Discovery, Characterization, and Manufacture of Peptide Radiopharmaceuticals: Webinar FAQs

1. **Can you expand a little on the appropriate choice of hit finding strategies for different target classes?**

For traditional molecular target classes, such as membrane receptors, transporters, enzymes and ion channels, selection of a peptide-based hit-finding platform should take several points into consideration, such as those listed below.

Both DNA Encoded Library (DEL) and Phage Display (alternatively mRNA-display) hit-finding platforms are well adapted for:

1) any protein which can be stably expressed as a fusion protein, with affinity tag(s), for in vitro immobilization and affinity-based selections
2) membrane-integrated receptor proteins such as GPCRs, which can be engineered as stable nanodisc forms, or functional extra-cellular domains (ECDs)

Phage Display platform works better for cell-based selections which are especially useful for:

1) Membrane-integrated receptor proteins that are stably overexpressed on cells with structural integrity consistent with function

Phage Display platform works better for in vivo selection:

1) Tissue- / organ-specific target ligand discovery
2) Tumor-specific target ligand discovery
3) Target validation

The availability of high-resolution structures of the molecular target raises the possibility of using Computer Assisted Drug Design (CADD) principles for structure-based or ligand-based compound design, or for using virtual screening methods to identify chemical matter that binds the target.

Consideration should also be given to whether the desired chemical matter needs to be (orally)bioavailable and/or passively permeable to cell membranes. If so, smaller 'unnatural' peptide-based macrocycles or constrained structures, discovered by a platform such as DEL, are likely a better choice than larger, linear peptide-based ligands.

2. **How many rounds of peptide design optimization, synthesis and testing might be required to arrive at a tractable therapeutic?**

Two, three or more peptide design-make-test-analyze (DMTA) iterative cycles are often required to optimize for the following:

1) In vitro affinity, functional potency and ADME optimization
2) In vivo stability, potency and DMPK optimization

Target compound profiles requiring passive membrane permeability, (oral)bioavailability, specific clearance goals, etc., typically require a greater number of DMTA cycles

3. **Which radioisotopes are most favored for radiopharmaceuticals?**

Diagnostic radioisotopes such as F18, Ga68, In111, Tc-99m, and therapeutic radioisotopes such as Lu177, I131, Y90 are widely used in the clinic; some of these agents have been approved by the FDA. Additionally, Cu64 and Zr89 are widely used in research settings. Alpha particle-emitters such as Ac225 and Pb212 are currently common in therapeutic settings, with some agents generating strong data in clinical trials.

4. **Besides cost, what advantages do peptides have over antibodies in this space?**

Both antibody and peptides possess high affinity and target specificity. Some advantages of peptides over antibodies in radiopharmaceutical space are:

1) Faster pharmacokinetics, especially suitable for diagnostic imaging
2) Almost no immunogenicity, compared to antibodies
3) Better cell permeability for cellular uptake, internalization and tumor penetration

Antibodies, being large proteins having Molecular Weight (MW) ~ 150 kDa, cannot pass through intact cellular or subcellular membranes in living cells, which leads to incomplete tumor penetration and inferior intra-tumoral distribution. The engineered smaller antibody fragments have improved rate of tumor uptake, however, they are often accompanied by more rapid clearance with less-optimal tumor accumulation, limiting renal dose and cross-reactivity. Peptides (also Beyond Rule of Five small molecules and antibody fragments) are typically MW ~ 2 kDa, in contrast. Peptide-based systems are often internalized into cells whereas antibody- (and Ab fragment-based) radioligands may internalize or remain on the cell surface, inducing a so-called cross-fire effect.

5. **How can different peptide structures affect radiochemical purity? (We have seen a lot of degradation in peptides due to radiolysis).**

Radiolysis is the dissociation of molecules by ionizing radiation, which can lead to the formation of radicals and degradation of peptide. Peptide and peptide-chelator conjugate stability is essential. There are several approaches to identify and increase radiochemical purity by minimizing radiolysis:

1) It is generally understood that amino acids with aromatic rings, such as phenylalanine, tyrosine, and tryptophan, are most susceptible to radiation; additionally, amino acids with sulfur-containing side chains, such as cysteine and methionine, may also be more sensitive to radiolysis
2) Radiolysis can be a major factor for peptides in solution with high radioactivity concentrations
3) Optimization of radiolabeling conditions to minimize the formation of radicals using lower labeling temperature or radioprotectants or lower radioactivity concentration will also help
4) Implementation of multiple QC methods such as radio-TLC and HPLC analysis to identify radiolysis soft spots for further structural modification
5) Generally, structural modifications for greater thermo- and acidic stability of peptide is key for tolerance of radiolabeling procedures

Ideally, isotopes and chelators will not otherwise bind to the portion of the peptide that binds the molecular target.

6. **Do you think multicyclic peptides will be the trend for future directions?**

Multicyclic peptides with structurally defined, rigid (constrained) scaffolds possess high stability, excellent affinity and selectivity; typically, the higher the degree of constraint (multiple rings), the higher these parameters rise. Platforms that prepare and screen compounds that already contain these features provide an advantage over platforms that identify linear or monocyclic peptide-based binders, that then typically need to undergo substantial optimization to build-in these constraints and stabilizations. Multicyclic peptides allow simultaneous presentation of several different binding loops to achieve superior target specificity and/or dual or higher dimensional targeting. Disulfide-directed multicyclic peptide libraries and trivalent scaffold peptide libraries have been developed in both academic and industrial settings and utilized in phage display selection techniques successfully.

7. **What is the capacity of C14, I125, or F, or H4 radioisotope incorporation?**

C14 and H3 with a longer half-life are widely used in ADME and Quantitative Whole Body Autoradiography (QWBA) studies. I125 labeled compounds can be used in bio-distribution studies. F18 is widely used for PET-CT imaging, which has better sensitivity and spatial resolution compared to SPECT. Approximately nine 18F-related radiopharmaceuticals have been approved by the FDA, thus far.

Compounds labeled with radioisotopes are prepared in microgram or milligram scales. The injected dose of radiopharmaceuticals containing ‘cold molecule’ is micro scale.

8. **How important is peptide thermostability for the radiochemical labeling step?**

For some macrocyclic chelators, the energies of activation for chelation of metal ions are significantly higher than those for acyclic chelators. The temperature for radiolabeling can be as high as 90 degrees C. Hence, the thermostability of peptides is very important. For some macrocyclic chelators, the pKa values for deprotonation of the coordinating donor atoms are also substantially lower than those for acyclic chelators, which requires acidic pH; hence, the thermostability under acidic pH is also important in this case.

9. **How dramatic is the change from a simple peptide to a peptide-chelator conjugate (regarding binding affinity and physicochemical properties)?**

Peptide affinity can be changed once it is connected by linker to the chelator. Usually, the linker will play a key role in order to maintain the affinity of peptide. Although most chelators are amino acid-based moieties, the physicochemical properties will also change due to incorporation of multiple lipophilic carbon chains in the chelators. When it is not ideal to modify chelators, pharmacokinetic-modifying linkers can be designed to improve the “target-to-background” ratio.