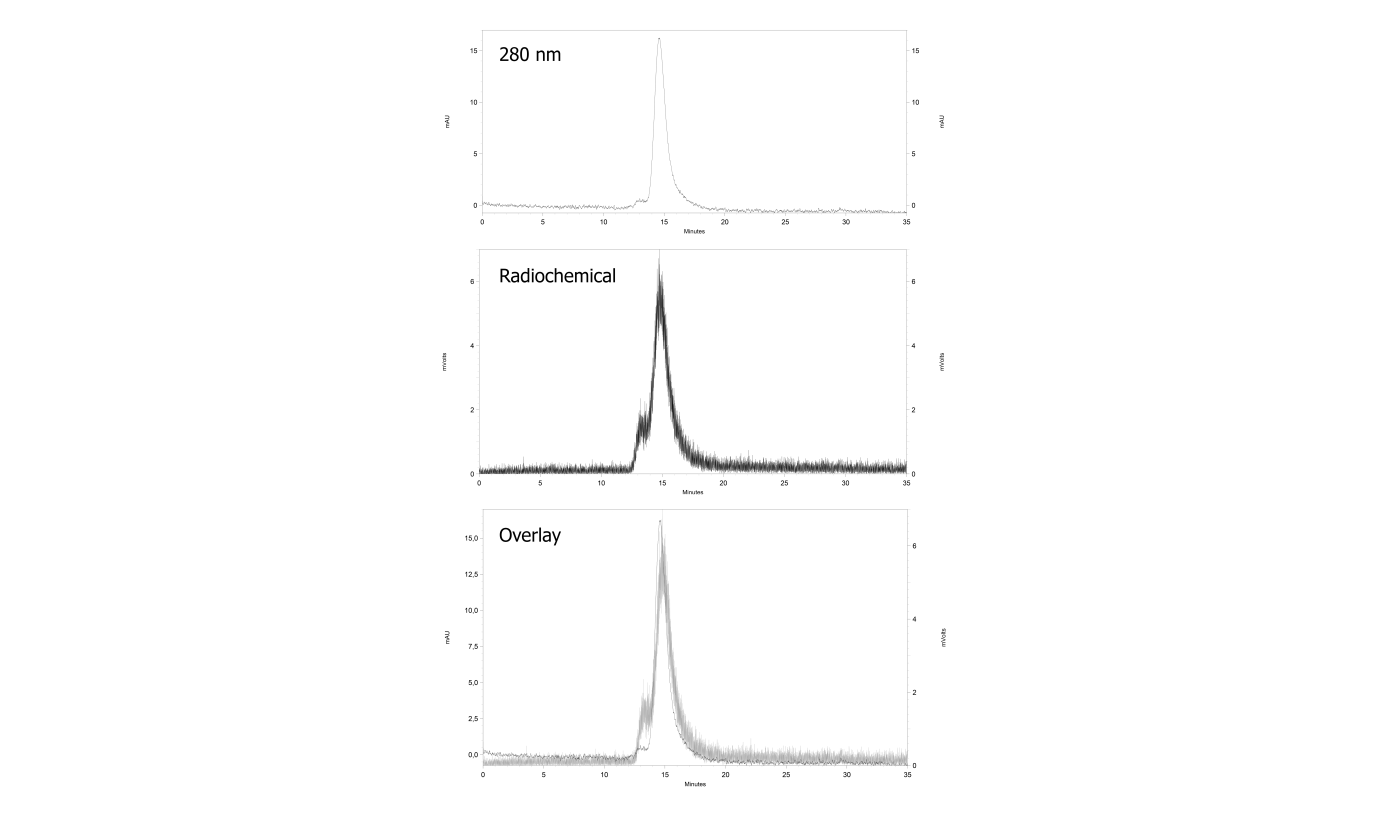
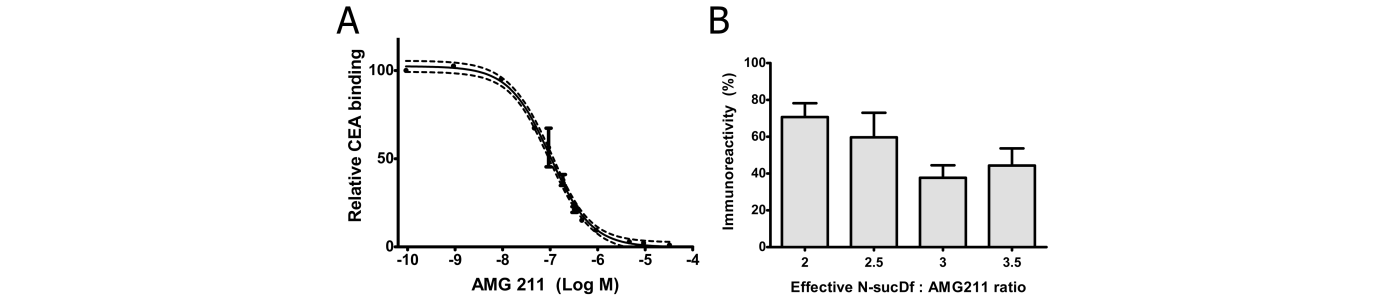
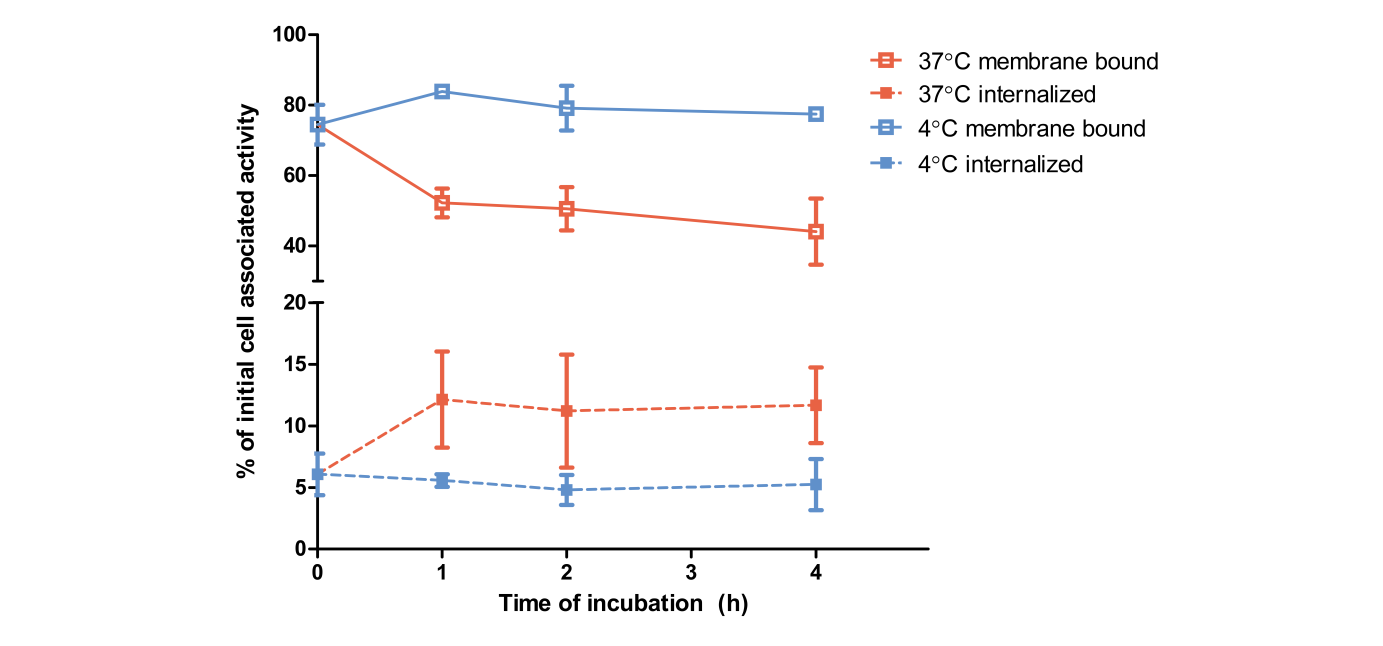
C:\Users\waaijersjh\Dropbox\AMG211 resubmission\Tiff figures\Sup Fig S1.tif **Figure S1.** Flow chart of the drug substance (N-sucDf-AMG211) manufacturing process and drug product (89Zr-AMG211) formulation and filling process.

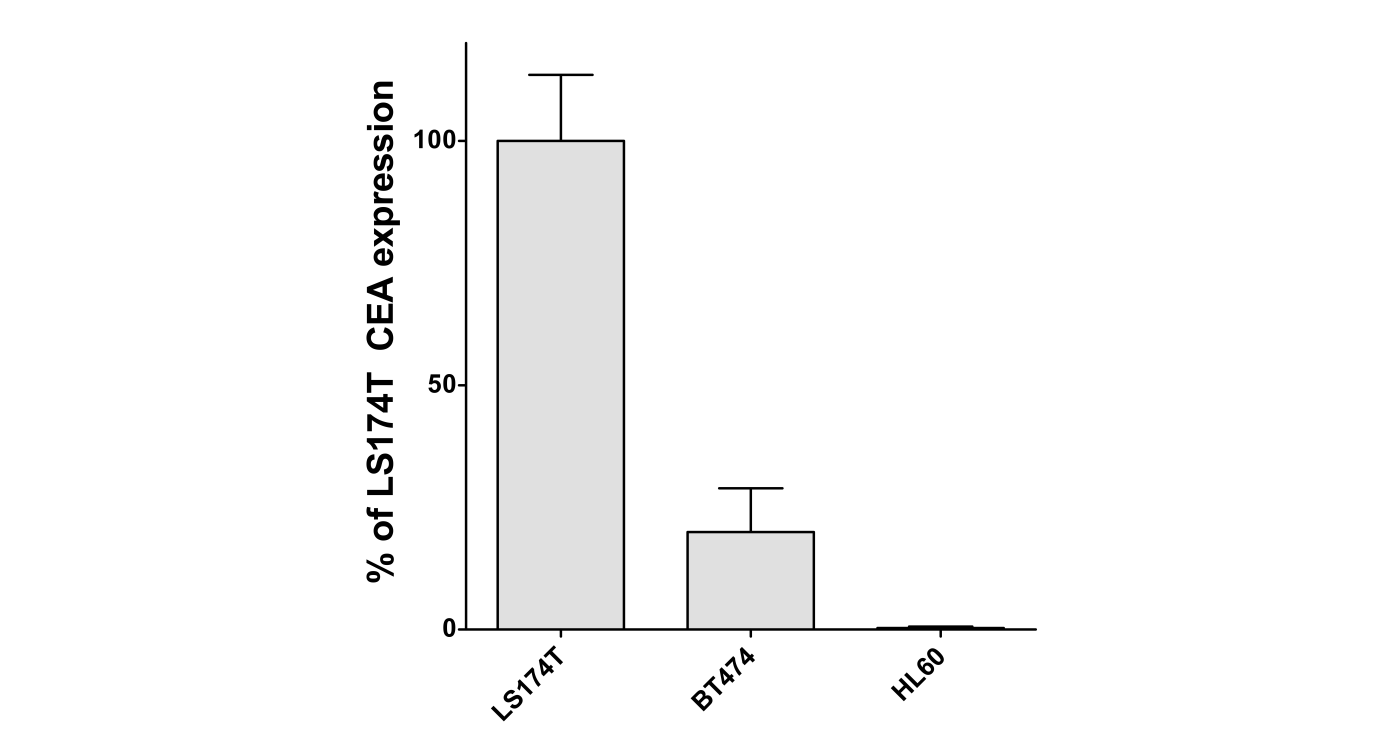
 **Figure S2.** Quality control of 89Zr‑AMG211. Representative size exclusion high performance liquid chromatography chromatogram of 89Zr‑AMG211 with 280 nm signal (top panel), radiochemical signal (middle panel) and the overlay (lower panel).

 **Figure S3.** Immunoreactivity of 89Zr‑AMG211. A) Representative competition assay using an effective N‑sucDf:AMG 211 ratio of 2:1. Curve fit with 95% confidence interval is visualized. B) Immunoreactivity towards CEA of different ratios N‑sucDf:AMG 211. Data are mean ± SD**.**

**Figure S4.** Membrane binding and internalization of 89Zr‑AMG211 after CEA binding on LS174T cells (*n* = 3). Membrane bound and internalized 89Zr-AMG211 are expressed as percentage of initial cell associated activity. Data are mean ± SD.



**Figure S5.** Expression of CEA on LS174T, BT474 and HL‑60 cell lines (*n* = 3). Membrane expression is expressed as percentage of LS174T signal. Data are mean ± SD.



**Table S1. GMP manufacturing of N‑sucDf‑AMG211 and 89Zr‑AMG211**. Release criteria are fulfilled for batch 1, 2, and 3. In addition stability are shown for N‑sucDf‑AMG211 stored at ‑80°C for 6 months, all quality criteria are still met.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Test** | **Specification** | **Batch 1** | **Batch 2** | **Batch 3** | **Batch 3**  **After 6M at -80°C** |
| **N-sucDf-AMG211** | | | | | |
| **Appearance** | Colourless to light yellow | Colourless | Colourless | Colourless | Colourless |
| **Conjugation ratio**  **of N-sucDf: AMG211** | 1.5-2.5 | 1.6 | 1.7 | 1.5 | Not applicable |
| **Impurities of  N-sucDf-AMG211 after conjugation** | ≤ 5% | 2% | 3% | 2% | Not applicable |
| **Filter integrity** | ≤ 20% | 15% | 14% | 19% | Not applicable |
| **Impurities of  N-sucDf-AMG211 end product** | ≤ 5% | 2% | 3% | 3% | 3% |
| **Signal reduction at 430 nm** | > 40% reduced | 78% | 68% | 80% | 84% |
| **Concentration** | 1.5-2.5 mg/ml | 1.59 mg/mL | 1.80 mg/mL | 1.65 mg/mL | 1.73 mg/mL |
| **pH** | pH 4.0 - 6.0 | 5.6 | 5.7 | 5.9 | 5.8 |
| **Endotoxins:** | ≤ 2.5 EU/ml | 0.687 EU/mL | 0.659 EU/mL | 0.542 EU/mL | Not determined |
| **Sterility:** | Sterile | Sterile | Sterile | Sterile | Not determined |
| **Residual solvents (ACN)** | < 410 ppm | < 100 ppm | < 100 ppm | < 100 ppm | Not determined |
| **Immunoreactivity towards CD3** | Preserved\* | Preserved | Preserved | Preserved | Preserved |
| **89Zr-AMG211** | | | | | |
| **Appearance** | Colourless to light yellow | Colourless | Colourless | Colourless | Colourless |
| **Radiochemical purity pre-purification** | ≥ 70% | 94.0% | 92.5% | 91.4% | 93.4% |
| **Radiochemical purity post-purification** | ≥ 95% | 96.8% | 98.1% | 97.6% | 98.2% |
| **pH** | pH 5.0-8.0 | 6.1 | 6.2 | 6.1 | 6.1 |
| **Filter integrity** | ≤ 20% | 19% | 14% | 14% | 16% |
| **Impurities of  89Zr-AMG211** | ≤ 5% | 1.5% | 2.4% | Not detectable | 2.2% |
| **Bacterial endotoxins** | ≤ 2.5 EU/mL | 0.450 EU/mL | 0.412 EU/mL | 0.532 EU/mL | Not determined |
| **Sterility** | Sterile | Sterile | Sterile | Sterile | Not determined |
| **Immunoreactivity towards CEA** | Preserved\* | Preserved | Preserved | Preserved | Preserved |

\* Preserved immunoreactivity indicates more than 50% binding compared to unconjugated for CD3 and more than 50% immunoreactivity for CEA