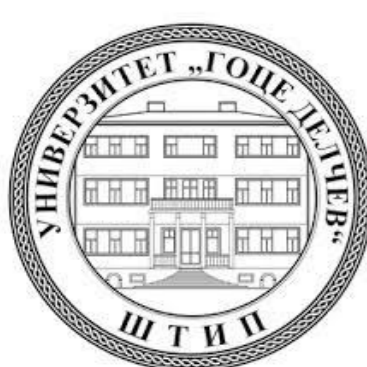


DEVELOPMENT OF READY TO USE KIT FORMULATION FOR TRASTUZUMAB RADIOIMMUNOCONJUGATES AND IDENTIFICATION OF RADIOCHEMICAL PURITY AS THE FIRST STEP IN QUALITY CONTROL OF THE FINAL PRODUCT

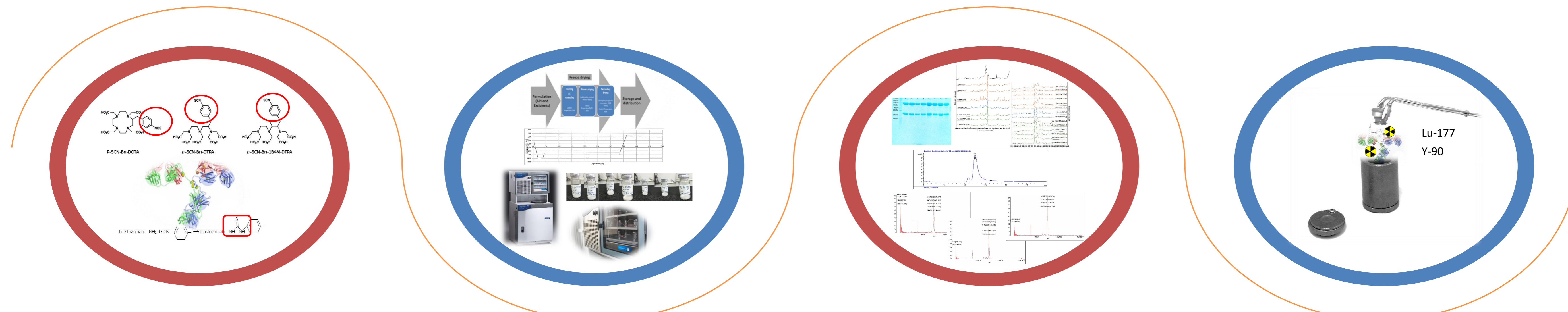
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The aim of this study is to present the part of our project dedicated to obtaining a stable, ready to use freeze dried kit formulation of antibody radioimmunoconjugates (trastuzumab immunoconjugates labelled with ⁹⁰Y and ¹⁷⁷Lu).



Conjugation of antibodies 4 °C during 18 hours

Purification by ultrafiltration (6 times)
with 0.05 M ammonium acetate (pH 7)
Immunoconjugate concentration 1 mg/mL

Freeze-drying of immunoconjugates

I - 40°C, 1°C/min, 5 hours
II -25°C, 0.15°C/min, 28 hours, 0.133 mbar
III-25°C, 0.2°C/min, 14 hours
1% mannitol

Chemical Identification

Infrared and Raman spectroscopy
MALDI-TOF MS (average number of BFCAs)
Integrity - HPLC-UV
Integrity - SDS-PAGE

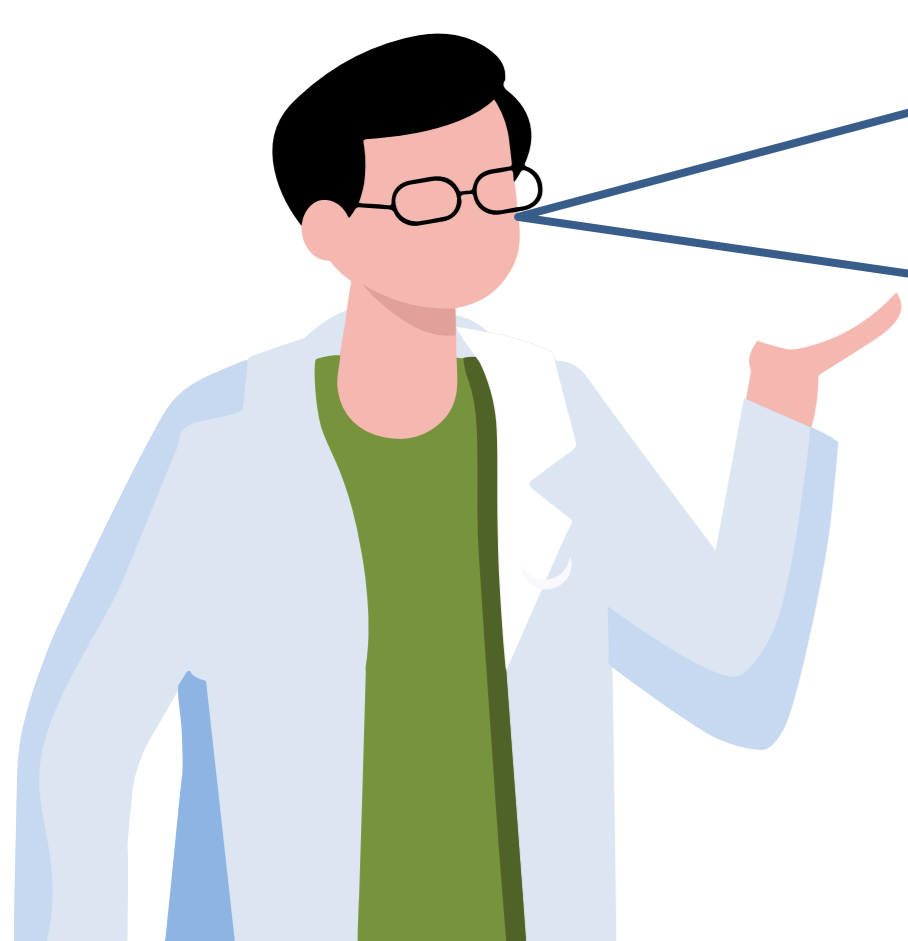
Radioactive labeling with Lu-177 / Y-90

BFCAs in the ratio 1:20
activity ⁹⁰Y 1,425 mCi, pH 4.5-5
activity ¹⁷⁷Lu 8.15 mCi, pH 6
Tr-DTPA, Tr-1B4M (30 min, room temp.)
Tr-DOTA (40 °C, 1 hour)

MATERIAL AND METHODS

Radiochemical purity - was tested with ITLC-SG using three mobile phases: 0.9% NaCl, 0.4 M methanol/sodium-acetate (1:1) and 0.1 M acetic buffer.

Stability - was tested in 0.9% NaCl (¹⁷⁷Lu) and 0.4 M methanol/sodium-acetate (1:1) (⁹⁰Y), after incubation at room temperature for 1, 24, 48 and 72h.



As the first step in on-going *in vitro* stability of the final product and radiochemical purity determination, we used ITLC-SG method with different mobile phases.

RESULTS

Stability

| | Incubation time [h] | Radiochemical Purity [%] | Release of ⁹⁰ Y and ¹⁷⁷ Lu[%] |
|-------------------------------------|---------------------|--------------------------|---|
| ⁹⁰ Y-DOTA-Trastuzuamab | 1 | 96.32 | 3.68 |
| | 24 | 92.40 | 7.6 |
| | 48 | 88.15 | 11.85 |
| | 72 | 82.83 | 17.17 |
| ⁹⁰ Y-DTPA-Trastuzuamab | 1 | 96.08 | 3.92 |
| | 24 | 88.96 | 11.04 |
| | 48 | 83.24 | 16.76 |
| | 72 | 74.99 | 25.01 |
| ⁹⁰ Y-1B4M-Trastuzuamab | 1 | 96.28 (m/a) | 3.72 |
| | 24 | 90.93 | 9.07 |
| | 48 | 87.66 | 12.34 |
| | 72 | 84.90 | 15.1 |
| ¹⁷⁷ Lu-DOTA-Trastuzuamab | 1 | 100 | 0 |
| | 24 | 99.14 | 0.86 |
| | 48 | 98.97 | 1.03 |
| | 72 | 98.52 | 1.48 |
| ¹⁷⁷ Lu-DTPA-Trastuzuamab | 1 | 99.29 | 0.71 |
| | 24 | 98.07 | 1.93 |
| | 48 | 97.88 | 2.12 |
| | 72 | 97.10 | 2.9 |
| ¹⁷⁷ Lu-1B4M-Trastuzuamab | 1 | 99.15 | 0.85 |
| | 24 | 98.55 | 1.45 |
| | 48 | 97.15 | 2.85 |
| | 72 | 96.87 | 3.13 |

Radiochemical Purity

| | | | |
|---------------------------------------|--------------------------------------|--------------------------------------|-------------------------------------|
| 96.32 % ⁹⁰ Y-DOTA-Tr | 96.08 % ⁹⁰ Y-DTPA-Tr | 96.28 % ⁹⁰ Y-1B4M-Tr | → 0.4 M methanol/ sodium acetate |
| 100.00 % ¹⁷⁷ Lu-DOTA-Tr | 99.29 % ¹⁷⁷ Lu-DTPA-Tr | 99.15 % ¹⁷⁷ Lu-1B4M-Tr | |

Conclusion

Radiochemical purity of used kit formulation (ITLC-SG) shows:

- high radiolabeling efficiency (>95%), using both isotopes.
- radioactive yield with ¹⁷⁷Lu (99%) was higher compared with ⁹⁰Y (>96%).
- radiolabeled conjugates are stable after 72 hours of incubation,
- small amount of free radioisotopes was released from radioimmunoconjugates (<5% of ¹⁷⁷Lu and <25% of ⁹⁰Y).