Overview/abstract

Introduction: The success of directly-labeled, monoclonal antibody (mAb) radiopharmaceuticals has been hindered by the pharmacokinetics (PK) of mAbs that lead to dose-limiting irradiation of marrow cells. Pretargeting resolves this issue by decoupling the delivery of the mAb from the radionuclide to make mAb-based radiotheranostics safe and effective. We are developing a host:guest pretargeting platform, utilizing cucurbit[7]uril (CB7) "hosts" and adamantane (Adma) radioligand "guests," that is the first to meet the critical prerequisites for effective, translatable pretargeting, including: 1) being nonimmunogenic, 2) being modular and tunable to any mAb and radionuclide, and 3) allowing for extended "lag times" that negate the need for clearing agents (**Fig. 1**). There remains a *critical need* for mAb-based radiotheranostics that are safe and effective, and our platform can be established to meet that need using elementally matched isotopes of Pb.

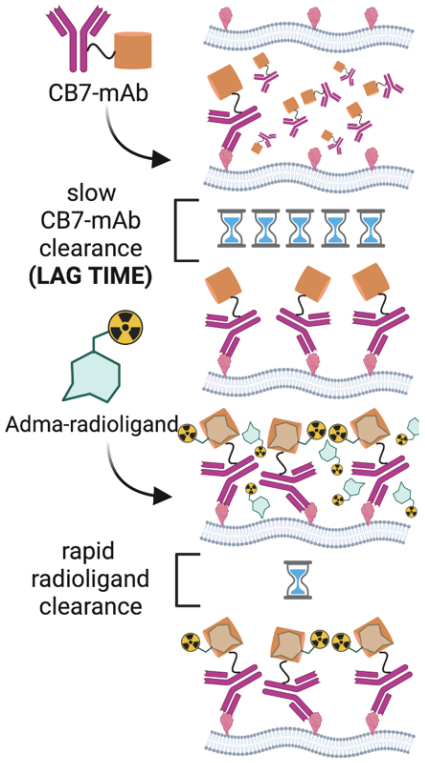

Figure 1. CB7-Adma pretargeting.

Our long-term goal is to develop an effective, safe, and translatable alternative to conventional mAb-based theranostics using CB7-Adma pretargeting for receptor-targeted single-photon emission computed tomography (SPECT) and α-particle targeted radionuclide therapy (α-TRT). The *overall objective* of this proposal is to develop CB7:Adma pretargeting with the elementally matched isotope pair ^{203}Pb and ^{212}Pb, identifying a lead radioligand, optimizing our pretargeting approach, and assessing the dosimetric advantages of that approach relative to a direct-labeling strategy. By doing so, we will set the stage for future IND enabling studies. Our *central hypothesis* is that the combination of therapeutic, high linear-energy transfer emissions from ^{212}Pb, convenience of elementally matched ^{203}Pb for treatment planning via SPECT, and improved safety of pretargeting can be combined to provide an ideal alternative to directly-labeled mAb radiopharmaceuticals. Our *rationale* is based on preliminary studies showing that CB7:Adma pretargeting achieves high tumor:non-target uptake ratios and an astonishing reduction in radiation dose to marrow as well as the established effectiveness of ^{212}Pb for α-TRT and ^{203}Pb for assessing its PK, biodistribution, and dosimetry. We will pursue the following specific aims.

Specific Aim 1 (SA1): Development and evaluation of $^{203/212}$Pb-labeled radioligands for pretargeting.

In *SA1* our goal is to develop a lead radioligand for developing our platform. First, we will prepare a library of ^{203}Pb-labeled, Adma-bearing "guest" radioligands that incorporate one of four chelators that are appropriate for Pb isotopes, including: DOTA, DO3A, PSC, and TCMC. Then, we will characterize ^{203}Pb-labeled radioligands' pharmacological profiles by assessing their Log D, serum stability, *in vivo* blood half-life, and CB7 binding affinity. The *in vitro* and *in vivo* stability of the ^{212}Pb-labeled radioligands will be similarly assessed. Finally, we will perform pilot pretargeted SPECT imaging and biodistribution studies with a carcinoembryonic antigen (CEA) targeting immunoconjugate (CB7-M5A) and the ^{203}Pb-labeled radioligands in BxPC3 xenograft bearing mice. We will select the radioligand with the best pharmacological profile and pretargeting performance as our lead radioligand.

Specific Aim 2 (SA2): Optimization and dosimetry of $^{203/212}$Pb host:guest pretargeting.

In Specific Aim 2, we will us the ^{203}Pb-labeled radioligand to iteratively optimize the pretargeting variables, including the lag time, injected radioligand:CB7-M5A stoichiometry, and injected mass of CB7-M5A via *in vivo* SPECT and biodistribution in the same xenograft model to determine the optimal pretargeting parameters with our Pb-labeled radioligands. With the optimal *in vivo* approach established, we will perform serial biodistribution studies and SPECT imaging with the best pretargeting parameters as well as with directly-labeled ^{203}Pb-M5A. The data will be used to determine the dosimetry and therapeutic index for both approaches to validate the dosimetric advantages of pretargeting. These studies will provide the preliminary data for more extensive studies investigating the imaging and therapeutic applications with the elementally matched Pb isotopes.

Expected outcomes: We will take the first steps toward developing our pretargeting platform for SPECT to be used as a probe to support theranostic α-TRT that yields high therapeutic efficacy and reduced radiation toxicity. These studies will lay the groundwork for more extensive studies investigating theranostic applications of our host:guest pretargeting platform in a wide array of diseases as well as IND enabling studies. *The data acquired will be used to support multiple applications for internal funding via the NIH (R21 and R01), DoD (Impact Award), and American Cancer Society (Research Scholar Grant) during the award period.*