## 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 mM 30' at 95°C | 30' blocking  solution at RT | M7300 (ser240) Dako 1/400 ON at 4°C | 15' blocking  solution at RT | rabbit anti-mouse-biotin E0354 Dako 1/400 + 1/25 NHS 30' at RT | streptavidin-HRP P0397 Dako  30' at RT |
| PTEN | Tris-HCl 0,01 M pH9 + EDTA 1 mM 1u at 90°C | 1h blocking  solution at RT | M3627 clone 6H2.1 Dako  1/200 ON at 4°C | / | Envision mouse HRP K4001 Dako 30' 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 Biotechnology 1/50 1h at 37°C | 15' blocking  solution at RT | goat anti-rabbit-PO 111-035-003 Jackson Immunoresearch 1/100 + 1/25 NHS 30' at RT | / |
| ERBB2 | Tris-HCl 0,01 M pH9 + EDTA 1 mM 10' microwave  ≈ boiling point | 30' blocking  solution at RT | A0485 Dako  1/300 2h at RT | 15' blocking  solution at RT | goat anti-rabbit-PO 111-035-003 Jackson Immunoresearch 1/100 + 1/25 NHS 30' at RT | / |
| EGFR | HCl 0.01 M + pepsin 0.04%  10' at 37°C. | 30' blocking  solution at RT | clone 31G7 Zymed 1/100  ON at 4°C | 15' blocking  solution at RT | rabbit anti-mouse-biotin E0354 Dako 1/400 + 1/25 NHS 30' 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 \*\*\*.