



DETERMINATION OF AVERAGE NUMBER OF CHELATORS CONJUGATED TO TRASTUZUMAB USING A MALDI-TOF MS

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INTRODUCTION

The importance of trastuzumab (Tr) conjugation with bifunctional chelators (BFCs) in different molar ratio are presented with this study. A successful radiolabeling can be achieved after binding of optimal number of BFCs (4-5) per monoclonal antibody (mAb). The aim of our examination is to show that using a different BFCs in different molar ratio (*p*-SCN-Bn-DTPA – 1:10, 1:20, 1:50; *p*-SCN-Bn-DOTA – 1:20; *p*-SCN-Bn-1B4M-DTPA – 1:10, 1:20, 1:50) will achieve enough binding of the BFCs to Tr and formulation of immunoconjugates for further radiolabeling.

MATERIAL AND METHODS

Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) was employed for determination of average number of BFCs. Resuspension solution (30% ACN/70% 0.1 TFA) was added to the samples and an aliquot of 1 μ l of the final sample was applied to the well plate template and mixed with 1 μ l of matrix (20 mg/ml Sinapinic acid in 50% ACN/50% 0.1 TFA). Acquisition mass range of the instrument is 100-300000 Da.

RESULTS

An optimal number of BFCs were calculated in all samples. There was no significant difference in the number of the attached chelating groups by higher excess of the chelators: *p*-SCN-Bn-DTPA-Tr [1:10 (5 groups); 1:20 (4.8 groups); 1:50 (5.3 groups)]; *p*-SCN-Bn-DOTA-Tr 1:20 (4.9 groups); *p*-SCN-Bn-1B4M-DTPA [1:10 (4.9 groups); 1:20 (4.5 groups); 1:50 (4.3 groups)].

CONCLUSION

MALDI-TOF MS calculations have shown successful conjugations and formulated immunoconjugates are protentional compounds for further ⁹⁰Y and ¹⁷⁷Lu radiolabeling.