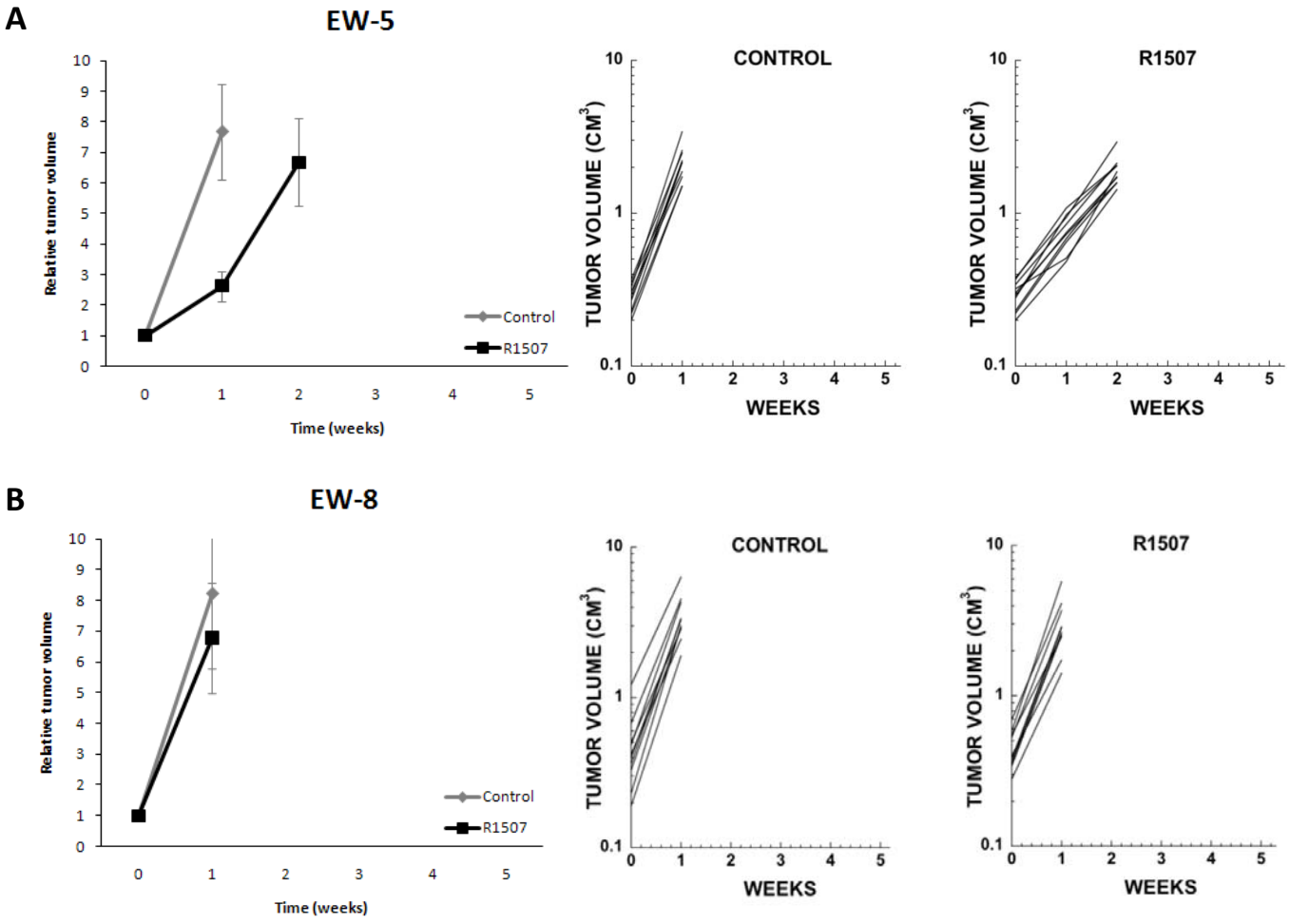
Supplementary Data

Supplemental Figure 1. Response of Ewing sarcoma xenografts to R1507 therapy



Response of EW-5 **(A)** and EW-8 **(B)** xenografts to R1507 therapy. Tumor-bearing mice were treated with 6 mg/kg R1507 twice weekly for a planned 6 consecutive weeks. Tumor diameters were measured weekly. Left panel: relative tumor volumes (RTV) (gray, control; black, R1507). Values are presented as mean RTV ± SD. Middle panel: growth of individual control tumors. Right panel: growth of individual R1507 treated tumors.

Response of Ewing sarcoma xenografts to R1507 therapy

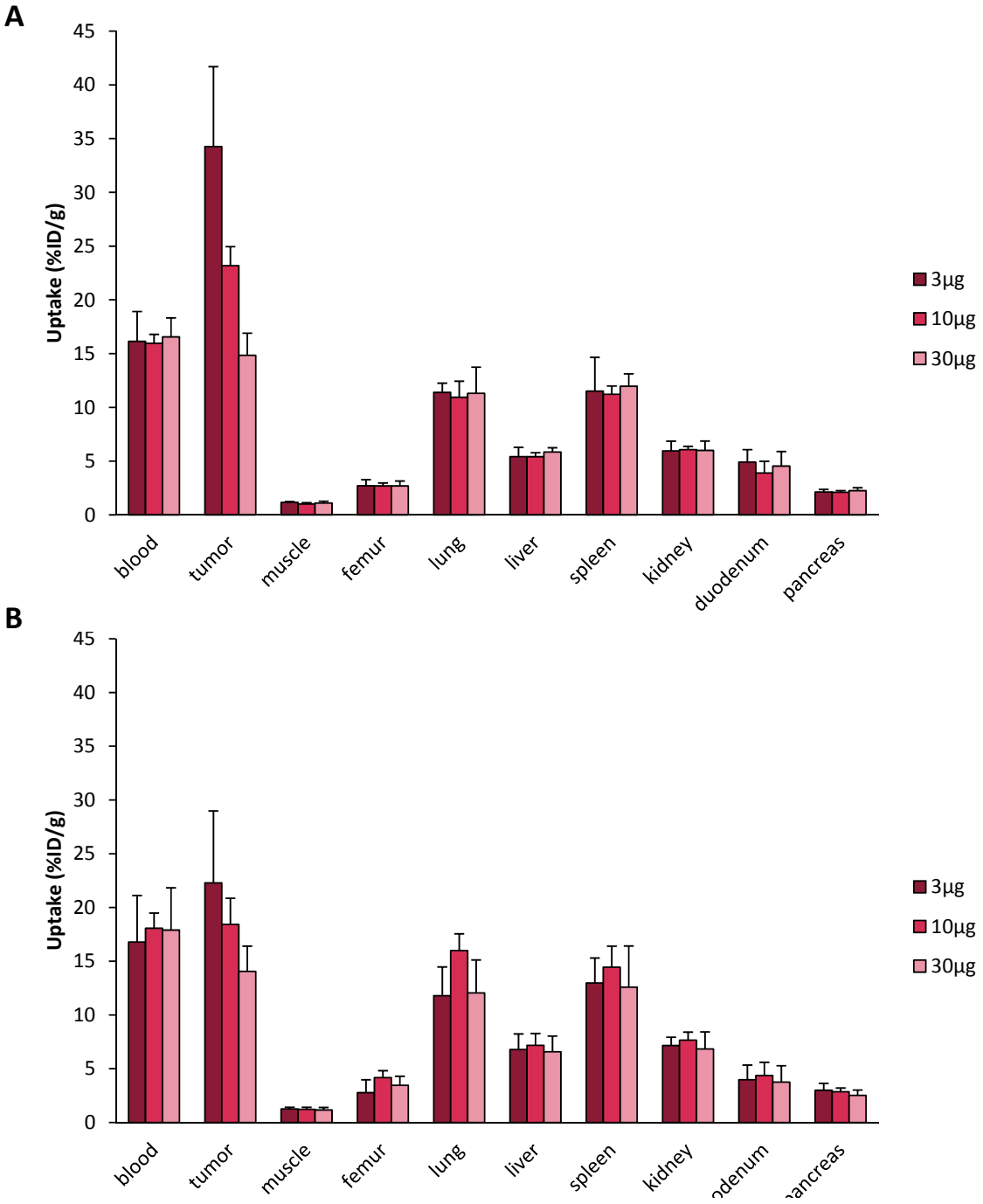
Material and methods: R1507 therapy experiments in EW-5 and EW-8 tumors were performed similarly as described by Kolb et al (11). In short, female CB17scid mice (6-8 weeks old) were s.c. implanted with EW-5 or EW-8 xenografts, and experiments started when tumors reached a volume between 200 and 500mm^3. Mice were subsequently randomized into control or treatment groups, each group consisting of 8-10 mice. Tumor-bearing mice from treatment groups received an intraperitoneal (ip) injection with 6 mg/kg R1507 twice weekly for 6 consecutive weeks. Tumor diameters were measured weekly. Tumor volumes were assumed to be spherical, and calculated from the formula $(\pi/6) \times d^3$, where d represents the mean diameter. Subsequently, relative tumor volumes (RTV) were calculated for control (C) and treatment (T) groups, and responses were determined by dividing the mean RTV of the treatment group by the mean RTV of the control group (T/C). All mice were maintained under barrier conditions, and these experiments were conducted using protocols and conditions approved by the institutional animal care and use committee of the Albert Einstein College of Medicine of Yeshiva University.

Results: Supplemental Figure 1A clearly demonstrates that although EW-5 tumors show progression in response to R1507 therapy, tumor growth is significantly delayed compared to the control group. We designated this tumor growth delay a moderate (intermediate) response (T/C = 34% at week 1). Supplemental Figure 1B demonstrates that there is no growth delay in EW-8 tumors in response to R1507 treatment. We therefore considered the EW-8 tumors to be non-responsive to R1507 therapy.

Supplemental Figure 2. **Dose-escalation study of ^{111}In-R1507 in OS-1 and EW-5 xenografts in CB17scid mice**

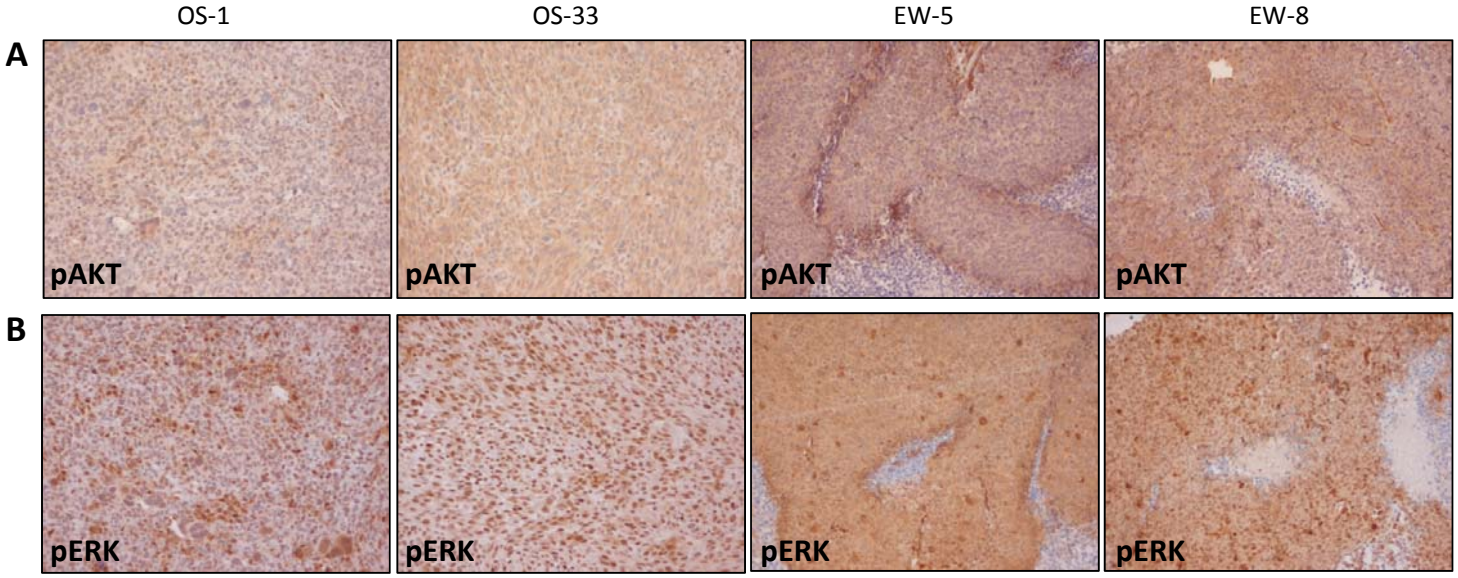
OS-1 **(A)** or EW-5 **(B)** tumor-bearing CB17scid* mice were injected with 3, 10 or 30 µg ^{111}In-R1507 (0.2 MBq). Dose-escalation studies were performed 3 days post injection. Values are presented as mean %ID/g ± SD (*n=6* per group).

* Other imaging experiments were performed in BALB/c nude mice.



Supplemental Figure 3. Activated downstream pathways in bone sarcoma xenografts

Representative images of pAKT **(A)** and pERK **(B)** expression in OS-1, OS-33, EW-5 and EW-8 xenografts. At least 3 different xenografts from each tumor type were used for each staining; representative images are shown. All images are x100 magnification, haematoxylin counterstain.



Activated downstream pathways in bone sarcoma xenografts

Material and methods: Tumor xenografts were stained immunohistochemically to determine pAKT and pERK expression. The xenografts were fixed in 4% formalin and subsequently embedded in paraffin. Tumor sections (4 µm) were deparaffinized in xylol and rehydrated through a graded ethanol into water series. Antigen retrieval was performed by microwave heating of slides in a 10 mM sodium citrate buffer, pH 6 for 10 min at 100 °C. Endogenous peroxidase activity was blocked with 3% H_2O_2 for 10 min at room temperature (RT), and nonspecific binding was prevented by blocking with 20% normal goat serum in tris-buffered saline (TBS) for 30 min at RT. Subsequently, sections were incubated with monoclonal rabbit anti-pAKT (1:50, Cell Signaling Technology) or monoclonal rabbit anti-pERK (1:100, Cell Signaling Technology) overnight at 4°C. Substitution of the primary antibody by TBS served as a negative control. Sections were then incubated with a goat-anti-rabbit biotinylated secondary antibody (1:200, Vector Laboratories) for 30 min at RT. Finally, avidin-biotin-enzyme complex (1:50, Vector Laboratories) was added for 30 min at RT, followed by an incubation for 5 min at RT in 3,3'-diaminobenzidine to visualize pAKT and pERK expression. Slides were counterstained with haematoxylin, dehydrated and coverslipped.

Results: Supplemental Figure 3 demonstrates that pAKT and pERK is present in all bone sarcoma xenografts. pAKT expression was predominantly cytoplasmic in all xenografts, although nuclear staining was detected as well occasionally. pERK localization varied between the different xenografts. OS-1 xenografts demonstrated both nuclear and cytoplasmic staining, while in OS-33 xenografts pERK was predominantly located in the nucleus. In both EW-5 and EW-8 xenografts, apparent cytoplasmic expression of pERK was demonstrated.