

Assessment of Early Treatment Response Using a Fast and Robust MRI Protocol in Genetically Engineered Mouse Lung Cancer Models

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Introduction

Lung cancer is a leading cause of death throughout the world, and there is a pressing unmet need for more efficacious therapies. BIBW2992 is a irreversible dual EGFR/HER2 inhibitor that has been shown to have antitumor activity in several lung tumor models (1, 2). For the purposes of pathology characterization and the evaluation of new treatment effects in vivo, a simple, fast and robust imaging protocol with high throughput is essential. Lung imaging of small animals using MRI is challenging, due to cardiac and respiratory motion, low proton density in the lung, and large local magnetic susceptibility around air-filled alveoli. These factors result in motion artifact and low SNR. Although cardiac and respiratory gating approaches can reduce motion artifact, they lead to variable imaging contrast due to varying repetition time which depends on the animal's respiratory rate. Also, gating approaches add complications to the imaging protocol. Another approach is to use ultrafast gradient echo sequences without gating, but they are time consuming because they require a large number of averages and acquire only one slice at a time. In this study, we have developed a robust MRI protocol for imaging mouse lungs in a fast and simple manner. We applied this new method to assess the early effect of BIBW2992 treatment in a genetically engineered mouse lung cancer model.

Methods

Five mice with conditional expression of EGFR deletion exon 19 (del 19) mutations were imaged before being given doxycycline-impregnated (doxy) food and once a week after doxy food to monitor tumor growth. When mice became symptomatic (panting, decreased oxygen saturation), they were started on BIBW2992 and imaged daily. All MRI experiments were carried out on a Bruker 7T BioSpec MRI system. Mice were anesthetized with 1-1.5% isoflurane in O₂. A modified FLASH sequence running in a steady-state condition with saturation slice refocusing navigators was used to acquire images without cardiac triggering or respiratory gating. This self-gated method (IntraGate) provides very high temporal resolution and retrospective reconstruction. The parameters were as follows: TR/TE=67/1.7ms, FOV=30x30mm, matrix=256x256, slice thickness=1mm, NEX=16, total scan time=2min19s. For image quality comparison purposes, a fast gradient-echo sequence with signal averaging (SNAP) was also used. SNAP has been used in several studies on lung disease in rats and mice (3). The parameters used for SNAP were the same as above except the TR=14ms, NEX=8, and total scan time =12min48s. The SNR and SNR efficiency were calculated for both techniques. The tumor volume was quantitated using 3D slicer. MRI results were compared with O₂ blood saturation (SO₂) and clinical symptoms.

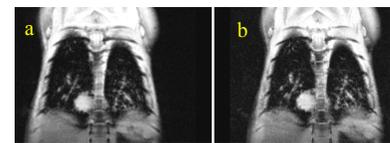


Fig.1. Comparison of the two techniques: a. IntraGate, b. SNAP.

Results

Figure 1 shows an example of a coronal image acquired using the two techniques: IntraGate (1a) and SNAP (1b). Tumors were clearly visualized using both imaging protocols with comparable image quality and with the absence of major motion artifacts. The SNR of the tumor was 99 and 28, while the SNR efficiency was 66 and 8, for the IntraGate and SNAP techniques, respectively. Sustained doxy induction resulted in the growth of adenocarcinomas that were visualized on MRI before the development of clinical symptoms (respiratory distress, hypoxemia). The volume of tumors

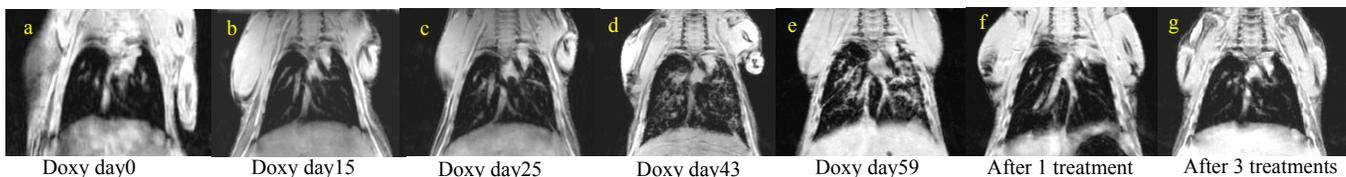


Fig.2. Tumor progression over time and after treatment.

measured by MRI correlated with the SO₂ level. Figure 2 demonstrates the progression of adenocarcinomas over time, as well as the effects of BIBW2992 treatment. There were no adenocarcinomas observed on the pre-doxy baseline images and the SO₂ was 98% (Fig. 2a). A small number of adenocarcinomas were apparent after 15 days of doxy food (Fig. 2b) with an SO₂ of 96%. Figs. 2c -2e shows progression of adenocarcinomas over 2 months of doxy induction with progression to diffuse adenocarcinomas (Fig. 2e) and a substantial drop in SO₂ (55%). The clinical symptoms included panting, paleness, crouching, and unresponsiveness to touch. Treatment with BIBW2992 at a dose of 40 mg/kg resulted in dramatic tumor reduction after 1 treatment (Fig. 2f) and 3 treatments (Fig. 2g), respectively, with the SO₂ increasing to normal (98%). The mice became more active and clinical symptoms resolved. The treatment was continued on a daily basis for 2 weeks and no new tumors appeared. Figure 3 shows the percentage of tumor reduction for all 5 mice after one treatment of BIBW. The early effect of treatment (after 1 dose) with BIBW2992 resulted in an average of 74% tumor volume reduction.

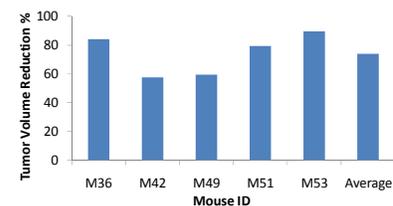


Fig 3. Tumor volume reduction after 1 treatment.

Discussion

We have demonstrated that the IntraGate technique used in this study is a fast and simple way to image mouse lungs without the complication of cardiac and respiratory gating. The MRI lung imaging protocol established here showed increased SNR efficacy. We used this protocol to monitor the progression of adenocarcinomas and for evaluating the efficacy of the irreversible EGFR/HER2 inhibitor BIBW2992. Objective response to treatment with BIBW2992 was apparent even after a single treatment dose.

References: 1) D Li, et al. *Oncogene*, 27(34), 4702-11, 2008; 2) S Perera, et al, *PNAS*, 106, 474-79, 2009; 3) N Beckmann et al, *Eur J Radio*, 64, 381-96, 2006.