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Review

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Advancing Novel Molecular Imaging Agents from Preclinical Studies to First-in-Humans Phase I Clinical Trials in Academia—A Roadmap for Overcoming Perceived Barriers

Raymond M. Reilly,*[,†,‡,§,∥] Karen Lam,[†] Conrad Chan,[†] and Mark Levine[⊥]

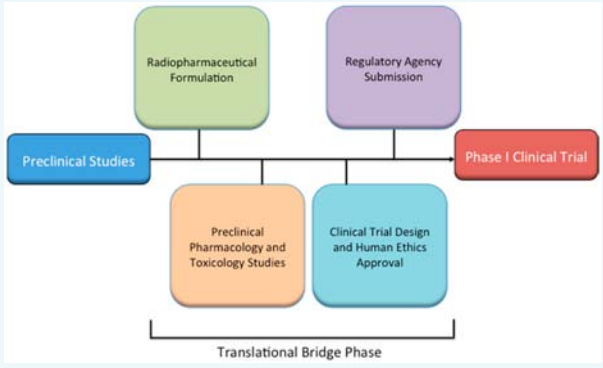
[†]Department of Pharmaceutical Sciences, Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, Ontario, Canada M5S 3M2

[‡]Department of Medical Imaging, University of Toronto, Toronto, Ontario, Canada M5T 1W7

[§]Toronto General Research Institute and [∥]Joint Department of Medical Imaging, University Health Network, Toronto, Ontario, Canada M5G 2M9

[⊥]Department of Oncology, Juravinski Cancer Centre, McMaster University, Hamilton, Ontario, Canada L8S 4L8

ABSTRACT: There is a critical need to advance promising novel molecular imaging (MI) agents for cancer from preclinical studies to first-in-humans Phase I clinical trials in order to realize their full potential for cancer detection and for predicting or monitoring response to targeted ("personalized") cancer therapies. Steps to clinical translation include radiopharmaceutical formulation, preclinical pharmacology and toxicology studies, clinical trial design and human ethics approval, and regulatory agency submission. In this Topical Review, we provide a "roadmap" to advancing one class of novel MI agents to Phase I trials in academia and illustrate the processes that we have successfully applied for ^{111}In-labeled pertuzumab, a MI probe for monitoring response of HER2-positive breast cancer to treatment with trastuzumab (Herceptin). We hope that our experience will encourage other academic radiopharmaceutical scientists to embrace this challenge.



INTRODUCTION

Molecular imaging (MI), especially single photon emission computed tomography (SPECT) and positron emission tomography (PET), is a powerful tool for detecting cancer and for characterizing the biological properties of tumors. Revealing tumor biology in an individual patient could aid in the optimal selection of molecularly targeted (i.e., "personalized") cancer therapies.[1] MI can also trace the delivery of anticancer agents to tumors[2] and probe their mechanisms of action which may predict tumor response.[3,4] Furthermore, the downstream effects of treatment on tumor viability can be probed by MI which reports on the effectiveness of treatment.[5,6] Thus, there has been rapid growth in radiopharmaceutical research aimed at the discovery of novel MI agents for cancer. A Pubmed (http://www.ncbi.nlm.nih.gov/pubmed) search combining the terms "molecular imaging" and "SPECT" and "cancer" yielded almost 1000 publications. Interrogating the terms "molecular imaging" and "PET" and "cancer" yielded an additional 2500 publications. However, within these "hits", selecting the article type as "Clinical Trial" revealed only 24 reports (2.4%) of SPECT probes and 95 reports (3.8%) of PET probes that were formally investigated in clinical trials in humans. Radiolabeled monoclonal antibodies (mAbs) represent one class of MI agents that are gaining increasing attention due to the success of antibody-based cancer therapy.[7] However, searching the ClinicalTrials.gov registry (https://clinicaltrials.gov/ct2/home) by combining the terms "SPECT" and "cancer" and "monoclonal antibodies" revealed only 8 clinical trials of tumor imaging with radiolabeled mAbs. Interrogating ClinicalTrials.gov for the terms "PET" and "cancer" and "monoclonal antibodies" identified another 6 trials of PET imaging with radiolabeled mAbs. Taken together with the PubMed search data, it is evident that only a small proportion of MI agents studied preclinically for tumor imaging have been advanced to clinical trials in patients.

The barriers preventing translation of promising MI agents from the "bench to the bedside" are not clear. One barrier may be financial. A report published almost 10 years ago estimated that the cost of development of a new diagnostic imaging agent from preclinical studies to regulatory approval was $100−200 million, while the market for a "blockbuster" imaging agent was only $200−400 million, making it difficult to recoup development costs.[8] It was further estimated that about a decade was

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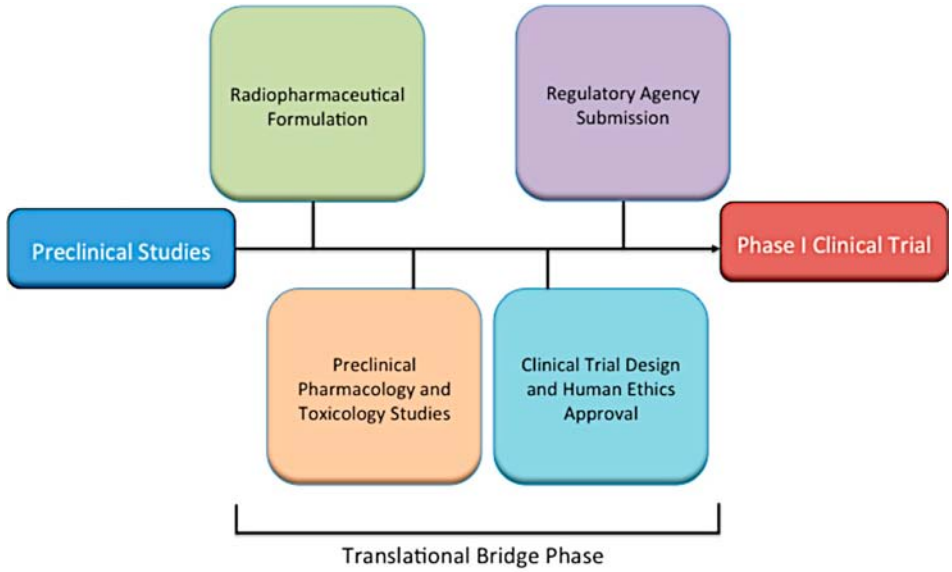

Figure 1. "Roadmap" demonstrating the four steps in the translational bridge phase required to advance novel MI agents from preclinical studies to Phase I clinical trial.

required for a new imaging agent to reach regulatory approval and be marketed.[9] However, these represent the total costs and length of time for clinical development of an imaging agent from preclinical studies through all stages of clinical trials (Phase I to III) to final regulatory approval and marketing. Based on our experience, we have found that novel MI probes can be advanced to first-in-humans Phase I trials in academia at a much lower cost (about $1 million) and requiring only a few years following completion of preclinical imaging studies. If successful, the results of these Phase I trials may "de-risk" further development by the radiopharmaceutical industry and encourage investment which should accelerate and expand the portfolio of probes reaching the clinic for the benefit of cancer patients.

To achieve translation of a novel MI agent to Phase I clinical trial in academia requires a very good understanding of the steps required to advance an imaging probe from a "molecular entity" studied in mouse tumor xenograft models to a "MI radiopharmaceutical" that meets the expected high quality and safety standards for human investigation. Radiopharmaceutical scientists may perceive major barriers to clinical translation in academia including a lack of expertise and insufficient resources. Unfortunately, only a few articles in the literature inform on the processes for advancing novel MI probes to human studies.[10−13] In this Topical Review, we describe the approach that we have successfully applied in our academic radiopharmaceutical research laboratory at the University of Toronto to overcome these perceived barriers. To illustrate, we describe the steps taken to advance ^{111}In-labeled pertuzumab (^{111}In-BzDTPA-pertuzumab), a novel MI probe for detecting response of HER2-positive breast cancer (BC) to treatment with trastuzumab (Herceptin) from preclinical studies[3] to a Phase I clinical trial in patients with metastatic HER2-positive BC (PETRA; ClinicalTrials.gov identifier: NCT01805908). We hope that this "roadmap" to clinical translation will provide confidence to other academic radiopharmaceutical scientists who are similarly interested in advancing novel MI agents to Phase I clinical trials.

ROADMAP TO CLINICAL TRANSLATION OF NOVEL MI PROBES

In 2009, we reported that microSPECT/CT imaging with ^{111}In-labeled pertuzumab detected decreased HER2 expression in subcutaneous human BC xenografts in athymic mice as early as 3 days after commencing treatment with trastuzumab and before changes in tumor size were noted.[3] Downregulation of HER2 is one of the proposed mechanisms of action of trastuzumab.[14] Imaging with ^{111}In-labeled pertuzumab was able to monitor decreased HER2 expression during treatment with trastuzumab because it binds to a different epitope than trastuzumab (domain II vs IV, respectively) and thus does not compete for receptor binding. In contrast, a radiolabeled trastuzumab imaging probe would compete with therapeutic trastuzumab, and thus, it would not be possible to differentiate trastuzumab-mediated HER2 downregulation from receptor blocking caused by treatment with trastuzumab. Trastuzumab-mediated decreased HER2 expression was correlated with a histopathologically documented good response to treatment at 3 weeks. These encouraging results prompted us to propose advancing ^{111}In-labeled pertuzumab to a Phase I clinical trial (PETRA trial; ClinicalTrials.gov identifier: NCT01805908) as a potential MI probe for monitoring response to trastuzumab in BC patients. The four major steps in the "roadmap" to clinical translation (Figure 1) for ^{111}In-labeled pertuzumab or for any MI probe are (i) radiopharmaceutical formulation, (ii) preclinical pharmacology and toxicology studies, (iii) clinical trial design and human ethics approval, and (iv) regulatory agency submission and approval.

The first three steps are closely linked and should not be considered in isolation. For instance, the injected radioactivity and mass amount to be administered to humans in the Phase I trial must be selected first in order to design the radiopharmaceutical formulation. Moreover, regulatory agencies require preclinical toxicology studies at scaled multiples of the proposed human injected radioactivity and mass amounts for the Phase I trial and using the actual radiopharmaceutical formulation to be administered in the trial. The injected radioactivity amount to be selected is dependent in part on the

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radiation dosimetry in order to minimize the radiation absorbed dose to patients from imaging studies in the trial. The radiation dosimetry in humans is projected from preclinical biodistribution and pharmacokinetic studies that are conducted at an injected radioactivity amount and mass amount that is scaled from the proposed human dose. Previously published literature with analogous MI probes that have been evaluated in humans are helpful to guide the selection of the injected radioactivity and mass amounts. In the case of ^{111}In-BzDTPA-pertuzumab, we selected 5 mg for the PETRA trial based on the range of mass amounts (10−100 mg) that were previously employed for other ^{111}In-labeled mAbs.[15−17] The PETRA trial protocol required three SPECT/CT imaging studies: (i) a baseline study prior to commencing trastuzumab treatment, (ii) a study at 1 week to evaluate trastuzumab-mediated HER2 downregulation compared to baseline, and (iii) an imaging study at one month to evaluate further decreases in HER2 expression and/or possible therapeutic response to trastzuzumab. Each imaging study required a separate administration of the radiopharmaceutical. Most previously reported imaging studies with ^{111}In-labeled mAbs employed a single injected radioactivity amount of 150−185 MBq. In order to minimize the radiation dose to patients for three administrations of ^{111}In-BzDTPA-pertuzumab, a lower injected radioactivity amount of 111 MBq was chosen. We then proceeded to formulate the radiopharmaceutical and conduct preclinical pharmacology and toxicology studies, based on the administration of 111 MBq (5 mg) of ^{111}In-BzDTPA-pertuzumab in the PETRA trial.

RADIOPHARMACEUTICAL FORMULATION

In order to advance ^{111}In-BzDTPA-pertuzumab to Phase I clinical trial, the first step in the roadmap was radiopharmaceutical formulation (Figure 2). A radiopharmaceutical kit is an attractive formulation for preparing novel MI agents for human studies because (i) many quality control tests can be performed in advance of preparing the final radiopharmaceutical for patients, (ii) the kits are stable and can be stored and labeled only when needed for a patient study, and (iii) the kits utilize radiometal chelation chemistry that robustly provides high labeling efficiency (≥90%) and does not require post-labeling purification. For imaging purposes, a labeling efficiency ≥90% for a kit (equivalent to final radiochemical purity) is considered acceptable by regulatory agencies, whereas therapeutic applications of radiopharmaceuticals (not discussed in this review) may require higher radiochemical purity (≥95%). These properties of kit formulations simplify radiopharmaceutical preparation and reliably ensure high quality for patient studies. There are several examples of kit formulations reported in the literature for preparation of MI probes.[18−21]

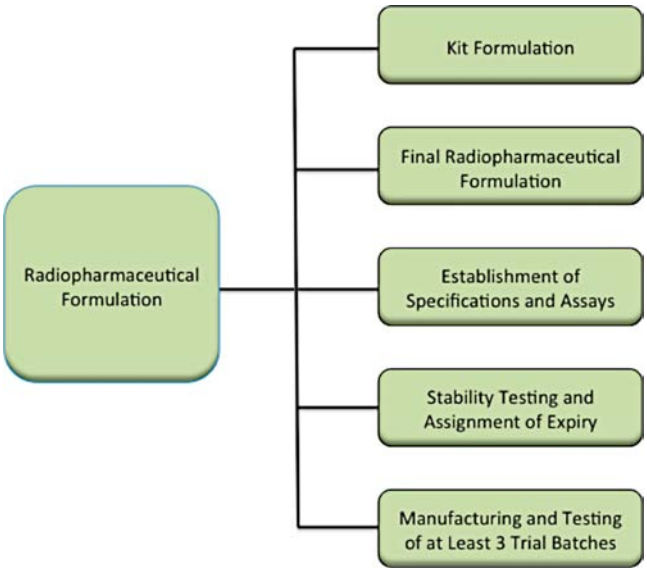

Figure 2. Radiopharmaceutical formulation step includes formulation of a kit and final radiopharmaceutical as well as establishment of specifications and quality control assays for raw materials, intermediates (including the kit), and the final radiopharmaceutical. The stability of the kit and final radiopharmaceutical need to be evaluated by testing against specifications over time, and this data is then used to establish expiry times. At least three independent lots of kits and final radiopharmaceutical are manufactured and tested against specifications to ensure that these will be reliably met.

We designed a kit for preparation of ^{111}In-BzDTPA-pertuzumab that consisted of a unit-dose vial containing 5.0 mg of pertuzumab modified with 2-(4-isothiocyanatobenzyl)-diethylenetriaminepentaacetic acid (p-SCN-BzDTPA) formulated in 0.5 mL of ammonium acetate buffer, pH 6.0.[22] p-SCN-BzDTPA was used instead of DTPA dianhydride previously employed for preclinical studies since this chelator provides a more stable ^{111}In complex and avoids cross-linking of the mAb.[23] The mean labeling efficiency of the kits with ^{111}In (110−150 MBq) was 95.8 ± 2.7%. Other quality control parameters for the radiopharmaceutical kit included protein concentration, volume, pH, appearance, BzDTPA substitution level, purity, and homogeneity. HER2 immunoreactivity was evaluated for each lot of kits and did not need to be measured for each lot of the final radiopharmaceutical product. For a full description of the specifications and assays for these parameters, the reader is referred to our recently published report.[22] Testing for sterility was performed by the USP Sterility Test at the clinical microbiology laboratory at Mount Sinai Hospital (Toronto, ON, Canada). The USP Bacterial Endotoxins Test was performed in our laboratory using a commercially available colorimetric limulus amebocyte lysate (LAL) assay (QCL-1000 End point Chromogenic LAL Assay, Lonza, Walkersville, MD). Quality specifications for ^{111}In-BzDTPA-pertuzumab included limits for total radioactivity and radioactivity concentration, specific activity, pH, radiochemical purity (>90%), radionuclidic purity, appearance, and sterility (retrospective).[22] Retrospective USP sterility testing was performed on a randomly selected sample (5%) of lots of ^{111}In-BzDTPA-pertuzumab after radioactive decay for 30 days. Retrospective sterility testing assures that the radiopharmaceutical administered to patients was sterile, but importantly that the method for its aseptic preparation will reliably result in a sterile product. Endotoxins testing was not routinely performed on the final radiopharmaceutical but a validation study was conducted by testing several pilot lots of ^{111}In-BzDTPA-pertuzumab for endotoxins to demonstrate that the product would meet USP requirements for bacterial endotoxins.

Stability testing was performed on one randomly selected vial from at least 3 independent lots of kits and final radiopharmaceutical by testing these lots monthly against the established specifications which included protein concentration, purity and homogeneity, pH, clarity and color, labeling efficiency or radiochemical purity but not sterility and apyrogenicity. These studies showed that the kits were stable when stored at 4 °C, and a 4-month expiry was assigned. ^{111}In-

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BzDTPA-pertuzumab was stable up to 24 h at room temperature (20 °C) but an 8 h expiry was assigned since this was adequate to allow shipping to the clinical trial site for patient administration.

GOOD MANUFACTURING PRACTICES (GMP)

Good Manufacturing Practices (GMP) are often perceived as a barrier to advancing novel MI probes to Phase I clinical trials in academia. There is frequently a misconception that GMP is focused only on the environment for pharmaceutical manufacturing, when in fact, GMP is a much broader quality assurance system that documents in detail the production of a pharmaceutical from raw materials through intermediates to final product, as well as the assays and specifications that have been implemented to ensure its quality.[24] In our experience, regulatory agencies do not apply full GMP requirements for novel MI agents to be investigated in a Phase I trial, but do require that many components of GMP are in place to ensure the quality and safety of the radiopharmaceutical for these first-in-humans studies. Raw materials used in the preparation of a novel MI agent for clinical investigation need to be pharmaceutical quality. Examples of raw materials include buffer salts and acids, radiometal chelators, mAbs or peptides, sterile water and normal saline, glass vials, and the radioisotope (e.g., ^{111}In). Pharmaceutical quality is most easily assured by purchasing pharmacopeial grade (e.g., USP) materials whenever available. Particularly important is to employ Sterile Water for Injection USP or Sodium Chloride Injection USP for formulation of any pharmaceutical buffers or for preparing kits or the final radiopharmaceutical. Since trace-metal contamination may decrease the efficiency of radiometal labeling, it is important to purify all pharmaceutical buffers by cation exchange chromatography on a column of Chelex-100 resin (BioRad, Hercules, CA). In the case where no pharmacopeial grade material is available, American Chemical Society (ACS) grade chemicals may be used but a high purity (>95%) should be selected. For any nonpharmacopeial raw materials, it is necessary to obtain an individual lot Certificate of Analysis (COA) from the supplier that certifies a high level of purity and lists and provides limits for trace impurities present. In addition, raw materials should be identity tested on receipt by analytical methods (e.g., NMR or testing for key functional groups) to ensure that the correct material has been received. Macrocyclics, Inc. (Dallas, TX) provides custom GMP synthesis of radiometal chelators, but in our experience, chemical grade chelators with high purity accompanied by an individual lot COA and identity testing on receipt are sufficient to meet regulatory requirements.

Small molecule precursors or peptide raw materials used to prepare MI agents may be synthesized in-house but require high purity (>95%) and full analytical characterization. Radioimmunoimaging agents present special challenges since regulatory agencies often require that the manufacturing process be fully elaborated including complete descriptions of any host cell banks and expression vectors used. Recombinant mAbs must also be tested for adventitious virus and endotoxins contamination.[25] In our experience, we have found that these challenges are most easily overcome by using a mAb that is already an approved pharmaceutical product. In such cases, the manufacturer may provide a letter to permit the regulatory agency to consult the manufacturing information on file for the mAb. The rapid growth in cancer immunotherapy has generated many pharmaceutical quality mAbs that recognize a wide range of molecular targets that could be developed as novel MI agents.[7] A Materials Transfer Agreement (MTA) needs to be established between the university and the company that describes the conditions for transfer of the material, including any intellectual property (IP) protection considerations. It may be possible for the university to negotiate shared IP in the MTA for any improvements in the material such as development into a novel MI agent. ^{111}In-BzDTPA-pertuzumab was prepared from pertuzumab (Perjeta; Hoffman La Roche, Mississauga, ON, Canada) which is an approved pharmaceutical mAb used for the treatment of HER2-positive metastatic BC.[26] The manufacturer provided a letter of authorization to Health Canada to access the information on file for pertuzumab and provided the raw material through a MTA to prepare ^{111}In-BzDTPA-pertuzumab for the PETRA trial.

Quality control assays and specifications for concentration, pH, clarity and color, and sterility and apyrogenicity must be established for all pharmaceutical buffers used to prepare the kit and final radiopharmaceutical. In some cases, pharmacopeial assays may be adapted to assay the concentrations of buffer salts (e.g., sodium phosphate, acetate, or bicarbonate). Kits and the final radiopharmaceutical must be terminally sterilized, usually by filtration through a 0.22 μm filter. The integrity of the filter is tested using the "bubble point test" which involves passing air through the filter after use and assuring that there is strong resistance. Kits must be tested for quality parameters (see Radiopharmaceutical Formulation) including protein concentration, volume, pH, appearance, chelator substitution level, purity and homogeneity, and immunoreactivity or receptor-binding. Sterility and apyrogenicity are determined by the USP Sterility Test and USP Bacterial Endotoxins Test, respectively, to ensure that the product is pharmaceutically acceptable for injection. The final radiopharmaceutical is tested for radiochemical purity (≥90%) and several other key quality parameters including radioactivity concentration, pH, and sterility (performed retrospectively on a randomly selected 5% sample of vials). The specifications and assays for pharmaceutical buffers, kits, and ^{111}In-BzDTPA-pertuzumab are outlined in our published report.[22] Standard operating procedures (SOPs) should be developed to provide written detailed processes for manufacturing and quality control of pharmaceutical buffers, kits, and the final radiopharmaceutical. Complete records on raw materials, intermediates (including kits), and the final radiopharmaceutical that are traceable by a lot numbering system must be maintained to investigate any quality issues. A lot release and recall procedure should be in place. In Canada, the results of quality control testing of radiopharmaceutical kits must be FAXed to Health Canada with a request for authorization of individual lot release prior to use for radiopharmaceutical preparation for patients in the trial.

The environment for kit and radiopharmaceutical manufacturing should meet GMP standards for air quality.[24] Sterile products must be prepared in a grade A air environment in a room with a minimum of grade C air. Grade A air contains <3520 particles per m^3 with diameter ≥0.5 μm and <20 particles with diameter ≥5 μm. A Class A laminar air flow cabinet (biosafety cabinet) provides grade A air since the High Efficiency Particulate Air (HEPA) filter removes all particles >0.45 μm. Ideally, the cabinet should be located in a "clean room" which has a HEPA-filtered air supply that meets at least grade C. At operation, i.e., during kit or radiopharmaceutical preparation, grade C air contains <3 520 000 particles per m^3

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with diameter ≥0.5 μm and <29 000 particles per m^3 with diameter ≥5 μm. These limits are 10-fold lower when there are no operations taking place (i.e., at rest). When no clean room is available, a dedicated room for pharmaceutical manufacturing in a clean facility may provide grade C air, but this requires testing by a certified air quality testing service to ensure that these standards are met. The biosafety cabinet requires testing and recertification annually. The cabinet and any equipment or supplies placed in the cabinet should be disinfected with 70% alcohol and sterile plasticware and syringes should be used for pharmaceutical formulation. Nonsterile equipment must be sterilized by autoclaving or gas sterilization. Equipment used in kit or radiopharmaceutical preparation ideally should be dedicated to avoid contamination, and should be maintained in a high state of cleanliness and in very good operating condition and regularly calibrated. Individuals preparing radiopharmaceuticals should be qualified and trained in sterile product preparation, and should wear a clean lab coat, face mask, and head covering whenever conducting manufacturing operations.

PRECLINICAL PHARMACOLOGY AND TOXICOLOGY STUDIES

The next steps in the roadmap to clinical translation are preclinical pharmacology and toxicology studies (Figure 3).

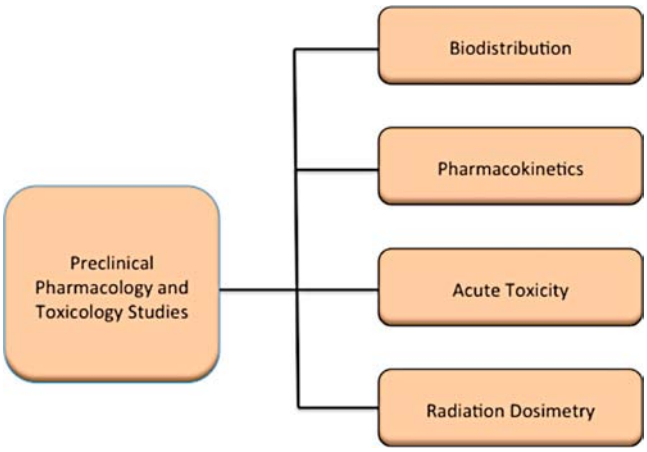

Figure 3. Preclinical pharmacology and toxicology studies required to advance a novel MI agent to Phase I clinical trial. These studies include evaluation of the normal tissue biodistribution at several time points and determination of the pharmacokinetics of elimination from the blood. The acute toxicity of the MI agent is examined at multiples of the proposed human injected radioactivity and mass amounts and includes evaluation of any adverse effects on the hematological system, liver, kidneys, and other normal organs. The radiation absorbed doses in humans are projected from preclinical biodistribution data using OLINDA/EXM dosimetry software.

Normal tissue biodistribution studies and evaluation of the pharmacokinetics of elimination from the blood are used to predict the radiation absorbed doses to humans for the Phase I trial. Biodistribution and pharmacokinetic studies for ^{111}In-BzDTPA-pertuzumab were performed in groups of four non-tumor-bearing Balb/c mice at several time points up to 7 days postinjection. This data was used to estimate the cumulative radioactivity in each source organ ($\tilde{A}$, $Bq \times h$) which was then applied to predict the radiation absorbed doses ($\bar{D}$; mSv) to target organs in humans using the Organ Level INternal Dose Assessment (OLINDA) software. OLINDA employs the Medical Internal Radiation Dose (MIRD) formalism which estimates target organ doses as $\bar{D} = \tilde{A} \times S$; where S is the dose ($mSv/Bq \times h$) to a target organ per unit of cumulative radioactivity in a source organ].[27] These studies estimated that each administration of ^{111}In-BzDTPA-pertuzumab (111 MBq) would deliver a whole body radiation absorbed dose of 5.6 mSv in a 50 kg adult human female. The total dose for three administrations of ^{111}In-BzDTPA-pertuzumab (16.8 mSv) for the PETRA trial was comparable to that for ^{111}In-capromab pendetide (Prostascint; 16 mSv)[28] or ^{18}F-2-fluorodeoxyglucose (^{18}F-FDG; 7−14 mSv).[29]

Acute toxicity studies were performed in groups of 10 female, non-tumor-bearing mice administered ^{111}In-BzDTPA-pertuzumab at 10 times the planned injected mass amount for patients in the PETRA trial and at 23 times the planned injected radioactivity amount scaled from the human to the mouse on a mg/kg or MBq/kg basis, respectively. Body mass was measured every few days over a 15-day period. Other measurements included complete blood cell counts (CBC), hemoglobin, hematocrit, serum creatinine (Cr), and serum alanine aminotransferase (ALT) at 2 days and 15 days postinjection. Mice were sacrificed and a comprehensive panel of tissues were collected after 15 days that were then examined histopathologically by a clinical pathologist. The pathologist provided a detailed written report. The controls for toxicity studies of ^{111}In-BzDTPA-pertuzumab were groups of 10 mice administered normal saline or unlabeled BzDTPA-pertuzumab. These studies demonstrated that there were no serious toxicities associated with the administration of ^{111}In-BzDTPA-pertuzumab at multiples of the planned human injected radioactivity and mass amounts. Regulatory agencies may require toxicology studies to be performed in a nonrodent as well as a rodent species, but this was not required in our case by Health Canada for ^{111}In-BzDTPA-pertuzumab since this was prepared from an approved pharmaceutical product (pertuzumab). The main purpose of the toxicology studies was to assess if the toxicity of pertuzumab was increased by labeling with ^{111}In. In the CTA to Health Canada, we provided all available literature on previous preclinical and clinical studies of pertuzumab that documented its safety at therapeutic doses that greatly exceeded the injected mass amount planned for the PETRA trial. For more details on the preclinical pharmacology and toxicology studies conducted for ^{111}In-BzDTPA-pertuzumab, the reader is referred to our published report.[30]

CLINICAL TRIAL DESIGN AND HUMAN ETHICS APPROVAL

The next step in the roadmap to clinical translation is the design of the Phase I clinical trial protocol and informed consent document as well as obtaining human ethics approval (Figure 4). To design a Phase I trial for ^{111}In-BzDTPA-pertuzumab as well as conduct the trial under Good Clinical Practices (GCP), we partnered with the Ontario Clinical Oncology Group (OCOG; http://www.ocog.ca) at McMaster University (Hamilton, ON, Canada). OCOG is an academic clinical trials organization that provides a multidisciplinary team of oncologists, biostatisticians, clinical trialists, human ethics and regulatory affairs specialists, information technology programmers, clinical research coordinators, and data monitoring and management assistants. OCOG collaborates with radiopharmaceutical scientists, nuclear medicine physicians,

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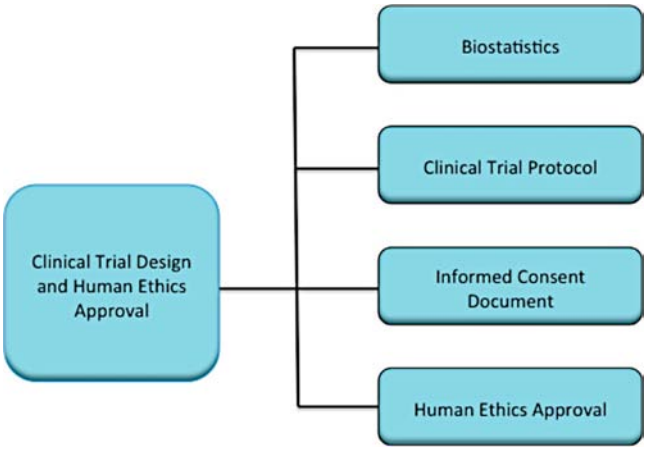

Figure 4. Clinical trial design and human ethics approval step required for advancing a novel MI agent to Phase I trial. Consultation with a biostatistician is required to estimate the patient sample size and to calculate the statistical power of the trial to test the hypothesis of tumor imaging with the agent. This phase includes the design of the clinical trial protocol and informed consent documents, as well as application for human ethics approval.

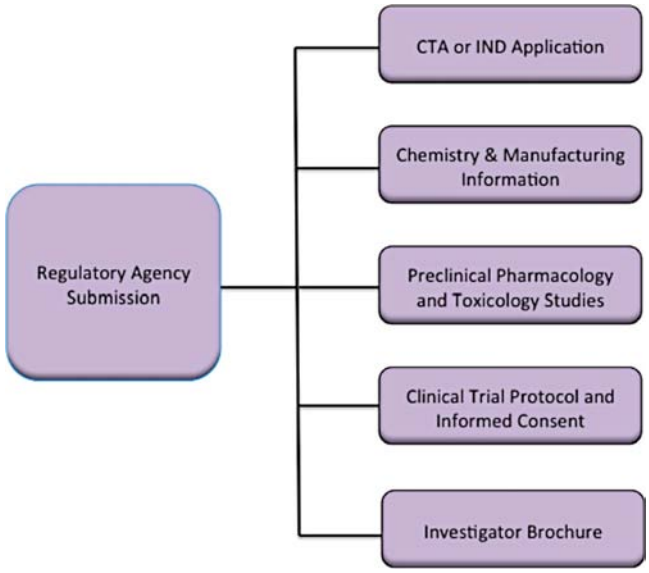

Figure 5. Final step required for advancing a novel MI agent to Phase I clinical trial is regulatory agency submission which includes completion of a CTA (Canada) or IND application (USA) that is supported by information on the chemistry and manufacturing of the probe, results of preclinical pharmacology and toxicology studies, clinical trial protocol and informed consent document, and the investigator's brochure (IB). Regulatory agency review normally takes 30 days.

medical physicists, and oncologists to conduct clinical trials of innovative MI agents for cancer. In collaboration with our group, OCOG designed a Phase I trial (PETRA; ClinicalTrials.gov identifier: NCT01805908) to study SPECT/CT imaging with ^{111}In-BzDTPA-pertuzumab to predict the response of patients with metastatic HER2-positive BC to treatment with trastuzumab combined with chemotherapy. In addition, OCOG submitted an application for human ethics approval for the trial to the Ontario Cancer Research Ethics Board (OCREB) which is a province-wide review board for clinical trials in cancer patients. Finally, OCOG provided regulatory support for the CTA submission to Health Canada for the trial and functioned as the liaison between Health Canada and our group as well as the trial investigators. Forming a partnership with interested and committed oncologists is essential to advance a novel MI agent to Phase I clinical trial. Partnering with an academic clinical trials organization such as OCOG assures that GCP are incorporated into the design and conduct of the trial.

■ REGULATORY AGENCY SUBMISSION

The final step in the roadmap to clinical translation is regulatory agency submission (Figure 5). In Canada, a Clinical Trial Application (CTA) must be submitted to Health Canada. Guidelines for compiling a CTA are available on the Health Canada Web site (http://www.hc-sc.gc.ca). A CTA is analogous to an Investigational New Drug (IND) submission to the U.S. Food and Drug Administration (FDA) and the process for radiopharmaceuticals in the U.S. has been published.[31] Overviews of the U.S. FDA regulations for PET radiopharmaceuticals (21 CFR part 212) are also available.[10,11,32] The USP provides further guidance on production of PET radiopharmaceuticals for human use (USP Chapter ⟨823⟩).[33] The CTA for a Phase I clinical trial in Canada requires three supporting modules: (i) chemistry and manufacturing, (ii) preclinical pharmacology and toxicology, and (iii) clinical trial protocol, investigator's brochure (IB), and informed consent. Information in these modules is compiled from the steps to clinical translation described (Figure 1). The IB is a product monograph for an investigational agent that summarizes the product information for the trial investigators. This information includes details of the formulation, the radioactivity and mass amount to be administered, radiation dosimetry, summaries of all previous preclinical or clinical studies, and any precautions regarding possible adverse effects from the investigational agent. In addition to the three Supporting Information modules, in Canada several forms must be completed for the CTA including the Drug Submission Application (Form HC3011), Clinical Trial Site Information form, and Quality Information Summary − Radiopharmaceuticals (QIS-R). In the case of biologics such as radiolabeled mAbs, the Quality Information Summary − Biologics (QIS-B) form must also be completed. The QIS-R and QIS-B forms summarize the standards and specifications for all steps in the manufacture of the radiopharmaceutical from raw materials through intermediates including the kit, to the final radiopharmaceutical. These forms further provide information on quality testing results for all lots of the kit and final radiopharmaceutical to date, including any pilot formulation development batches. Following submission, Health Canada reviews the CTA and will provide a "No Objection Letter" (NOL) within 30 days if the application is deemed satisfactory. Upon receipt of the NOL, the trial may proceed. In some cases, Health Canada requests additional information during the CTA review that must be provided in a timely manner, often within 48 h. We received the NOL for the PETRA trial within the 30 day review period on February 14, 2013. There are similar processes for review and approval of IND applications by the U.S. FDA including a 30-day review period (http://www.fda.gov). Health Canada and the U.S. FDA both provide an opportunity for pre-CTA/IND meetings to discuss the requirements for individual agents.

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CONCLUSIONS

Two patients have been enrolled to date in the PETRA trial. Both patients demonstrated good uptake of ^{111}In-BzDTPA-pertuzumab in metastatic lesions in the liver (example shown in Figure 6) and there were no serious adverse reactions that were

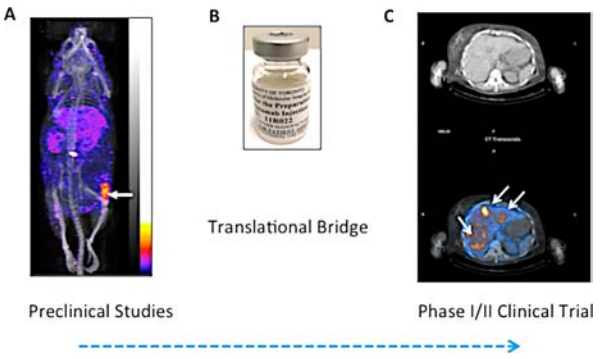

Figure 6. ^{111}In-labeled pertuzumab was advanced from completion of preclinical imaging studies in tumor-bearing mice that showed localization (arrow) in a HER2-positive human breast cancer (BC) xenograft (A; arrow) through translational bridge studies including the formulation of a kit and final radiopharmaceutical (B) to Phase I clinical trial in patients with metastatic HER2-positive BC (C), in academia in two years and requiring an investment of about $1 million. Clinical images demonstrate good tumor localization in metastases to the liver in a patient with HER2-positive BC at 48 h postinjection of ^{111}In-BzDTPA-pertuzumab (arrows). A transaxial CT image is shown at the top and the CT images were coregistered with the SPECT images in the bottom panel.

atributable to administration of the radiopharmaceutical. These patients are being treated with trastuzumab, and trastuzumab has been associated with some adverse effects in patients, particularly when combined with certain chemotherapeutic agents.[34] The total cost of development of ^{111}In-BzDTPA-pertuzumab including all preclinical studies, translational bridge studies including the conduct of the Phase I clinical trial is estimated at $1 million and was funded entirely by peer-reviewed research grants from the Ontario Institute for Cancer Research (OICR), a provincial cancer research funding agency. Only two years were required to advance ^{111}In-BzDTPA-pertuzumab from completion of preclinical studies to Health Canada regulatory approval of the CTA permitting the clinical trial to proceed. These first-in-humans studies of ^{111}In-BzDTPA-pertuzumab demonstrate the feasibility of advancing novel MI agents to Phase I clinical trial in an academic setting.

In the movie Apollo 13, after the disastrous explosion in space, Gene Kranz, the Mission Control Director gathers the NASA scientists together and takes chalk and marks an "X" on the chalkboard half way between the moon and the earth, and says, "You're telling me that you can only get our guys to here? That's unacceptable, gentlemen—I want our guys all the way back to earth with time to spare. Failure is not an option." We would argue that failure is not an option in the advancement of promising MI probes from preclinical studies to clinical evaluation in humans—we must reach for the moon in envisioning the future of MI, but we also need to get all the way back to the earth by taking up the challenge to move these probes forward to first-in-humans Phase I trials. Only by embracing this challenge will we be able to realize their full potential for the future benefit of cancer patients.

AUTHOR INFORMATION

Corresponding Author

*Tel. 1-416-946-5522. FAX: 1-416-978-8511. E-mail: raymond.reilly@utoronto.ca.

Notes

The authors declare the following competing financial interest(s): Pertuzumab was provided to R.M.R. through Materials Transfer Agreements with Genentech, Inc. for the preclinical and translational bridge studies and to R.M.R. and M.L. by Hoffman La Roche, Ltd. for the PETRA Phase I clinical trial.

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ABBREVIATIONS

$\tilde{A}$, cumulative radioactivity in a target organ; ACS, American Chemical Society; ALT, alanine aminotransferase; BC, breast cancer; BzDTPA, benzyl-diethylenetriaminepentaacetic acid; CBC, complete blood count; COA, Certificate of Analysis; CTA, Clinical Trial Application; Cr, serum creatinine; $\bar{D}$, Radiation absorbed dose to a target organ; FDA, Food and Drug Administration; ^{18}FDG, ^{18}F-2-fluorodeoxyglucose; GCP, Good Clinical Practices; GMP, Good Manufacturing Practices; HEPA, High Efficiency Particulate Air; HER2, human epidermal growth factor receptor-2; IB, Investigator's Brochure; IND, Investigational New Drug Submission; IP, Intellectual Property; LAL, limulus amebocyte lysate; mAb, monoclonal antibody; MI, molecular imaging; MTA, Materials Transfer Agreement; NOL, No Objection Letter; OCOG, Ontario Clinical Oncology Group; OCREB, Ontario Cancer Research Ethics Board; OICR, Ontario Institute for Cancer Research; OLINDA, Organ Level INternal Dose Assessment; p-SCN-BzDTPA, 2-(4-isothiocyanatobenzyl)-diethylenetriaminepenta-acetic acid; QIS-B, Quality Information Summary – Biologicals; QIS-R, Quality Information Summary – Radiopharmaceuticals; S, Radiation absorbed dose per unit cumulative radioactivity in a source organ; SOP, Standard Operating Procedure; USP, United States Pharmacopeia

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