ONLINE SUPPLEMENT

We used the Norton-Simon modeling approach to examine various intermittent dosing and scheduling of lapatinib with and without capecitabine in breast cancer xenografts to test hypothesis that the intermittent high dose lapatinib (1) is tolerable and as efficacious as a continuous dosing and (2) can be given in combination with capecitabine.

Eight-week-old female athymic nu/nu mice were implanted with 0.72 mg/90 days release β-estradiol pellets (Innovative Research of America, Sarasota, FL) and subsequently injected subcutaneously with 10 million HER2- overexpressing BT474 cells (known be sensitive to lapatinib and capecitabine) together with Matrigel (BD Biosciences). Mice were treated and housed in accordance with the in accordance with guidelines approved by the Memorial Sloan-Kettering Cancer Center Institutional Animal Care and Use Committee and Research Animal Resource Center.

Lapatinib 100mg/kg was used for continuous dosing (1). Norton-Simon modeling method was used to derive intermittent drug dose schedules and has been previously described for capecitabine (2). The following intermittent lapatinib dose-schedules were examined ("on" = days of active drug dosing, "off" = days of rest): 3-on/11-off, 5-on/9-off, 7-on/7-off at 100mg/kg; 3-on/4-off and 3-off/11-off at 400mg/kg, and 800mg/kg (Supplemental Figure 1). Capecitabine (7on/7off at 500mg/kg) was based on Norton-Simon modeling as published (2-4). Mice were observed daily throughout the treatment period for signs of morbidity/mortality. Tumors were measured twice weekly using calipers, and volume was calculated using the formula: length x width^2 × 0.52. Body

weight was also assessed twice weekly. Decrease in body weight was used as a surrogate measure of toxicity.

Anti-tumor efficacy was assessed by the mean tumor volume with standard error of the mean. Relative changes in tumor volume were calculated based on the tumor volume at the time of tumor implantation and at the end of drug treatment. Significance testing for between-group comparisons was conducted using a mixed effect model for repeated measures. The p-values reflect differences in the trajectories of change by treatment groups. All the analysis was performed using SAS version 9 (SAS Institute, Cary, NC).

Three different intermittent dosing schedules were examined at a dose of 100mg/kg (Figure 1a). The relative changes in tumor volume were similar for the three schedules. No statistically significant difference in the trajectories of the changes were noted among the intermittent schedules, but the continuous dosing was more efficacious than the intermittent schedules ($p< 0.001$).

The “3 day-on” lapatinib was further evaluated at 100mg/kg, 400mg/kg and 800 mg/kg (Figure 1b). Higher doses of intermittent lapatinib showed better efficacy compared to the continuous dosing: -18% (continuous at 100mg/kg), -50% (3-on/4-off, 400mg/kg), -36% (3on/11off, 400mg/kg), -69% (3 days on/4 days off, 800mg/kg), and -60% (3-on/11-off, 800mg/kg). The statistically significant difference in trajectory or weight change was noted for 3-on/4-off at 800mg/kg ($p=0.04$) when compared to the continuous. However, a qualitatively more intense regimen (4-off vs.11-off) was observed to be more debilitating to mice.

High dose lapatinib at 800mg/kg (3-on/11-off) was evaluated concurrently or sequentially with capecitabine (Figure 2). The concurrent administration was too toxic, as mice rapidly lost >20% body weight and had to be sacrificed (therefore, no data points shown). The sequential administration was feasible and tolerable with no significant difference in weight change compared to the single agent administration ($p=0.62$). A relative reduction in tumor growth was similar for both high dose lapatinib with or without capecitabine ($p=0.87$): -40% vs. -54%.

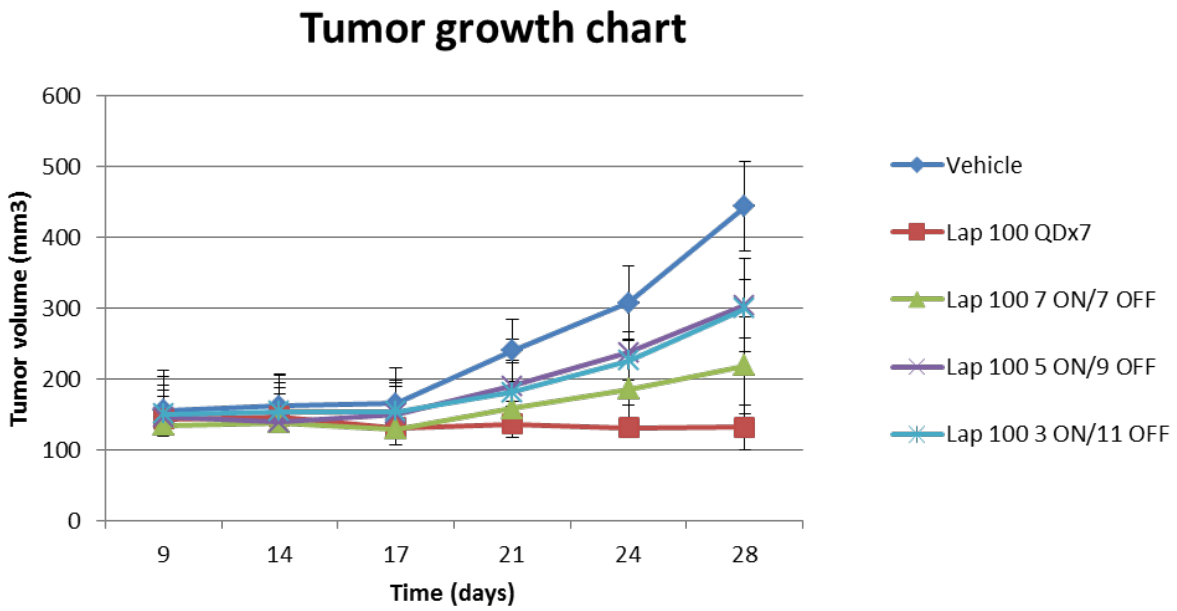
Figure Legends.

Figure 1a. Tumor growth chart comparing the three intermittent dosing schedules (3 days on, 5 days on, and 7 days on) in comparison to the continuous dosing at 100mg/kg.

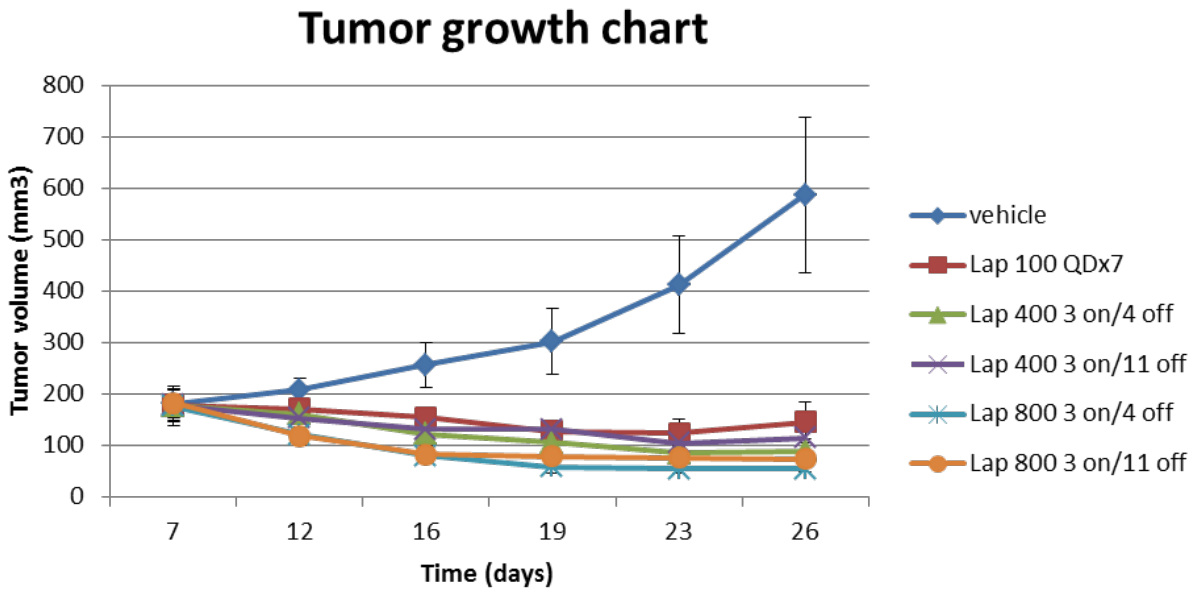
Figure 1b. Tumor growth chart comparing the high doses of lapatinib at 400mg/kg and 800mg/kg given intermittently compared to the standard continuous dosing at 100mg/kg.

Figure 2. Tumor growth chart comparing the high dose lapatinib at 800mg/kg on 3 days on/11 days off schedule with and without capecitabine given 7 days on/7days off at 500mg/kg as compared to capecitabine single agent given 7 days on/7days off at 500mg/kg. The concurrent high dose lapatinib and capecitabine group not shown due to the early treatment discontinuation from toxicity.

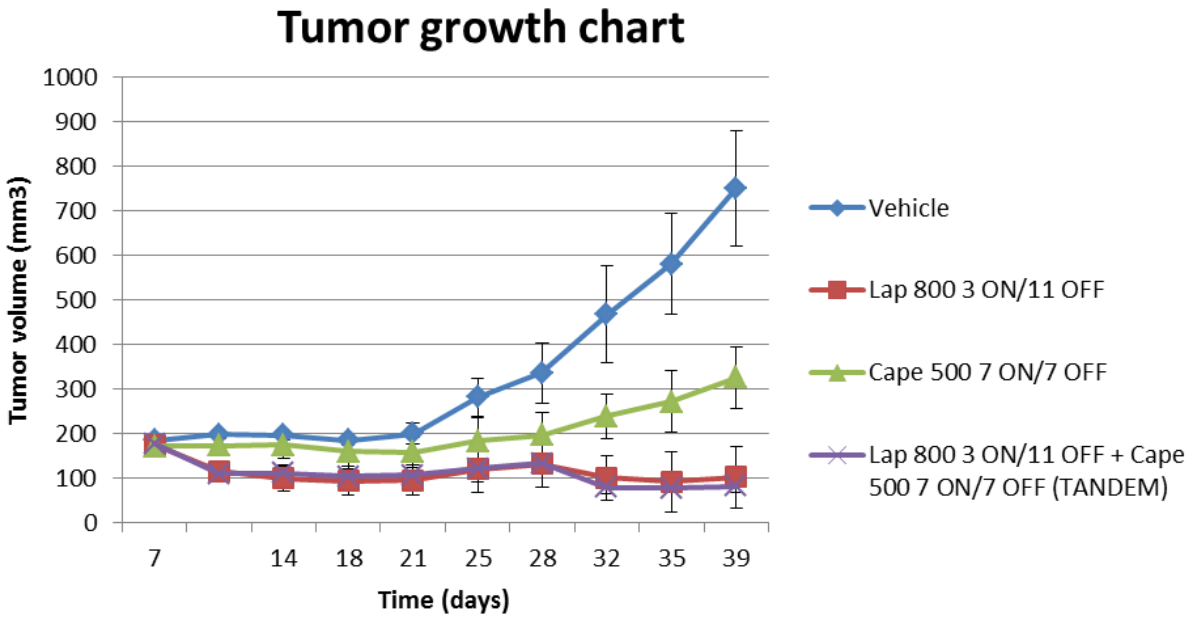
Supplement Figure 1a



Supplement Figure 1b



Supplement Figure 2



References:

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