

SUPPLEMENTAL DATA

It was observed that during fulvestrant treatment, the serum estradiol (E2) levels in patients as measured by fluorescent-immuno-assay increased. To determine whether this could be contributed to cross-reactivity between fulvestrant and the estradiol assay, the following experiment was performed. Increasing doses of fulvestrant (2, 5, 10, 20, 30, 50, 75, 100 nmol/L) were added in triplicate to different sets of serum from untreated patients with known E2 levels (0.03, 0.04, 0.09, and 0.13 nmol/L). Serum estradiol was measured by fluorescent-immuno-assay, before and after the addition of fulvestrant. Since the viscosity of fulvestrant makes it relatively difficult to precisely add the indicated doses, fulvestrant levels were measured by LC/MS/MS. The increase in measured E2 was thereafter compared to the measured fulvestrant levels by LC/MS/MS, which allowed construction of a calibration curve (Supplemental Fig. S2). We observed a ~0.05 nmol/L increase in apparent E2 for each 10 nmol/L increase in plasma fulvestrant.

Plasma fulvestrant levels in patients in our study were determined at day 28 and day 84, concurrently with FES-PET (Supplemental Fig. S1A). Serum estradiol levels were measured at baseline, day 28 and day 84 and corrected for cross-reactivity with fulvestrant as described above (Supplemental Fig. S1B).