## Supplementary tables

### Supplementary table 1: Patient and tumor characteristics

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **N (%)** | **Age (y) median (range)** | **Stage** **n (%)** | **Tumor size** **(cm)** **median** **(range)** | **Complete****resection****n (%)** | **Adjuvant chemo****n (%)** | **Adjuvant radio****n (%)** | **Adjuvant hormone****n (%)** | **Median PFS/OS (months)** |
|  |  |  | **I** | **II** | **III** | **IV** | **NA** |  |  |  |  |  |  |
| LMS | 153(52) | 57(27-90) | 47(49) | 7(7) | 21(22) | 20(21) | 58 | 9,7 (2-34) | 59/80(74) | 24/100(24) | 21/100(21) | 1/100(1) | 17/35 |
| STUMP | 15(5) | 46(24-77) |  |  |  |  |  | 8 (5-12) | 14/14(100) | 1/9(11) | 2/9(22) | 0/9(0) | 41/52 |
| LGESS | 68(23) | 50(20-79) | 34(64) | 6(11) | 5(9) | 9(15) | 14 | 4 (1,8-48) | 39/45(87) | 5/56(9) | 6/56(11) | 13/56(23) | 141/X |
| HGESS | 13(4) | 59(49-78) | 5(39) | 1(8) | 1(8) | 6(46) | 0 | 8,3 (4-20) | 5/9(56) | 2/9(22) | 1/9(11) | 0/9(0) | 26/22 |
| UUS | 26(9) | 60(29-80) | 9(38) | 1(5) | 5(24) | 7(33) | 4 | 11 (3-19) | 13/18(72) | 4/20(20) | 6/20(30) | 1/20(5) | 9/9 |
| LG AS | 13(4) | 65(31-85) | 8(73) | 0(0) | 1(9) | 2(18) | 2 | 5,2 (1,8-11) | 8/10(80) | 1/11(9) | 1/11(9) | 3/11(27) | X/X |
| HG AS | 4(1) | 76,5(65-84) | 0(0) | 0(0) | 2(67) | 1(33) | 1 | 14,3(8,5-15) | 2/2(100) | 1/3(33) | 1/3(33) | 0/3(0) | 5/15 |
| HG uSAR NOS | 5(2) | 69(57-80) | 3(75) | 0(0) | 1(25) | 0(0) | 1 | 9 (7,6-15) | 5/5(100) | 2/5(40) | 0/5(0) | 0/5(0) | 7/17 |

No stage was assigned to STUMP (smooth muscle tumor of uncertain malignant potential) patients and stage percentages were calculated with exclusion of missing data (NA: not available). Information on primary surgery was only available for those patients that had information on complete tumor resection. N: number of patients; LMS: leiomyosarcoma; LGESS: low-grade endometrial stromal sarcoma; HGESS: high-grade endometrial stromal sarcoma; UUS: undifferentiated uterine sarcoma; LG AS: low-grade adenosarcoma; HG AS: high-grade adenosarcomas; HG uSAR NOS: high-grade uterine sarcoma not otherwise specified; FIGO: International Federation of Gynecologic Oncology; chemo: chemotherapy at primary disease; radio: radiotherapy at primary disease; hormone: hormonal therapy at primary disease; X: not estimated.

### Supplementary table 2: Overview of **immunohistochemistry** methods

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|   | **Epitope retrieval** | **Pre-Ab block** | **Primary Ab incubation** | **Pre-Ab block** | **Secondary molecule** | **Tertiary molecule** |
| phospho-S6S240 | Tris-HCl 0,01 M pH9 + EDTA 1 mM30' at 95°C  | 30' blocking solution at RT | M7300 (ser240) Dako1/400ON at 4°C | 15' blocking solution at RT | rabbit anti-mouse-biotin E0354 Dako 1/400 + 1/25 NHS30' at RT | streptavidin-HRP P0397 Dako 30' at RT |
| PTEN | Tris-HCl 0,01 M pH9 + EDTA 1 mM1u at 90°C  | 1h blocking solution at RT | M3627 clone 6H2.1 Dako 1/200ON at 4°C | / | Envision mouse HRP K4001 Dako30' at RT | / |
| PDGFR | Tris-HCl 0,01 M pH9 + EDTA 1 mM 30' at 95°C  | 30' blocking solution at RT | sc-338 (C-20) Santa Cruz Biotechnology1/501h at 37°C  | 15' blocking solution at RT | goat anti-rabbit-PO 111-035-003 Jackson Immunoresearch1/100 + 1/25 NHS30' at RT | / |
| ERBB2 | Tris-HCl 0,01 M pH9 + EDTA 1 mM10' microwave ≈ boiling point | 30' blocking solution at RT | A0485 Dako 1/3002h at RT | 15' blocking solution at RT | goat anti-rabbit-PO 111-035-003 Jackson Immunoresearch1/100 + 1/25 NHS30' at RT | / |
| EGFR | HCl 0.01 M + pepsin 0.04% 10' at 37°C.  | 30' blocking solution at RT | clone 31G7 Zymed1/100 ON at 4°C | 15' blocking solution at RT | rabbit anti-mouse-biotin E0354 Dako 1/400 + 1/25 NHS30' at RT | streptavidin-HRP P0397 Dako 30' at RT |

EDTA: ethylenediaminetetraacetic acid; Ab: antibody; NHS: normal human serum; ON: overnight; RT: room temperature; HRP: horse radish peroxidase; PO: peroxidase.

### Supplementary table 3: **Immunohistochemistry** scoring system

|  |  |
| --- | --- |
| **Score for staining proportion** | **Score for staining intensity** |
| 0 | No cells | 0 | negative |
| 1 | <1% of cells | 1 | weak |
| 2 | 1-10% of cells | 2 | moderate |
| 3 | 11-33% of cells | 3 | strong |
| 4 | 34-66% of cells |  |  |
| 5 | 67-100% of cells |  |  |
| **Addition of proportion and intensity scores** |
| 0-5 | negative |
| 6-8 | positive |

### Supplementary table 4: Characteristics of patients from whom patient-derived xenograft models were established

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Model** | **Tumor used for implantation** | **Stage at diagnosis** | **Age at diagnosis** | **Previous treatment** | **Treatment after surgery** | **PFS (months)** | **OS****(months)** |
| EMC036 | Primary uterine tumor | Ib | 55 | none | unknown | 3,5 | 5 |
| EMC050 | Primary uterine tumor | IV | 59 | Tamoxifen and radiotherapy for other cancers | unknown | 0a | 3 |
| EMC041 | Primary lung metastasis | IVb | 61 | none | Response to chemotherapy but progression pancreatic cancer | 0a | 13b |
| EMC029 | Abdominal recurrence | Ib | 58 | none | Response to radiotherapy | 23 | >58 |
| EMC031 | Abdominal recurrence | / | 62 | No response to doxorubicin | none | 15 | 30 |

a: constant progression; b: patient died of pancreatic cancer

## Supplementary figure legends

### **Supplementary figure 1: Survival of uterine sarcoma patients according to tumor grade and histologic subtype**

Kaplan-Meier survival curves showing disease-specific survival (A,C) and progression-free survival (B,D) for the different uterine sarcoma subgroups. A and B display all patients according to their tumor grade. The number of patients in the analyses is indicated next to the curve with number of events between brackets. C and D display all patients according to their histological subtype. C: 242 patients, 117 events; D: 210 patients, 137 events.

### **Supplementary figure 2: Immunohistochemistry stainings of potential targets**

**A**

Representative images of strong positive immunohistochemistry stainings for the 5 selected targets (including negative staining for PTEN) in selected cases. Histologic subtypes and corresponding scores are indicated (<6 =negative, ≥6 =positive). Pictures were taken at 40X magnification (scale bar indicates 20 µm).

### **Supplementary figure 3: High-grade endometrial stromal sarcoma with YMHAE/NUTM2A/B fusion and cyclin D1 expression**

A: HGESS carrying the t(10;17)(q22;p13) translocation as shown by break-apart FISH. The green signal indicates 3’ probes RP11-100F18 and RP11-60C18 for YWHAE, while the red signal shows the 5’ probe RP11-22G12, shown separately in C and D. In the overlay (A), the yellow signal shows the normal allele, while separate red and green signals show a break in YWHAE (scale bar= 20 µm, total magnification= 100X). Extra magnifications of 1,7X are shown in the lower right corners. The YMHAE/NUTM2A/B fusion was confirmed by RT-PCR (see supplementary methods). B: Strong nuclear cyclin D1 staining in the same HGESS tumor (scale bar= 50 µm, total magnification= 50X).

**Supplementary figure 4: Combination of the mTORC1/2 inhibitor TAK-228 and the PI3K-α inhibitor alpelisib inhibits EMC041 tumor growth to the same extent as BEZ235**

EMC041 treatment experiment as shown in figure 2, with the addition of the treatment arm of combined mTORC1/2 inhibitor TAK-228 + PI3Kα inhibitor alpelisib. Trabectedin was excluded to clarify the figure. Tumor volumes were measured twice weekly and all growth curves were compared using two-way repeated measures ANOVA. Data points and error bars represent mean values and standard error of the mean. Growth curves significantly different from the placebo-treated group are indicated with \*\*\*.