Clinical and Pre-clinical Evaluation of Ac-225 Labeled Biomolecules for Targeted Alpha Therapy(TAT)

Shakera Khatoon Rizvi
Research Coordinator
IPD, PINSTECH

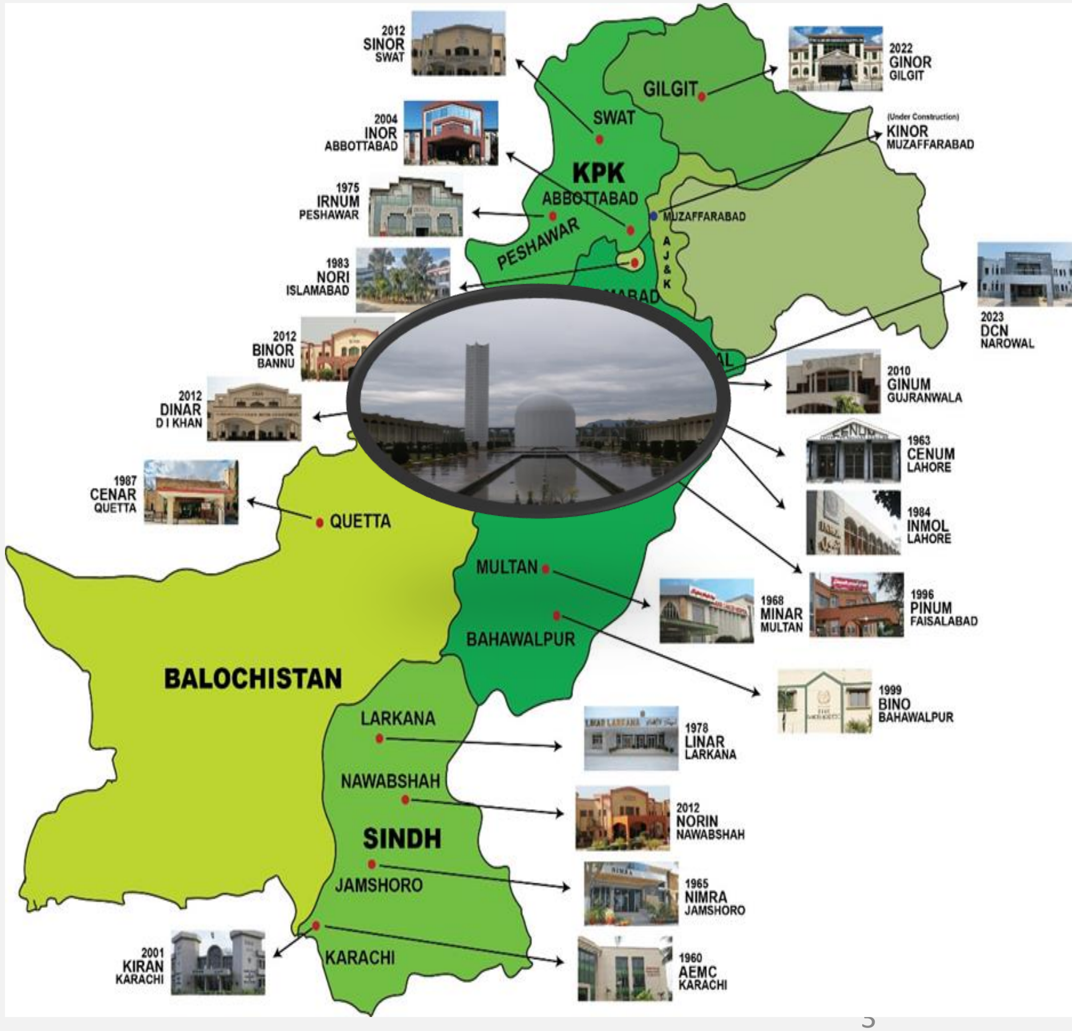
Content

- ❖Overview of PINSTECH facility
- ❖PAEC(PINSTECH)- MEDICIS collaboration
  Introduction of Modern Radioligand Therapies
- ❖Objectives of the project
- ❖Clinical Transformation of Ac-225
  - Methodology
  - Results
  - Future Work
- ❖Targeted Therapy with Tb-161

PAEC- PINSTECH

Our research reactor PARR-I at PINSTECH is the central facility to provide radiopharmaceuticals to all the public sector and private hospitals in the country

- Mo-99m Production facility
- ^{99m}Mo/Tc99m Generator
- I-131 production
- GMP facility for production of cold kits
- Established Quality Control Labs
  - Gamma Spectrometry
  - Alpha& Beta spectrometry
  - Radio HPLC
  - ITLC
  - ICP-MS
- Animal House



PINSTECH-MEDICIS Collaboration

- **Radionuclide Production**
  - **Cyclotron Production**
  - **Reactor Production**
- **Radiochemistry**

  Strong collaboration

  Mr. Irfan Tariq
- **Clinical Transformation of Radionuclides**
  - **GMP Production of Radiopharmaceuticals**
    - **Radiolabeling of Radionuclides with Bio-molecules**
    - **Quality Control**
  - **Preclinical Studies**
  - **Clinical Trials**

Introduction

Cancer Research Advances

Decades of research have resulted into the development of theranostics

Integration of therapeutics and diagnostics in a single management approach

Diagnosis ⟷ **Theranostic** ⟷ **Treatment**

Single compound is used for a variety of applications in patient-centered care radiolabeled with different radionuclide

Theranostic Radiopharmaceuticals

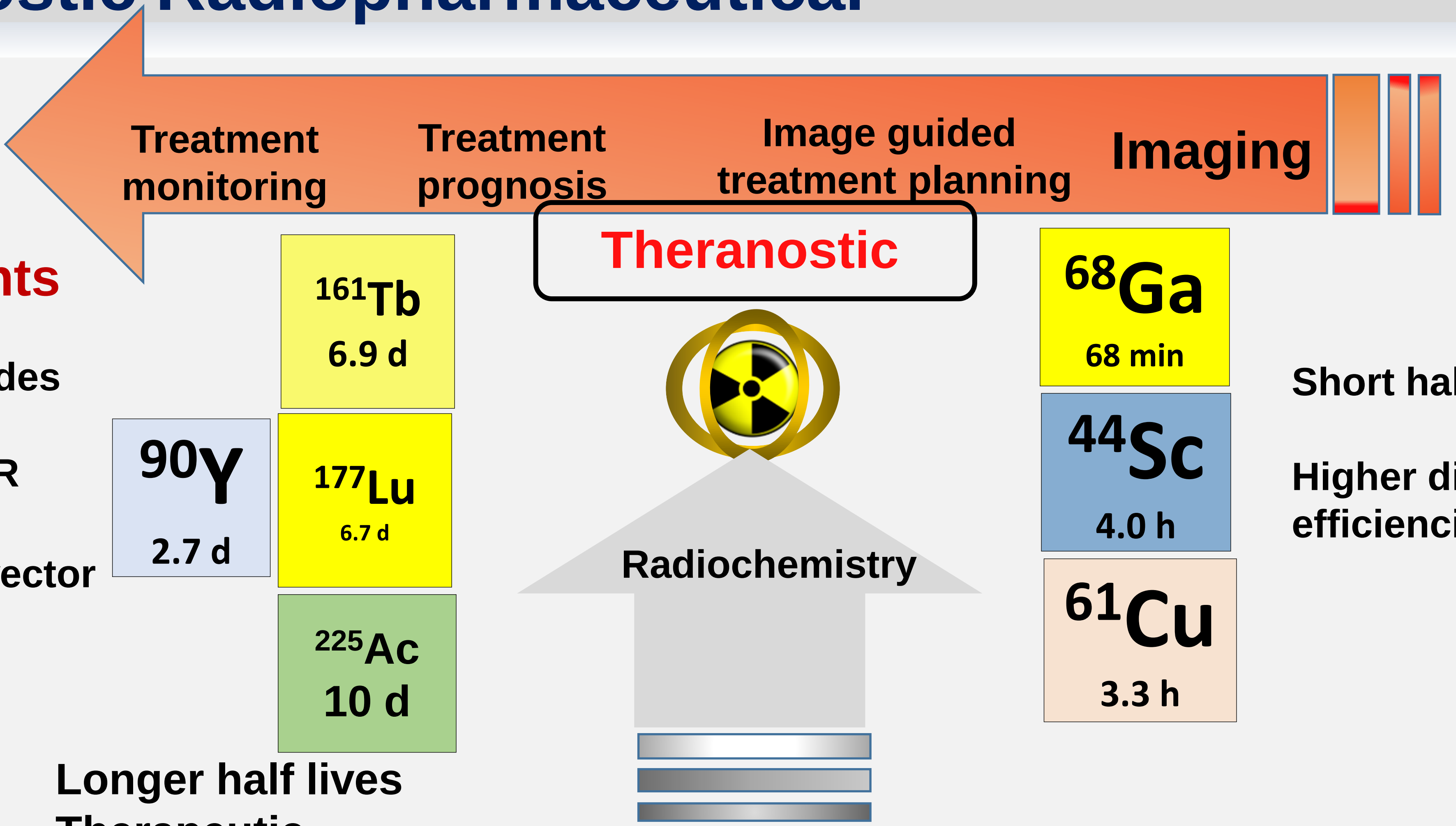
Peptide Receptor Radionuclide Therapy (clinical success)

binding SSTRs, which are expressed on the cell surface of the tumor cell, followed by internalization of the radionuclide-peptide complex

Theranostic Radiopharmaceutical

Components

1. Radionuclides
2. CHELATOR
3. Targeting vector



Short half lives

Higher diagnostic efficiencies

Longer half lives
Therapeutic potential

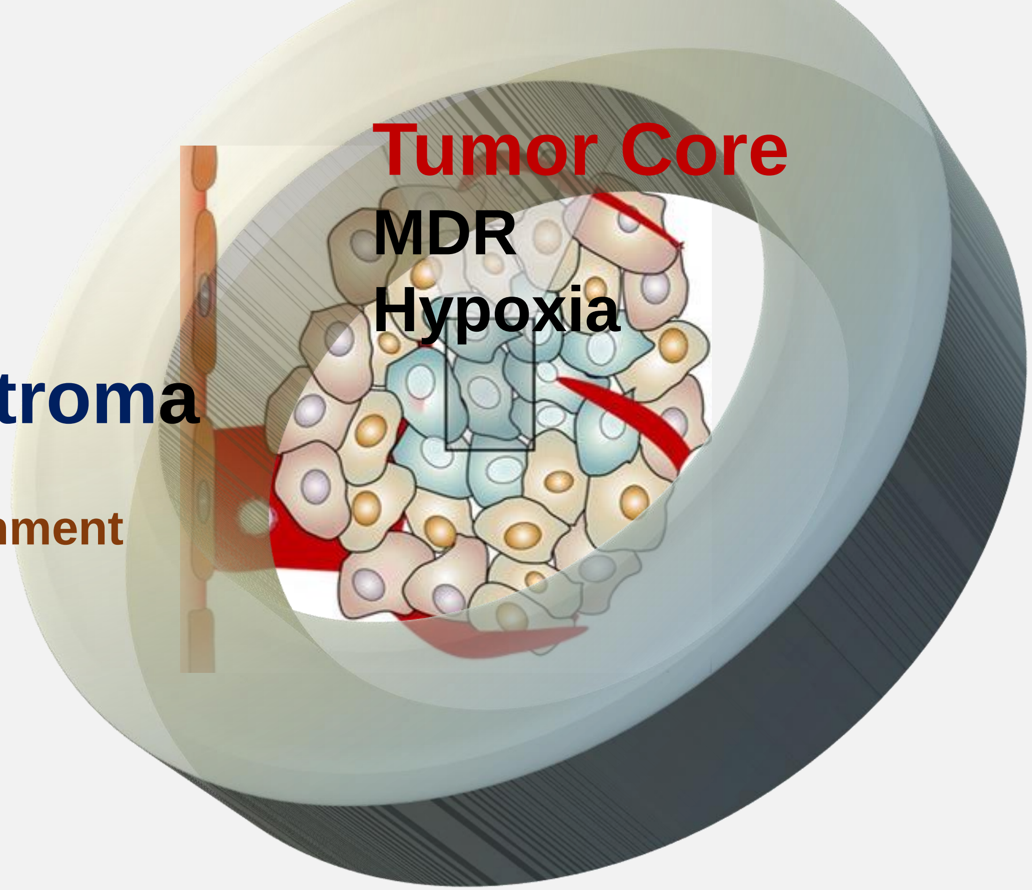
Lesser amount of pharmaceutical Maximum Therapeutic outcome

Clinical Transformation of Radionuclides

Targeted Radioligand Therapies

- **Radio Nuclide**
  - **Production**
  - **High specific Activity**
  - **Purification**
- **Radiopharmaceutical**
  - **Targeted**
  - **Stability in Vivo**
  - **Retention Time**
- **Oncological vector**
  - **Overexpressed Receptors (SSTR,CD44 etc)**
  - **Antibodies**
  - **FAPI**
- **Targeting Systems**
  - **Active**
  - **Passive**

Mix of Chemistry, Biology & Physics can help



Tumor Core

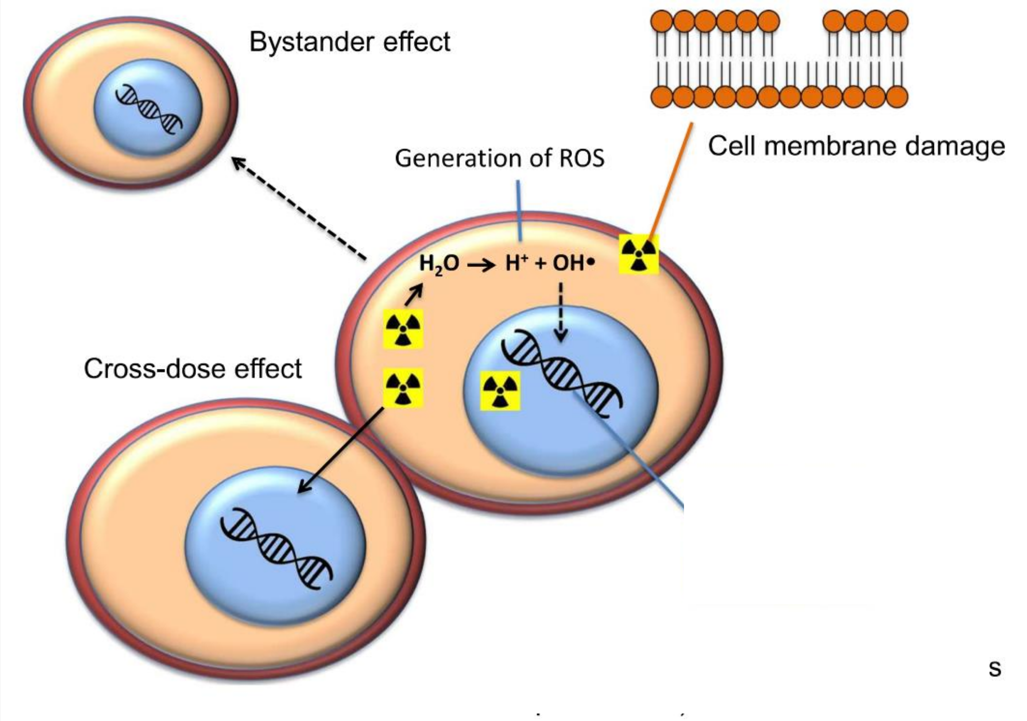
MDR

Hypoxia

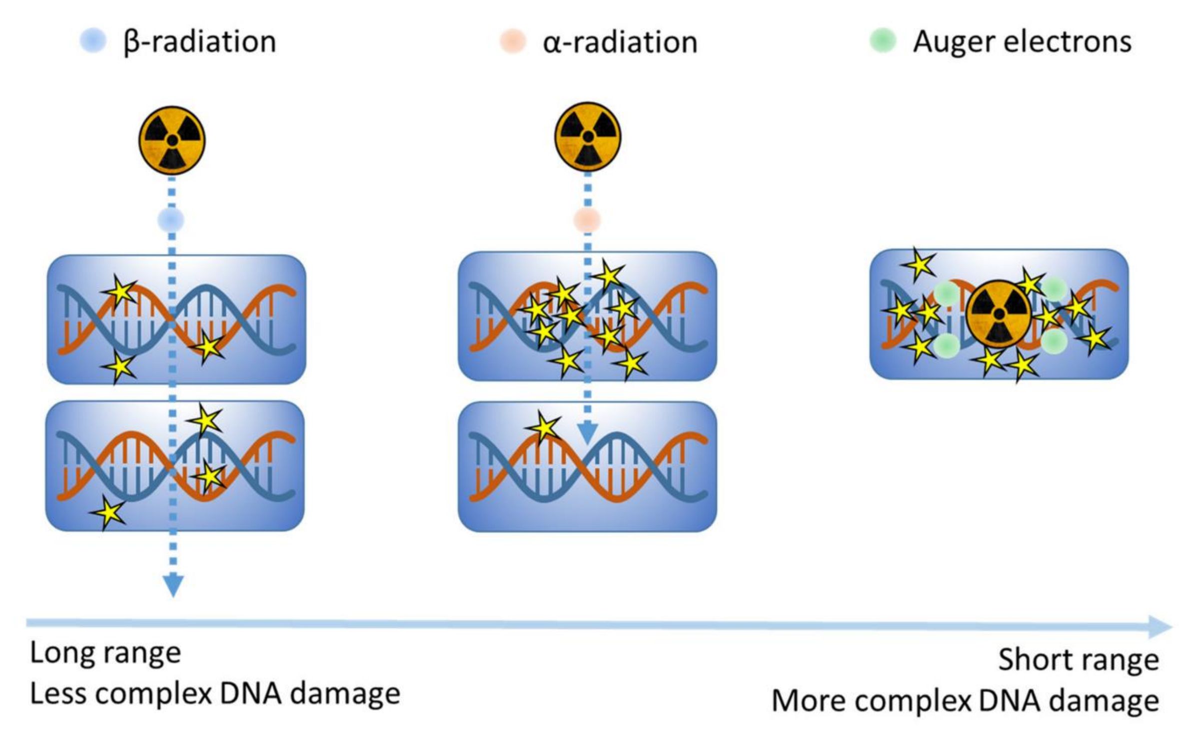
Tumor Stroma

Receptors
Micro environment
Protiens,
pH,
EPR

Targeted Alpha& Beta Therapies



Ku et al. EJNMMI Radiopharmacy and Chemistry (2019)



Cancers **2020**, *12*(8), 2098; https://doi.org/10.3390/cancers12082098

DNA is considered the main target for causing radiation-induced cell death and indeed, the greater the unrepaired DNA damage the higher the incidence of lethality

PRRT in Pakistan

- ^{177}Lu-DOTATATE for single patient dose has been optimized with radionuchemical purity of 98 ± 2% at pH 5 with 2M acetate buffer and 55mg of gensitic acid.

- Ppetide content varied from 250-300µg depending on the specific activity.

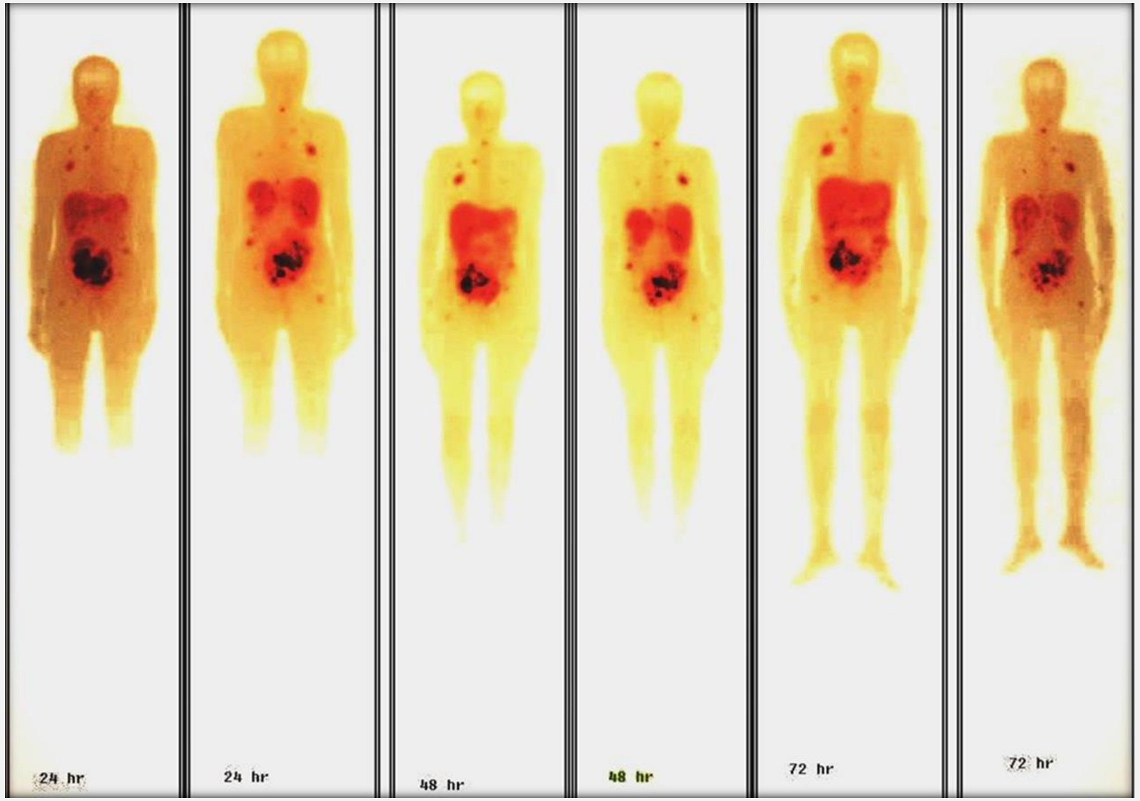
- Radiolabelling carried out by heating the mixture at 90^{o}C for 30minutes
- 50mg of Ascorbic Acid to avoid radiolysis

- Total volume of the 740MBq dose is optimized at 2ml for dispensing at hospital pharmacy

Theranostic radiopharmaceuticals in Pakistan

Development of theranostics pharmaceuticals started in 2017 with start of 1st national project for production of ^{177}Lu-DOTATATE for treatment of neuroendocrine tumors

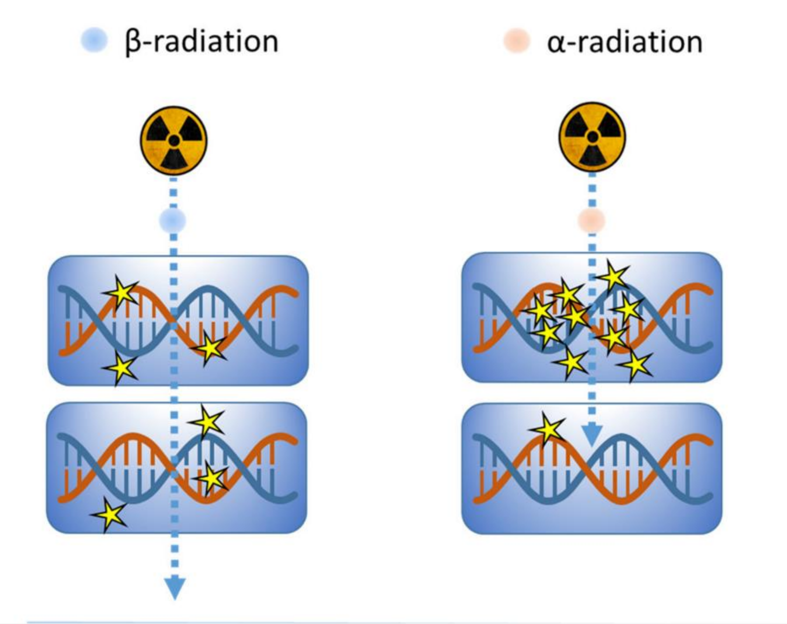
- Registered Product of PINTHERA [^{177}Lu][Lu-DOTATAE] & [^{177}Lu][Lu-PSMA]
- More than 100 doses have been delivered to patients



180mCi ^{177}Lu-DOTATATE on 7/03/2020

Targeted Alpha Therapy

Clinical Transformation of AC-225



Objectives

- Development and Optimization of Ac-225 Radiopharmaceuticals for targeting specific oncological targets.
- Evaluate the stability, radiochemical purity and biological efficacy of Ac-225 labeled pharmaceuticals in vitro.
- Preclinical Evaluation to assess bio distribution, pharmacokinetics, and therapeutic efficacy in animal models.
-
- Determine the maximum tolerated dose (MTD) and dose-limiting toxicities (DLTs).
- Clinical trials to evaluate safety, dosimetry, and preliminary efficacy in patients.

Ac-225 Pharmaceuticals

Recurrence of resistant disease after treatment with β- emitter like Lu-177

Decay cascade of six daughter Progeny

- Substantial amount of energy
  - Alpha & Beta combined
- High Linear Energy Transfer
- Suitable for micrometastasis
- Lower radionuclide doses

^{225}**Ac**

10d

- Emission **Therapeutic**	**4 α-**particle per decay, **6MeV, 7Mev,6Mev,** **3β$^-$** 659 keV, 198 keV, 444 keV,
γ Emissions **SPECT Imaging**	**218.0 keV, 440 KeV,**

Production of Ac-225

Source Received at IPD

Ra-225 source

Purified Ac-225 Source

Chemical Separation
GMP Facility available

Quality Control
&
use for radiolabelling

[^{225}Ac][Ac-Pharmaceutical] Production

Radiolabeling of Ac-225

- DOTATATE, DOTA-PSMA, FAPI, Hyaluronic Acid
- Quality Control

In-vitro Studies

- Stability Studies

Preclinical Studies In-vivo

- Biodistribution
- Cytotoxicity
- Dose Estimations

Clinical Studies

- PAEC Nuclear Medicine Hospitals

Results

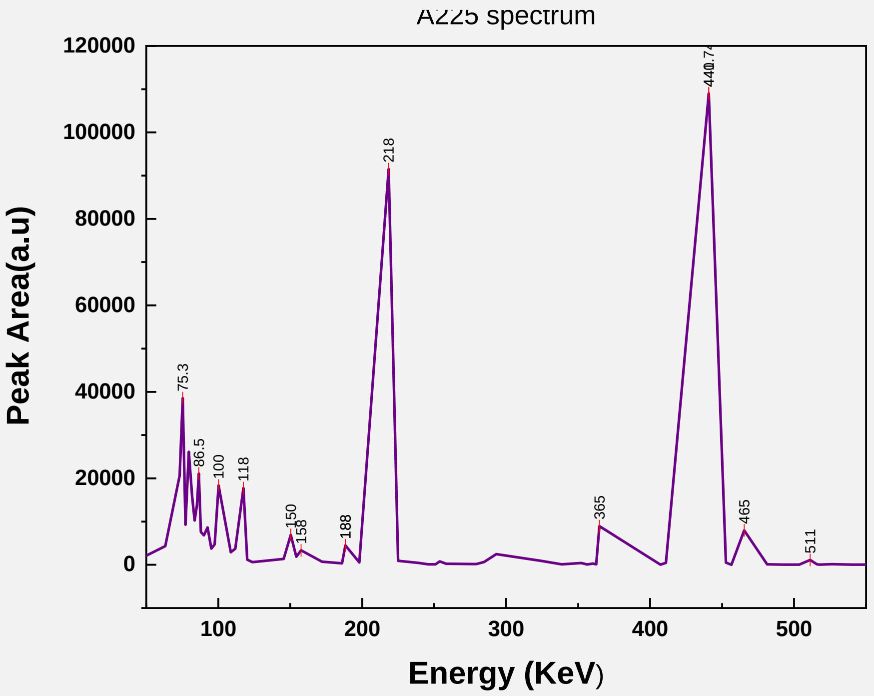
Quality Control of Ac-225 Received from MEDICIS-CERN

Radionuclide Purity

Gamma spectrometry

Ac-225 eluted from Ra-225/AC-225 Generator at PINSTECH

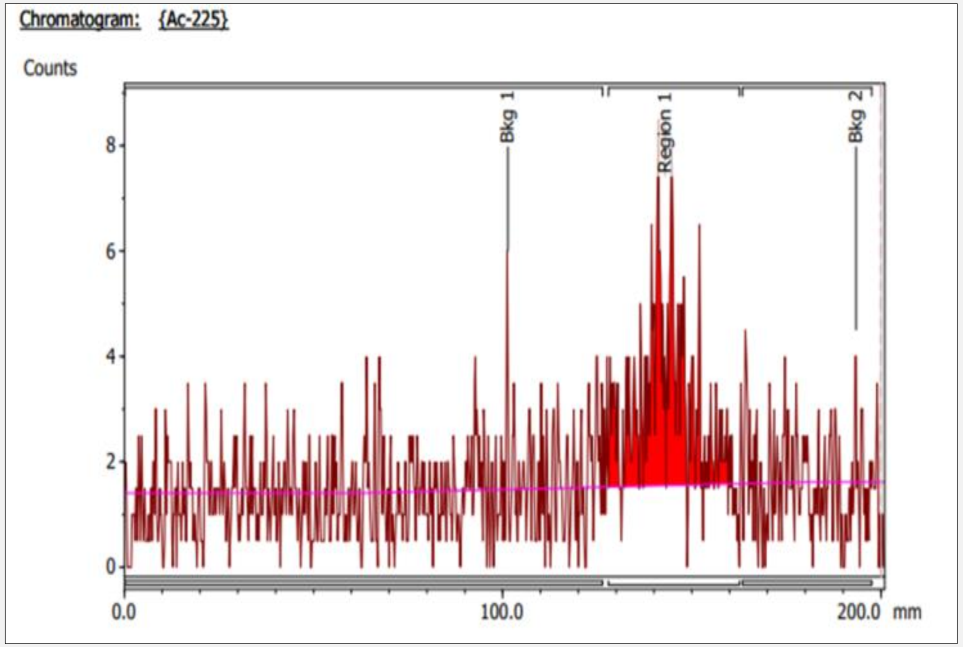
Nuclide	Energy (KeV)	Gamma Abundance (%)	Efficiency	Activity (Bq)	Error
^{221}Fr	218.12	11.4	0.76	4.02E+108	2.57E+112
^{213}Bi	440.5	25.94	0.42	2.69E+72	1.33E+73
^{225}Ac	99.8	1.0	1.13	36468	4574
^{225}Ac	150.1	0.6	0.97	27016	3325
^{225}Ac	157.3	0.32	0.94	24174	4701
^{225}Ac	188	0.45	0.85	28611	1320
^{225}Ra	40	30	0.92	19	4

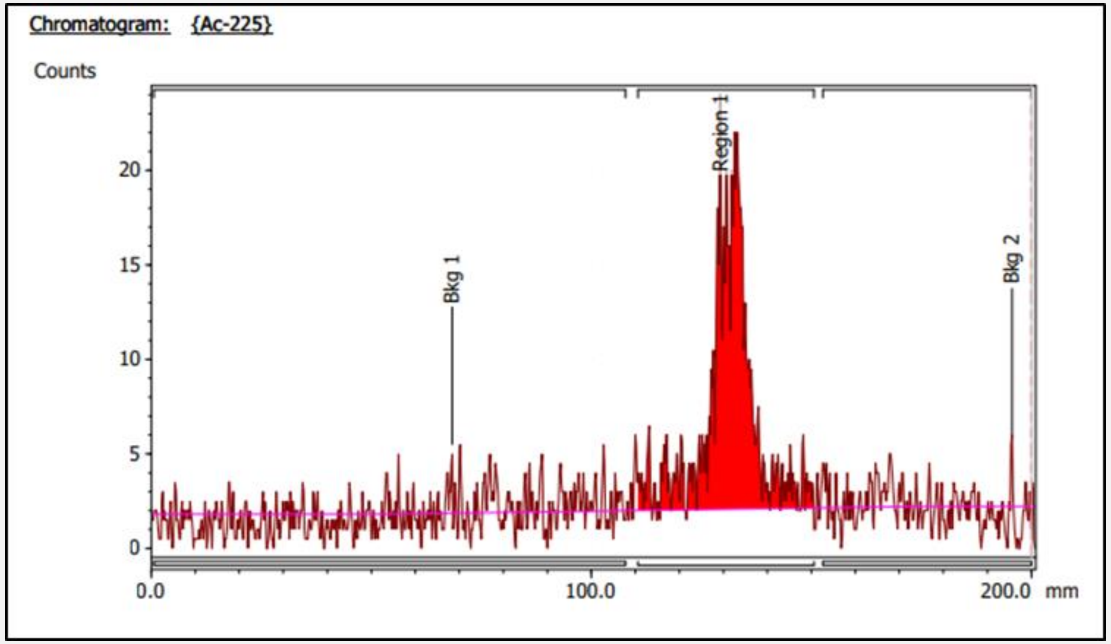


Radiochemical Purity

ITLC

ITLC-SG Chromatogram of Ac-225





Solvent: **0.1M Citrate Buffer, pH 5**

Solvent: Aqueous Acetonitrile

Rf = 0.8 - 1

Preparation of [^{225}Ac][Ac-DOTATATE]

Optimized conditions for preparation of [^{225}Ac][Ac-DOTATATE] with RCP >95%

- **30 KBq of Ac-225 in 100µl of 0.1M HCl**
- **200µg of DOTA-TATE**
- **2ml of Tris buffer pH 6.8**
- **1M Sodium Ascorbate 100µl**
- **Heating time: 120 minutes at 95^{o}C**

Future Works

- **In Vitro studies**

- **In vivo preclinical studies**

- **Clinical Trial for TAT of neuroendocrine patients**

Tb-Phramaceuticals

$^{161}_{65}$**Tb**

6.9 d

155 γ

Tb

65 5.32 d

SPECT Imaging

β⁻ Emission **Therapeutic**	**$E_{av.}$154 KeV**
γ Emissions **SPECT Imaging**	**25.65 (23%),48.91 (17%), 74.56 (10%)**
Electron energies	**~ 12.4 e−, 46.5 keV per decay**

- **Tb has four medically relevant isotopes, covering all major nuclear medicine modalities**
- **Enable theranostics with chemically identical radiopharmaceuticals.**
- **^{155}Tb and ^{161}Tb may be more available**

Quality Control [Radiochemical Purity Tb-161]

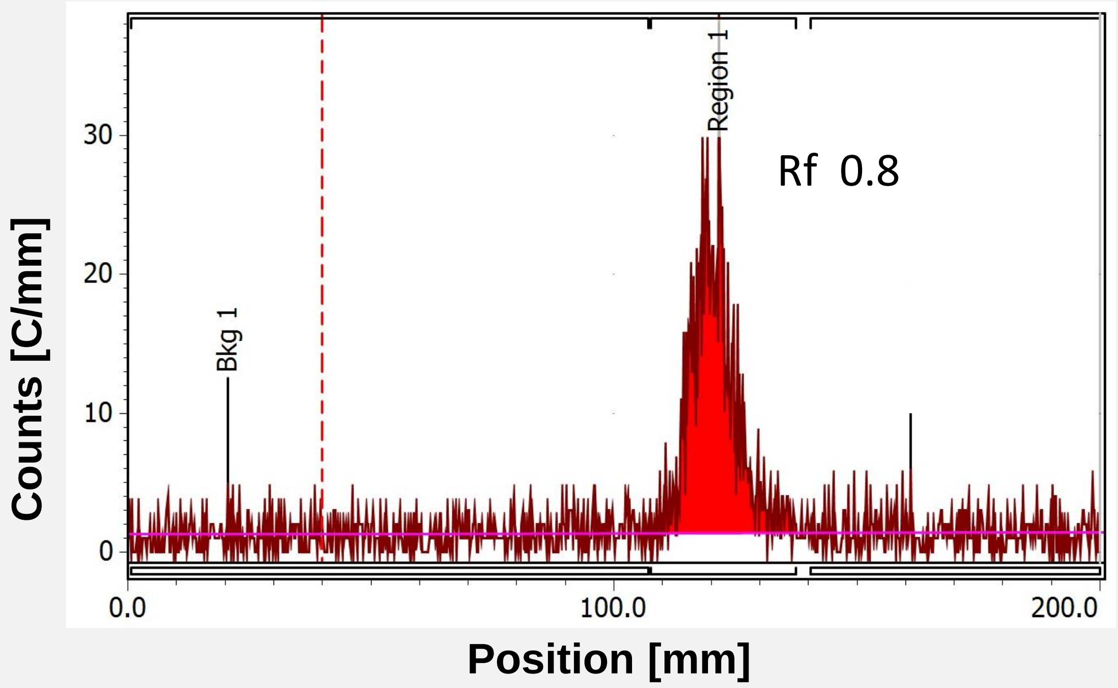
Radiochemical purity of the $^{161}TbCl_3$ solution was determined through Radio Instant Thin layer Chromatography

Radiochemical purity achieved
> 99%

The absence of the R_f 0 fraction

- No species of Tb forming colloids in Solution
- Only $^{161}Tb^{3+}$ in the final product making complex with citrate

Radio Thin Layer Chromatogram of $^{161}TbCl_3$



Solvent System: 0.1 M sodium citrate (pH 5)

Thank You