**Supplementary Figures**

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**Supplementary Figure 1:** Serum levels of AMA-MMAE.

Serum levels of AMA-MMAE in mice after a single injection of 1mg/kg (blue) or 10mg/kg (orange) AMA-MMAE show that this ADC has dose-linear pharmacokinetic properties in the 1- 10mg/kg range (A). Comparison between the pharmacokinetics of ADC and naked antibody show that the decreased clearance of AMA-MMAE is within two-fold of the value for the naked antibody AMA (B).



**Supplementary Figure 2:** Correlation plot of tumor growth inhibition versus specific tumor uptake**.**

A correlation plot of tumor growth inhibition versus specific tumor uptake (AMA : gD ratio) revealed a correlation (r2= 0.823).

**Supplementary Table 1:** Tumor growth inhibition study of mice bearing OVCAR3-X2.1.

**Table 1:** Tumor growth inhibition study of mice bearing OVCAR3-X2.1 cells comparing the efficacy of 5mg/kg and 20mg/kg doses (n=6 mice per group).

|  |  |  |
| --- | --- | --- |
|  | **5mg/kg AMA-MMAE** | **20mg/kg AMA-MMAE** |
| **Day** | **Tumor size (mm3)** | **SD** | **Tumor size (mm3)** | **SD** |
| 0 | 351 | 82 | 330 | 76 |
| 5 | 408 | 112 | 315 | 64 |
| 10 | 272 | 69 | 183 | 62 |
| 14 | 205 | 66 | 109 | 50 |
| 18 | 158 | 35 | 70 | 28 |

**Supplementary Table 2:** Overview results.

**Table 2:** Overview results of tumor growth inhibition studies (5mg/kg), tumor tracer uptake of 89Zr-AMA and 89Zr-anti-gD 6 days after tracer injection. Tumor-to-blood ratios of 89Zr-AMA and 89Zr-anti-gD, AMA:gD ratio and mesothelin expression as determined by FACS analysis and immunohistochemistry are also presented. \*indicates a significant difference (*p* < 0.05) between 89Zr-AMA and 89Zr-anti-gD.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Tumor** | **% Tumor growth inhibition** | **89Zr-AMA****(%ID/g)** | **89Zr-anti-gD****(%ID/g)** | **Tumor-to-blood ratio****89Zr-AMA** | **Tumor-to-blood ratio****89Zr-anti-gD** | **AMA:gD ratio** | **Mesothelin expression by FACS** | **Mesothelin expression by IHC** |
| OVCAR-3X2.1 | 92 | 10.4 ± 0.8\*  | 4.9 ± 0.7 | 2.0 ± 0.4\* | 0.7 ± 0.1 | 2.1 | 3+ | 3+ |
| Capan-2 | 67 | 8.8 ± 1.0\* | 4.2 ± 0.6 | 1.4 ± 0.3\* |  0.6 ± 0.1 | 2.1 | 2+ | 2-3+ |
| HPAC | 66 | 6.0 ± 1.2\* | 3.7 ± 0.9 | 1.8 ± 0.3\* | 0.6 ± 0.1 | 1.6 | 2+ | 3+ |
| AsPC-1 | 39 | 6.0 ± 0.7 | 4.9 ± 0.4 | 0.8 ± 0.1 | 0.5 ± 0.1 | 1.2 | 2+ | 3+ |
| HPAF-II | 20 | 5.9 ± 0.3 | 6.1 ± 0.7 | 1.0 ± 0.1 | 0.8 ± 0.1 | 1.0 | 1-2+ | 2+ |
| MSTO-211H | 0 | 9.0 ± 2.8 | 8.3 ± 1.5 | 1.1 ± 0.3 | 1.3 ± 0.3 | 1.1 | 0.5+ | 1+ |