Lu-177 LABELLED RITUXIMAB - NEW APPROACH TO HAVE SUITABLE RADIOPHARMACEUTICAL

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Rituximab 144 is a chimeric anti-CD20 B-cell specific monoclonal antibody approved for the treatment of low-grade non-Hodgkin's lymphoma that has shown significant antitumor response and improved progression free survival either given alone or given as radioimmunoconjugate. CRP has been designed to focus on the preparation of 177Lu-labeled Rituximab as a therapeutic radiopharmaceutical for the treatment of lymphomas.

Conjugation of SCN-Bn-DTPA with Rituximab and radiolabelling with ^{177}Lu and ^{177}Lu-DTPA-Bn-Rituximab, QC and biodistribution studies in mice.

ARGENTINA

Evaluation and preclinical studies of ^{177}Lu-labelled Rituximab in normal and tumor mice.

BRAZIL

QC and Cytotoxicity to inhibit cell proliferation in cell lines for radioimmunoconjugates of ^{177}Lu/^{90}Y-DOTA-h-R3/Trastuzumab

CZECH REPUBLIC

Production of high specific activity, clinical grade ^{177} Lu, preparation and QC of ^{177}Lu-Reditux® and ^{177}Lu-labelled MabThera®, as well as ^{177}Lu-DOTA-TATE.

INDIA

Pre-clinical in vitro screening of kit formulations of ^{177}Lu-labelled Rituximab, QC and binding assays, as well as small animal studies

ITALY (ROME)

Development of a freeze-dried kit based on DOTA-Rituximab for labelling with ^{177}Lu, standardization and further development.

POLAND

Development of radiolabelled Rituximab using two conjugation approaches: with p-SCN-Bn-DTPA and p-SCN-Bn-DOTA and optimization of the labelling technique

SYRIA

Aim

Development and preclinical evaluation of a sterile kit formulation for Rituximab that would be suitable for in-house preparation of the radiolabeled MAb for RIT studies in patients.

IAEA

Coordinated Research Project

Development and preclinical evaluation of therapeutic radiopharmaceuticals based on 177Lu- and 90Y- labelled monoclonal antibodies and peptides

Expected inputs

1. To find the ways of extending the success of radiolabelled anti-CD20 antibodies in indolent non-Hodgkin's lymphoma to other forms of cancer
2. To improve significantly the efficacy and acceptability using radionuclides as- **Lutetium-177** with the lower energy of their emission, their relatively long half-life and good gamma emission.

The clinical preparation of Lu-177 somatostatin analogues and the development of novel radiopharmaceuticals based on other peptide analogues.

AUSTRIA

Conjugation of Rituximab with CHX-A"-DTPA and p-SCN-Bn-DOTA, QC, *in vitro* stability and preliminary biodistribution studies of the labelled conjugate.

CHINA

Conjugation of Rituximab with DTPA-CHX using different experimental conditions, RCP and in vivo assessment of tumor uptake.

CUBA

Development of conjugation/labelling procedure, optimization of the QC of the immunoconjugate and determining the stability of the labelled product

ITALY (MILAN)

Development of suitable animal models and in vivo preclinical assessment; safety-, kinetic-, excretion-, dosimetry-, efficacy results of the produced conjugates

HUNGARY

Developing ^{177}Lu-labelled bioactive compounds, peptides and antibodies and evaluation as therapeutic agents.

SAUDI ARABIA

Establishing an efficient freeze-drying procedure for developing a final kit formulation for simple antibody labelling and determining the toxicity and therapeutic efficacy of the produced kits.

REPUBLIC OF MACEDONIA

Materials

Monoclonal antibody (mAb)
Trade name: Rituxan, MabThera
(100 mg/10 ml; Roche)
Source: Chimeric monoclonal antibody
Target: CD20, primarily found on the surface of B cells
Chemical data: $C_{6416}H_{9874}N_{1688}O_{1987}S_{44}$, 144 kD
Chelators
1. **p-SCN-Bn-DOTA,** (Macrocyclics, B-205)
2. **DOTA-NHS-ester,** (Macrocyclics, B-280)
3. **DOTA** (SIGMA)
4. **DTPA** (SIGMA)

Lutetium-177

Methods

1. **mAb purification -** preconditioning/purification by ultrafiltration
2. **Conjugation -** using a bifunctional chelating agent and subsequent purification of the conjugate
3. **Purification of conjugated mAb**

^{177}Lu labeling of the DOTA conjugates

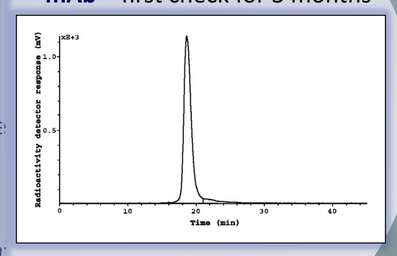
Quality control:
HPLC
TLC
Stability studies
Immunoreactivity
In vitro competitive binding assay,
Immunoreactive fraction assay,
Protein characterization by MALDI-ToF
Animal studies - **biodistribution studies** in normal mice and nude mice xenografts followed by **imaging studies**

Results

ICP-MS analysis for the determination and characterization of the complex using "not-radioactive" Lu

Freeze Drying Procedures
Volume of solution: 1ml
Filled into 2ml glass vial (fill depth = 0.75 cm)
Freeze Drier LABCONCO
Initial procedure
•Ramp from room temperature to -45^{0}C (ramp rate 1^{0}C/min)
•Hold for 2h, ramp to -20^{0}C (1^{0}C/min)
•Hold for 1h and return to -45^{0}C
•Maintain shelf temperature for 2 hours
•Primary drying was conducted at chamber pressure (Pc) of 57 mTorr and shelf temperature of -25^{0}C and +25^{0}C
•Chamber pressure (Pc) was constant for primary and secondary drying
•Primary drying for BSA was 4 min and 3 min for IgG and mAb
Secondary drying
Shelf temperature of 40^{0}C for 10 hours (increase 15-20 hours)
- (ramp rate 1^{0}C/min)

Stability studies:
-**IgG** – 6 month used freeze dried formulation
-**BSA** – 10 month
-**mAb** – first check for 3 months



CONCLUSION

The RIT involving the new radioisotopes, now as a mature technology, can and should enter in a phase of well designed and focused clinical developments that may be expected to afford significant therapeutic advances.
This proposed new radiopharmaceutical - Lu-177 labelled Rituximab can be one of the promising for the treatment of low-grade non-Hodgkin's lymphoma.