A comparison of $^{64}$Cu-labeled bi-terminally PEGylated A20FMDV2 peptides targeting integrin $\alpha_\nu\beta_6$

SUPPLEMENTARY MATERIALS

Supplementary Figure 1: HPLC chromatograms showing the $^{64}$Cu labeled radiotracers. Radio-HPLC analysis was performed with a mobile phase of water (0.1% TFA) and acetonitrile (0.1% TFA), 20–100% acetonitrile from 0 to 14 mins, and elution was run with a 1 mL/min flow rate.

Supplementary Figure 2: Radio-TLC chromatograms of $[^{64}\text{Cu}]\text{Cu-DOTA-(PEG28)}$-A20FMDV2 (A) and $[^{64}\text{Cu}]\text{Cu-PCTA-(PEG28)}$-A20FMDV2 (B) in 50 mM DTPA.
**Supplementary Figure 3:** Stability profiles in PBS and human serum at 37°C up to 24 h of radiotracers (A) $[^{64}\text{Cu}]\text{Cu-DOTA-(PEG28)}_2$-A20FMDV2 and (B) $[^{64}\text{Cu}]\text{Cu-PCTA-(PEG28)}_2$-A20FMDV2.

**Supplementary Figure 4:** Expression of $\alpha_v\beta_6$ on a variety of cell lines as determined by flow cytometry. The bar graph shows mean fluorescence units (MFU) when cells were incubated with either the 10D5 antibody ($\alpha_v\beta_6$-positive) or an IgG2a negative control isotype antibody.
## Supplementary Table 1: Tumor-to-organ ratios of $^{64}$Cu-labeled bi-terminally PEGylated A20FMDV2 peptides in $\alpha\beta\gamma$-positive CaSki tumor-bearing mice

| Tissue    | 1 h after injections | 4 h after injection | 24 h after injection |
|-----------|----------------------|---------------------|----------------------|
|           | DOTA | PCTA | DOTA | PCTA | DOTA | PCTA | DOTA | PCTA |
| Blood     | 4.87 | 1.48 | 2.98 | 0.29 | 4.80 | 0.94 | 3.09 | 1.83 |
| Lung      | 0.71 | 0.21 | 0.44 | 0.15 | 0.52 | 0.13 | 0.61 | 0.32 |
| Liver     | 0.90 | 0.18 | 0.24 | 0.06 | 0.45 | 0.11 | 0.34 | 0.30 |
| Spleen    | 5.81 | 0.77 | 2.56 | 0.76 | 3.11 | 0.47 | 2.28 | 1.39 |
| Muscle    | 1.36 | 0.30 | 1.33 | 0.08 | 1.71 | 0.19 | 2.57 | 2.15 |
| Heart     | 1.37 | 0.34 | 0.65 | 0.18 | 1.48 | 0.09 | 1.53 | 1.25 |
| Bone      | 3.36 | 2.59 | 3.20 | 1.09 | 5.51 | 1.89 | 5.22 | 2.88 |
| Kidney    | 0.02 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.02 | 0.02 |

## Supplementary Table 2: Tumor-to-organ ratios of $^{64}$Cu-labeled bi-terminally PEGylated A20FMDV2 peptides in $\alpha\beta\gamma$-positive BxPC-3 tumor-bearing mice

| Tissue    | 1 h after injections | 4 h after injection | 24 h after injection |
|-----------|----------------------|---------------------|----------------------|
|           | DOTA | PCTA | DOTA | PCTA | DOTA | PCTA | DOTA | PCTA |
| Blood     | 7.86 | 2.21 | 14.25 | 0.86 | 5.53 | 1.23 | 13.65 | 2.44 |
| Lung      | 1.21 | 0.40 | 1.51 | 0.21 | 1.12 | 0.14 | 1.71 | 0.27 |
| Liver     | 0.76 | 0.23 | 2.02 | 0.44 | 0.63 | 0.11 | 1.29 | 0.05 |
| Spleen    | 7.06 | 2.08 | 14.36 | 3.70 | 4.21 | 0.92 | 11.45 | 3.39 |
| Muscle    | 3.37 | 0.68 | 3.51 | 0.78 | 4.60 | 0.43 | 3.75 | 0.18 |
| Heart     | 2.06 | 0.57 | 2.04 | 0.32 | 2.62 | 0.21 | 2.72 | 0.25 |
| Bone      | 9.29 | 2.53 | 10.94 | 6.20 | 8.31 | 1.67 | 19.22 | 9.75 |
| Kidney    | 0.06 | 0.03 | 0.04 | 0.01 | 0.05 | 0.01 | 0.03 | 0.01 |

## Supplementary Table 3: Biodistribution profile of $^{64}$Cu-labeled bi-terminally PEGylated A20FMDV2 peptides in $\alpha\beta\gamma$-positive CaSki tumor-bearing mice (1 h and 1 h Block)

| Tissue    | 1 h Block | 24 h Block |
|-----------|-----------|------------|
| Blood     | 0.22 | 0.14 |
| Lung      | 1.37 | 0.36 |
| Liver     | 1.04 | 0.14 |
| Spleen    | 0.17 | 0.06 |
| Muscle    | 0.73 | 0.33 |
| Heart     | 0.71 | 0.24 |
| Bone      | 0.62 | 0.76 |
| Kidney    | 42.72 | 35.48 |
| Tumor     | 0.95 | 0.29 |
**Supplementary Table 4: Biodistribution profile of \(^{64}\text{Cu}\)-labeled bi-terminally PEGylated A20FMDV2 peptides in \(\alpha_\nu\beta_\nu\)-positive BxPC-3 tumor-bearing mice (1 h and 1 h Block)**

|               | DOTA       | PCTA       |
|---------------|------------|------------|
|               | 1 h        | 1 h Block  | 1 h         | 1 h Block  |
| %ID/g         | SD         | %ID/g      | SD          | %ID/g      | SD          | %ID/g      | SD          |
| Blood         | 0.28       | 0.07       | 0.24        | 0.07       | 0.27        | 0.03       | 0.27        | 0.11       |
| Lung          | 1.73       | 0.29       | 1.14        | 0.20       | 2.58        | 0.41       | 1.25        | 0.27       |
| Liver         | 2.79       | 0.59       | 2.59        | 0.36       | 1.94        | 0.20       | 1.68        | 0.20       |
| Spleen        | 0.30       | 0.06       | 0.34        | 0.10       | 0.28        | 0.04       | 0.24        | 0.05       |
| Muscle        | 0.62       | 0.22       | 0.18        | 0.06       | 1.13        | 0.24       | 0.28        | 0.12       |
| Heart         | 1.01       | 0.15       | 0.34        | 0.06       | 1.90        | 0.25       | 0.27        | 0.02       |
| Bone          | 0.25       | 0.12       | 0.28        | 0.14       | 0.38        | 0.31       | 0.66        | 0.92       |
| Kidney        | 38.04      | 5.47       | 27.60       | 2.79       | 105.39      | 13.58      | 80.64       | 15.50      |
| Tumor         | 2.13       | 0.83       | 0.71        | 0.16       | 3.86        | 0.58       | 0.75        | 0.09       |