaging development may be most efficient following their lead. These include biomarkers or

theranostics using true tracers of **drugs presently in clinical studies** radiolabeled with ^{11}C and ^{18}F (if the drug contains a fluorine atom). A second method is to monitor by external imaging a downstream effect of a drug. For example, drugs that inhibit HSP90 disrupt the expression of HER2 on the cell surface, which can be monitored using radiolabeled antibodies or fragments to HER2 [23],[24]. Another approach is developing diagnostics or biomarkers that target general disease control points (Table 2).

It is interesting to note that detailed guidelines have not been issued by the US FDA to date for biomarkers. Swanson has proposed guidelines for imaging biomarkers as opposed to diagnostic imaging. He proposes the establishment of regulatory criteria and related industry guidance for FDA approval for commercial marketing of radiotracers for strictly biomarker applications. To differentiate the indications, the FDA would permit the clinical use of radiotracers, which have been FDA-approved for biomarker applications, subject to labeling statements that preclude the use of the radiotracer for the independent diagnosis of a disease or condition [25].

Besides an emphasis on diagnostics or biomarkers based on pharma research, further emphasis should be given to targeted radiopharmaceutical therapy, which is unique to Nuclear Medicine. This is a concept that is being revisited as, for example, in an editorial by Divgi, who presented several design criteria [26]. His proposal outlines various key properties:

- o - *"the presence of a potential unmet therapeutic need in a disease with a dismal prognosis.*
- o - *a need for a companion diagnostic imaging biomarker to appropriately select patients for therapy and **monitoring therapeutic drugs** (Rx).*
- o - *the ability to quantify radiation-absorbed dose to the tumor (or normal tissue).*

- o - *the ability to detect all or a certain percentage of tumors*".

How does this shift the technical design goals of the radiopharmaceutical chemists, if the goal of diagnostic radiopharmaceuticals is shifting from determining a change in receptor density as a function of disease and treatment to determining the total number of sites of disease? This requirement is best applied to oncology, but could be equally applied to infection and inflammation. With that as the goal, then targeting small objects by external imaging is paramount. Counting statistics, spatial resolution blurring, and the target to-background ratio influence the detection of small volumes by external imaging. Here PET with its increase resolution would have an advantage. But instrument resolution is not the only factor. For example, endogenous ligands and their radiolabeled analogs and exogenous ligands used by mammalian cells, such as glucose, and their radiolabeled analogs often have standard uptake values (SUV) of 5 or lower for the cut-off between normal and abnormal lesions, making it a challenge to detect smaller volumes as a function of treatment [4]. Note that several radiolabeled "poisons [27]" such as ^{68}Ga labeled radiotracer for PSMA has a much higher SUV and target to non-target ratio than ^{18}F fluoromethylcholine, which led to higher detection of abnormalities by the PSMA targeted radioligand [28].

"Back to the Future" may be the theme for the ideal properties of a radiotracer as characterized by the earliest radiotracers (Table 1). Namely N-methyl spiperone (^{11}C N-MeSpip) and quinuclidinyl 4-iodobenzilate (^{123}I IQNB), had high affinities for their respective receptors and therefore the biodistribution was heavily weighted by delivery rather than receptor density [29]. However, they resulted in high SUVs and target to non-target ratios. In the following radiotracers (Fig 1) a lower affinity was most often chosen in order for the biodistribution to be more weighted by the target density than to the delivery. However, if the

goal is to find a maximal number of abnormalities, as high an affinity as possible is needed to counter the decreasing average target density in an imaging pixel. If detection (Dx) and destruction (Rx) are the goals, so intermediate affinities that increase identifiability of the target density are no longer a goal. The concept is that maximum target density averaged over an imaging pixel divided by the highest affinity ($B_{\max}/K_d$) will give the optimal SUV target to non-target ratio.

A new paradigm should be considered. One that reverses the technical development and the clinical application, putting the clinical application as the first design criteria as follows:

- Identifying an unmet clinical need and the “potential” to improve significantly patient outcome: This requires knowledge of the **SOC and emerging treatment paradigms** for the disease, especially the **current SOC imaging procedures and the current SOC** ex vivo tests (blood, urine, biopsy) or consultation with a physician treating the target disease.
- Technically, a high affinity and high $B_{\max}$ ($B_{\max}/K_d$), **which is related to BP**.
- These can be considered as necessary but by no means sufficient, but should increase the probability of clinical impact.

AIMS ANDSCOPE FOR NUCLEAR MEDICINE AND BIOLOGY

Nucl Med Biol has further defined the AIMS and SCOPE, which is published in each issue.

Realizing the challenge of assessing the potential clinical utility and impact of a novel radiopharmaceutical, we offer some thoughts and guidance. In general, diagnostic radiopharmaceuticals should meet one of the following broad clinical needs [30],[31], considered in the context of a general approach to biomarkers [32]. **We realize that no single**

diagnostic test alone detects disease or determines treatment, and therefore our goals emphasize roles that imaging agents may have in the context of overall patient care and disease management.

1. Disease detection, diagnosis, and determination of extent (staging) –

Radiopharmaceuticals can exploit the in vivo phenotype* of disease processes to identify diseases on the basis of their ability to retain radiopharmaceuticals at levels higher than normal tissue.

2. Disease characterization – Radiopharmaceutical imaging provides quantitative information on disease phenotypic features. This can be complementary to tissue assay in the imaging can characterize the entire disease burden, including disease heterogeneity, in a way that tissue sampling cannot. This includes the growing concept of companion diagnostics, where imaging can help determine the expression of targets matched to highly specific therapy.

** phenotype is used here as the cellular expression of the altered DNA; previously it was used to describe a particular method of screening for new drugs.*

3. Measuring therapeutic response versus disease progression – Appropriately targeted radiopharmaceuticals can provide a robust and early readout of therapeutic efficacy and importantly, determine when the disease has not responded to treatment.

4. A focus of radiopharmaceutical imaging related to radionuclide therapy, where a radiolabeled imaging agent matched to the therapeutic radiopharmaceutical is key to determining the targeting and/or radiation dosimetry of the targeted agent.

Other factors that will individually and collectively help determine the need for and likely impact of a new radiopharmaceutical:

1. Agents targeted to processes found in a range of diseases that can impact a large number of patients: Example includes aberrant glycolysis ($[^{18}\text{F}]\text{FDG}$), which is seen a number of diseases, including cancer, heart disease, neurologic disease, inflammation, and infection, among others. Another example is bone turnover ($^{99\text{m}}\text{Tc}$ -diphosphonates, $[^{18}\text{F}]\text{fluoride}$), common to a large number of skeletal disorders including cancer, trauma, infection, and metabolic disorders. Given the ubiquity of these processes, it is no accident that the tracers targeted to them are among the most commonly used agents in current nuclear medicine practice.

2. Specific targets in common diseases: The impact of radiopharmaceuticals highly specifically targeted to a limited number of diseases, may still have a significant clinical impact if those disease are common. Examples include agents for detecting abnormal proteins deposited in neurodegenerative disease, such as amyloid, and the use of radioiodine and analogs for thyroid disorders. Other emerging agents include targeted agents for prostate cancer (PSMA agents, e.g.) and breast cancer (estrogen receptor targeted agents, e.g.).

3. Specific targets for uncommon diseases, where there are no suitable alternatives: Examples include the use of somatostatin receptor imaging for neuroendocrine tumors. Although these tumors are rare, radiotracer imaging is a key component of care for almost all patients with advanced disease, making somatostatin imaging a modest component of most nuclear medicine practices. Another example is the use of $[^{131}\text{I}]\text{iodide}$ to treat thyroid cancer. Though the disease is rare, radioiodine for thyroid cancer remains a key component of therapy and is an important part of nuclear medicine practice [33].

4. Situations where the use of a radioactive imaging agent offers significant advantages over competing methods: In some cases, including some applications of

less targeted radiopharmaceuticals, radiotracer methods may offer an advantage over other competing imaging methods. One example is myocardial perfusion imaging, where the high contrast signal arising with the use of a trapped nuclear perfusion agent (e.g., [^{99m}Tc]sestamibi, $^{82}\text{RbCl}$) offers some advantages over competing modalities such as ultrasound, CT, and MRI, underlying the long-term viability of nuclear cardiology procedures. Another example is the use of [^{99m}Tc]sulfur colloid for lymphatic mapping, where high contrast and the ability of radioactive signals to direct surgical approaches in the operating room have made nuclear imaging a key component of lymphatic mapping procedures, and have spurred the development of agents more specifically targeted to lymph nodes [34].

We offer these thoughts not as an exhaustive list of clinical applications of new radiopharmaceuticals, but rather as a guide to considering the potential clinical impact of new agents as part of the Aims and Scope of NMB.

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Table Captions

Table 1. List of targeted radiopharmaceuticals from 1939 to 1985 that have been studied in humans by external imaging. Those with an asterisk have FDA approval.

Table 2. List of possible radiotracer opportunities in the class of general disease control points.

Figure Captions

Figure 1. Definition of the differences between phenotypic screening and molecular target screening. Taken from Zheng et al. Ref [3].

Figure 2. Molecular Imaging and Contrast Agent Database (MICAD) analyzed by target for the first 1400 chapters with the integrin receptors and epidermal growth factor receptor as the most studied. Y axis = number of chapters.

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Figure 1

Phenotypic approach = The molecular target does not need to be known and, in fact, multiple targets and pathways may be involved.

(b) Phenotypic screening approach:

Molecular targeting approach = target identification relevant to a disease of interest. For the drug industry, the number of targets has increased in the post-genomic era.

(a) Molecular target screening approach:

Figure 2

Table 1

Date	PET	SPECT
1939		[¹³¹I]iodide*
1977	[¹⁸F]FDG*	
1981		[^{123/131}I]MIBG*
1983	[¹¹C]N-MeSpip	[¹²³I]IQNB
1983	[¹⁸F]FDOPA*	
1984	[¹⁸F]cyclofoxy	
1985	[¹¹C]Raclopride	[^{99m}Tc]NGA*
1985	[¹¹C]Carfentanil	
1985	[¹¹C]Flumazenil	
>1985	Many	Many

Possibilities Include:

'Glucose' Metabolism

'Proliferation'

Angiogenesis

Inflammation

Apoptosis

P-gp

Hypoxia

pH change

lymph node mapping

tyrosine kinases?