Evaluating Dose Timing Effects of Gefitinib (an EGFR Inhibitor) in a Breast Cancer Model using ADC and T_1

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INTRODUCTION

An emerging paradigm for improving anti-tumor efficacy of breast cancer treatment is the combination of therapeutic agents. The combination of standard chemotherapy regimens with targeted agents capable of inhibiting signal transduction is showing promise for improving therapeutic efficacy. Gefitinib, an inhibitor of the epidermal growth factor receptor (EGFR) has demonstrated enhanced anti-tumor activity when co-administered with cytotoxic agents, in a range of human tumor xenografts [1]. There is evidence to suggest that gefitinib's ability to suppress survival signaling is transient and that prolonged application may reactivate cell survival signaling pathways [2]. Furthermore, gefitinib has demonstrated greater anti-tumor activity when applied in a pulsatile dose prior to paclitaxel over a daily dose in concert with paclitaxel or paclitaxel alone [2]. Thus, the timing of gefitinib administration might play an important role in treatment efficacy.

Breast cancer studies have employed techniques believed to reflect tumor physiology, including dynamic contrast enhanced MRI, to probe tumor microvasculature and characterize tumor response. The present study complements these efforts by using the apparent diffusion coefficient (ADC) of water, T_1 and tumor volume changes, to investigate possible differences between the anti-tumor effect of two-day pulsed versus daily gefitinib dosing. ADC is sensitive to parameters like cell organization, cell density, microstructure and microcirculation. Recent investigations have reported ADC changes in response to treatment as early as 2 days post treatment [3].

METHODS

We studied 17 nude mice bearing Her2/neu (erB2) breast tumor xenografts (200-400 mm^3). MRI was performed at 3 time-points over a 10 day period: day 0 (prior to first dose), day 4, and day 9, on a 1.5T GE system. Mice were randomly assigned to three groups: control (n=5), which received saline; daily dose (n=6), which received a daily dose (150 mg/kg) of gefitinib for 10 days; pulsed dose (n=6), which received two doses (1000 mg/kg) of gefitinib on day 2 and day 3. To understand the natural progression of our tumor model, we studied an additional 8 mice. These mice did not receive any agent and were imaged over 14 days (Figure A).

Diffusion weighted imaging acquisition was performed using: a three-axis diffusion sensitized SSFSE sequence, b = 0,600 s/mm^2, TR/TE = 9899/69 ms, matrix = 256x256, FOV = 100 mm and slice thickness = 3 mm. T_1 images were acquired with a 3D SPGR sequence: flip angle = 40,20,8°, TR/TE = 27/8 ms, matrix = 256x256, FOV = 100 mm, slice thickness = 1mm. ADC and T_1 maps were generated offline using in-house software. Non-viable tumor, defined as hyper-intense regions onT_2 weighted images, was excluded using a semiautomated clustering algorithm written in MATLAB. Means and quartiles within viable tumor were extracted from ADC and T_1 maps. To reduce intra-animal variability, each animal was normalized to its baseline value. Statistical difference in ADC and T_1 between groups was established in an ANOVA design with post-hoc testing. Student's t-tests were used to probe differences in tumor volume between groups.

RESULTS

The change in tumor volume in both treatment groups was significantly different from the controls ($p < .05$): the control group increased continuously; as did the daily dose group, albeit at a slower rate; and the pulsed dose group exclusively exhibited growth arrest at day 4 (Figure B). ADC and T_1 were statistically different between groups, $F(2,15) = 16.93$, $p < .001$ and $F(2,15) = 14.44$, $p < .0003$, and the treatment groups differed from the control group ($p < .05$). However, the pulsed and daily dose groups were statistically indistinguishable by ADC or T_1. ADC continuously decreased in the control group but increased in both treatment groups (Figure C). T_1 initially decreased in all groups (day 4), kept decreasing in the controls, but rebounded in both treatment groups (day 9). T_1 decrease was more pronounced (by ~80%) in the two treatment groups, and T_1 rebound was greater (by 62%) in the pulsed dose group than in the daily dose group (Figure D). In the "natural progression" experiment, ADC fluctuated and returned to baseline by day 14 while T_1 demonstrated an 11% decrease (day 2) which was sustained through day 14 (Figure A).

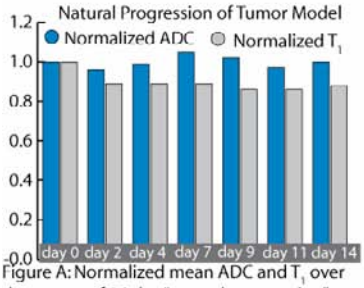

Figure A: Normalized mean ADC and T_1 over the course of 14 day "natural progression" study. Animals in this study received no agent. ADC fluctuated and returned to baseline by day 14. Initial 11% T_1 decrease (at day 2) is sustained through day 14

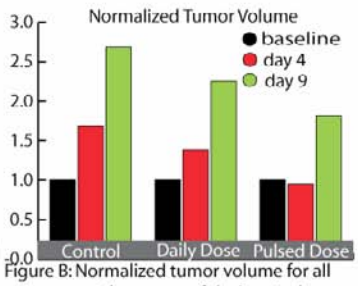

Figure B: Normalized tumor volume for all groups over the course of dosing study. Tumor volume continuously increased in controls, and at a slower rate in daily dose group. Pulsed dose group exclusively displayed growth arrest at day 4.

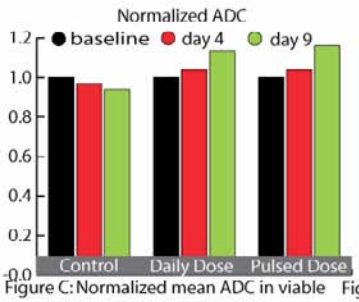

Figure C: Normalized mean ADC in viable tumor during dosing study. ADC decreased continuously in the control group but increased continuously in both dose groups.

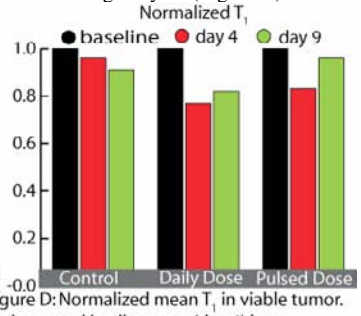

Figure D: Normalized mean T_1 in viable tumor. T_1 decreased in all groups, (day 4) kept decreasing in controls but rebouds in both dose groups (day 9). Decrease was greater (80% more) in both dose groups and pulsed group exhibited greater (62% more) rebound.

DISCUSSION

Our results demonstrate an effect due to gefitinib reflected by changes in ADC, T_1 and tumor volume as early as 3 days post gefitinib administration. We found significant ADC increases in both treatment groups whereas ADC decreased in the control group, suggesting reduced cell density in the treatment groups due to gefitinib. We observed greater T_1 decrease in the two treatment groups over the controls (~20% vs. 4%) and a T_1 rebound unique to both treatment groups. To further investigate this interesting finding, we studied an additional 8 mice to understand the natural progression of our tumor model. The "natural progression" study revealed an 11% initial T_1 decrease (still less than 20%), without rebound. These findings point to an effect (pronounced T_1 decrease and rebound) due to gefitinib in both treatment groups.

We were unable to distinguish between the two treatment groups based on mean ADC and T_1 changes - it should be noted that gefitinib was not followed up with a chemotherapeutic agent and this might explain why. These studies are proceeding and more comprehensive experiments are planned. Based on tumor volume changes, where the pulsed dose exclusively demonstrated tumor growth arrest (day 4), we speculate that pulsatile gefitinib dosing might be more effective than daily dosing. Our results also go towards establishing ADC, T_1 and tumor volume as sensitive parameters for characterizing tumor response to gefitinib in this breast cancer model.

REFERENCES

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