Production of ^{89}Zr -Pembrolizumab

In short, 5 mg pembrolizumab (0.2 mL, 25 mg/mL) was mixed with 770 µL 0.9% NaCl and the pH of the solution was adjusted to 8.9-9.1 with 0.1 M Na_2CO_3. Next, this solution was added to 20 µL of 5 mM (3 equivalents) NCS-Bz-DFO in DMSO (Macrocyclics, Boston, USA) and incubated for 30 min at 37˚C at 550 rpm in a thermomixer. After purification by size exclusion chromatography (PD10, GE Healthcare) to remove unreacted DFO-Bz-NCS, the product, DFO-pembrolizumab, was radiolabelled with zirconium-89 in a 2 mL reaction. The reaction mixture consisting of 200 µL 1M oxalic acid (containing the required amount of ^{89}Zr), 90 µL 2M Na_2CO_3, 1 mL 0.5 M Hepes and 0.71 mL DFO-Pembrolizumab (~1.7 mg) was reacted for 60 minutes at room temperature while slowly shaken. The product was isolated by size exclusion chromatography using a PD10 column. The product was eluted in 50 mM NaOAc + 200 mM Sucrose pH 5.5 ± 0.3 and formulated to arrive at an injection dose of 37 MBq – 2 mg – 20 mL [^{89}Zr]Zr-Pembrolizumab. The following quality controls were performed and met the pre-set specifications. pH: 5.7 ± 0.2; radiochemical purity: 99.1 ± 0.8% (spin filter) and 99.8 ± 0.9 % (SE-HPLC); protein integrity: 100.0 ± 0.1 %; immune reactive fraction (binding assay): 89.6 ± 4.4 %; endotoxin content: <0.2 EU/mL [1]. Size exclusion HPLC was performed using a superdex increase 200 10/30 GL size exclusion column (GE healthcare Life sciences) including a guard column using a mixture of 0.05 M sodium phosphate, 0.15 M sodium chloride (pH 6.8) and 0.01 M NaN_3 as the eluent at a flow rate of 0.5 mL/min. Sterility of each ^{89}Zr-pembrolizumab batch was assured by performing a media fill immediately after final filter sterilisation of each batch of [^{89}Zr]Zr-pembrolizumab.

References

1. Vugts DJ, Klaver C, Sewing C, et al: Comparison of the octadentate bifunctional chelator DFO*-pPhe-NCS and the clinically used hexadentate bifunctional chelator DFO-pPhe-NCS for (89)Zr-immuno-PET. Eur J Nucl Med Mol Imaging 44:286-295, 2017