**Supplementary Data**

Supplementary methods.

Supplementary Fig. S1. 89Zr-AMG 211 presence in healthy tissues at 3 hours expressed as %ID.

Supplementary Table S1. Calculated 89Zr-AMG 211 serum half-life.

Supplementary Table S2. Median 89Zr-AMG 211 SUVmean in kidneys, liver, spleen,

bone marrow, lung, and intestine per dosing cohort and per PET scan time point.

Supplementary Table S3. Quantifiable tumor lesions on 89Zr-AMG 211 PET.

**Methods**

**89Zr-AMG 211 administration**

The tracer 89Zr-AMG 211, with or without cold AMG 211, was administered over 3 hours, based on the maximum tolerated dose that was assessed in the AMG 211 phase 1 study at the time the protocol was written. If patients received only 200 µg 89Zr-AMG 211, this was administered in 3 hours. In the 200 µg 89Zr-AMG 211 + 1,800 µg cold AMG 211 group, cold AMG 211 was administered first in 162 minutes, followed by 89Zr-AMG 211 in 18 minutes, while this was 173 minutes and 7 minutes in patients receiving 200 µg 89Zr-AMG 211 + 4,800 µg cold AMG 211, respectively. When imaging was performed immediately after the end of the second AMG 211 treatment period, 200 µg 89Zr-AMG 211 infusion over 3 hours was started within 30 +/- 5 minutes after completion of AMG 211 continuous IV infusion.

**Calculations**

AMIDE output (activity concentration in Bq/cc) was used to calculate the standardized uptake value (SUV) of every VOI with the following formula:

$$SUV=\frac{Activity concentration \left(\frac{Bq}{cc}\right)x 0.001}{Injected activity \left(MBq\right)/ Weight patient (kg) }$$

Subsequently, for all VOIs, the percentage injected tracer dose per kilogram (%ID/kg) was calculated with the following formula:

$$\%ID/kg=\frac{Activity concentration \left(\frac{Bq}{cc}\right)x 0.001}{Injected activity \left(Bq\right) }\*100\%$$

Injected activity was corrected for decay between moment of tracer injection and time of scanning (under the assumption of a tissue density of 1 kg/L).

**89Zr pharmacokinetics**

Radioactivity was measured in 1 mL whole blood and 1 mL urine with a calibrated well-type gamma-counter (LKB Instruments). The SUV on PET in the blood pool was correlated to the calculated SUV in blood samples at each PET scan time point.

To assess binding of 89Zr-AMG 211 to immune cells, 4 mL of whole blood collected at each PET scan time point was separated by Ficoll-Paque PLUS. Plasma, buffy coat and remaining sample including erythrocytes and granulocytes were collected after centrifugation, and radioactivity was determined with a gamma counter. Buffy coat, containing platelets and most leukocytes, was isolated and washed with phosphate buffered saline (140 mM NaCl, 9 mM Na2HPO4, 1.3 mM NaH2PO4, pH = 7.4). Radioactivity in plasma, buffy coat and remaining blood was expressed as % of total radioactivity in blood.

To study 89Zr-AMG 211 integrity, Mini-PROTEAN®TGX™ Precast Gels (10%; Bio-Rad) were loaded with 5 µL plasma and 1 µL urine collected at each PET scan time point, together with 89Zr-AMG 211 as a positive control. Gels were exposed overnight to phosphor imaging screens (Perkin Elmer) in X-ray cassettes. The screens were read using a Cyclone Storage Phosphor System (Perkin Elmer) and Optiquant™ software version 3.00. Molecular weight was verified using ProSieve™ color protein maker (Lonza).

**Supplementary Fig. S1.** 89Zr-AMG 211 presence in healthy tissues at 3 hours expressed as %ID.Each dot represents one patient, and the different imaging dosing cohorts are represented by symbols either in blue (before AMG 211 treatment) or in green (during AMG 211 treatment): circle = 200 µg 89Zr-AMG 211; triangle = 200 µg 89Zr-AMG 211 + 1,800 µg cold AMG 211; square = 200 µg 89Zr-AMG 211 + 4,800 µg cold AMG 211; diamond = 200 µg 89Zr-AMG 211 after AMG 211 6,400 µg/day for 28 days; hexagon = 200 µg 89Zr-AMG 211 after AMG 211 12,800 µg/day for 28 days.

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| **Supplementary Table S1.** Calculated 89Zr-AMG 211 serum half-life |
|  | **200 µg 89Zr-AMG 211** |
|  | **Before treatment** | **During treatment** |
| Added cold AMG 211 dose (µg) | 0 | 1,800 | 4,800 | 0 | 0 |
| AMG 211 treatment dose (µg/day) | 6,400 | 6,400 | 6,400 | 6,400 | 12,800 |
| Number of patients | 2 | 4 | 2 | 1 | 1 |
| Serum half-life | 2.4 | 3.3 | 2.6 | 3.5 | 16.4 |
| Weight (kg)\* | 73 | 84 | 87 | 81 | 84 |
| eGFR (mL/min\*1.73 m2)\* | 107 | 88 | 90 | 82 | 70 |

**\*** In case *n* > 1 data is shown as mean.

**Supplementary Table S2.** Median 89Zr-AMG 211 SUVmean in kidneys, liver, spleen,
bone marrow, lung, and intestine per dosing cohort and per PET scan time point

|  |  |
| --- | --- |
|  | **200 µg 89Zr-AMG 211** |
|  | **Before treatment** | **During treatment** |
| **Added cold AMG 211 dose (µg)** | **0** | **1,800** | **4,800** | **0** | **0** |
| **AMG 211 treatment dose (µg/day)** | **6,400** | **6,400** | **6,400** | **6,400** | **12,800** |
| **Number of patients** | **2** | **4** | **2** | **1** | **1** |
| Kidney, 3 hours | 87.2\* | 89.0 | 94.1 | 52.7 | 30.1 |
| Kidney, 6 hours | 95.8 | 90.1 | 112.6 | 73.5 | 36.4 |
| Kidney, 24 hours | 75.7 | 96.0 | 111.2 | 99.6 | 41.7 |
| Liver, 3 hours | 4.5\* | 3.1 | 4.7 | 3.4 | 4.2 |
| Liver, 6 hours | 4.1 | 2.9 | 4.0 | 3.9 | 3.9 |
| Liver, 24 hours | 4.5 | 2.0 | 3.8 | 3.6 | 5.7 |
| Spleen, 3 hours  | 2.9\* | 3.2 | 4.4 | 3.4 | 3.4 |
| Spleen, 6 hours | 2.3 | 3.1 | 3.3 | 3.0 | 2.7 |
| Spleen, 24 hours | 1.5 | 1.4 | 2.7 | 2.5 | 1.8 |
| Bone marrow, 3 hours | 1.0\* | 1.8 | 1.6 | 1.6 | 2.6 |
| Bone marrow, 6 hours | 0.9 | 1.0 | 1.4 | 1.2 | 1.6 |
| Bone marrow, 24 hours | 0.9 | 1.0 | 0.7 | 1.0 | 2.0 |
| Intestine, 3 hours | 3.0\* | 1.9 | 1.1 | 3.9 | 1.6 |
| Intestine, 6 hours | 2.8 | 1.9 | 2.7 | 3.0 | 1.6 |
| Intestine, 24 hours | 3.8 | 2.5 | 3.2 | 3.4 | 0.9 |
| Lung, 3 hours | 0.5\* | 0.6 | 0.9 | 1.0 | 1.0 |
| Lung, 6 hours | 0.3 | 0.4 | 0.5 | 0.8 | 0.9 |
| Lung, 24 hours | 0.1 | 0.2 | 0.2 | 0.2 | 0.6 |

 \* This data is based on *n* = 1 patient since in the other patient in the same dosing

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| cohort PET imaging was not performed 3 hours post tracer infusion. **Supplementary Table S3.** Quantifiable tumor lesions on 89Zr-AMG 211 PET |
| **Patient** | **Lesion** | **Organ** | **Longest axis (mm)\*** | **Tumor uptake (SUVmax)** |
| **3 hours** | **6 hours** | **24 hours** |
| 1 | 1 | Lung | 25 | ND | 2.0 | 1.4 |
|  | 2 | Lung | 18 | ND | 1.7 | 2.6 |
|  | 3 | Liver | 40 | ND | 3.9 | 3.4 |
|  | 4 | Liver | 16 | ND | 2.6 | 4.2 |
|  | 5 | Liver | 52 | ND | 1.4 | 2.6 |
|  | 6 | Liver | 73 | ND | 3.5 | 4.5 |
|  | 7 | Liver | 38 | ND | 3.8 | 4.1 |
|  | 8 | Liver | 45 | ND | 4.6 | 5.8 |
|  | 9 | Liver | 42 | ND | 4.2 | 4.2 |
|  | 10 | Liver | 52 | ND | 5.0 | 3.7 |
|  | 11 | Liver | 36 | ND | 3.2 | 4.2 |
|  | 12 | Liver | 20 | ND | 4.2 | 4.0 |
|  | 13 | Bone |  | ND | 3.2 | 1.3 |
| 2 | 1 | Liver | 63 | 4.8 | 3.5 | 3.1 |
|  | 2 | Liver | 67 | 3.0 | 3.5 | 3.8 |
|  | 3 | Liver | 39 | 2.8 | 2.6 | 3.9 |
|  | 4 | Liver | 54 | 2.1 | 1.3 | 3.4 |
| 3 | 1 | Lung | 22 | 4.0 | 2.5 | 2.8 |
|  | 2 | Lung | 45 | 2.3 | 3.0 | 2.0 |
|  | 3 | Lung | 26 | 4.0 | 3.5 | 1.9 |
|  | 4 | Lung | 15 | 2.4 | 11.3 | 3.3 |
| 5 | 1 | Colon | 63 | 3.1 | 3.8 | 2.9 |
|  | 2 | Lymph node | 39 | 7.6 | 5.9 | 2.9 |
|  | 3 | Liver | 60 | 2.8 | 1.5 | 1.7 |
|  | 4 | Liver | 49 | 4.8 | 1.8 | 1.6 |
|  | 5 | Liver | 46 | 5.3 | 3.3 | 3.0 |
|  | 6 | Liver | 48 | 2.7 | 4.2 | 3.3 |
|  | 7 | Liver | 49 | 2.1 | 5.6 | 4.4 |
| 7 | 1 | Soft tissue | 63 | 1.4 | NE | 1.9 |
|  | 2 | Liver | 19 | 4.6 | NE | 3.4 |
|  | 3 | Liver | 26 | 4.0 | NE | 4.1 |
|  | 4 | Liver | 46 | 3.0 | NE | 2.5 |
|  | 5 | Liver | 34 | 4.4 | NE | 2.1 |
|  | 6 | Liver | 64 | 3.0 | NE | 1.4 |
|  | 7 | Liver | 27 | 4.4 | NE | 5.9 |
|  | 8 | Liver | 27 | 4.5 | NE | 3.0 |
| 8 | 1 | Liver | 49 | 3.8 | 2.7 | 1.1 |
| \* As measured on diagnostic CT. ND, not done; NE, not evaluable due to technical reasons. |