INTRODUCTION
Jak1/2 inhibition suppresses Stat3 phosphorylation that is characteristic of many tumor types. Activated Stat3 promotes the transcription of factors that enhance tumor growth, survival, and angiogenesis. AZD1480 is a novel small molecule inhibitor of Jak 1/2, which have been shown to be key mediators of Stat3 activation. Both DW-MRI and DCE-MRI have been used to monitor cancer treatment. This work seeks to assess the utility of DW-MRI and DCE-MRI in determining AZD1480 efficacy compared to the potent anti-angiogenic drug cediranib at early treatment time points.

MATERIALS and METHODS
Thirty mice were injected with Calu-6 lung cancer cells in the hind limb; once the tumors reached approximately 200 mm³, the mice were randomized into the following treatment groups: AZD1480 (50 mg/kg, p.o. q.d.), cediranib (6 mg/kg p.o. q.d.), and vehicle control. All animals were imaged at 9.4T at baseline, day 3 and day 5 post-treatment time points. DCE-MRI. Pre-contrast T₁ maps were obtained using an IR FLASH gradient echo sequence with eight inversion times with TR/TE/α= 12100 ms/ 3.44 ms/15° and NEX = 4, FOV = 35 mm², and matrix = 128² for fifteen 1 mm slices. The dynamic acquisition employed a SPGR sequence with TR/TE/α = 100 ms/2.83 ms/10°, and NEX = 4. A bolus of 0.1 mmol/kg Gd-DTPA was delivered via a jugular catheter using an automated syringe pump. A population derived VIF (Cp) was used to fit the tissue signal data (Ct) in the central slice for each mouse at each time point using a standard model. DW-MRI. A gated and navigated PGSE sequence was used with the following parameters: TR/TE/α= 2000 ms/42 s/15°, acquisition matrix = 128², FOV = (35 mm)², and NEX = 2 with A = 35.00 ms and δ =5.00 ms for b-values of 150.88, 500.2, and 800.22 mm²/s. The diffusion weighted signal at each b (S(b)) was fit to the following equation to extract the apparent diffusion coefficient (ADC):

\[ S(b) = S_0 e^{-ADCD} \]

The percent change from baseline measurements for Ktrans, ADC, and Vv were calculated for each time point. The Wilcoxon rank test identified significant changes in imaging parameters while H&E, CD31, cParp, and Ki-67 histology data validated the results.

RESULTS
Fig. 1 presents the resulting Ktrans (1a) and ADC (1b) changes for each treatment group. A significant decrease (indicated by *) in Ktrans was found at both day 3 and day 5 post-treatment time points for the cediranib group. Interestingly, no significant changes in Ktrans occurred for the AZD1480 group compared to controls, but a significant increase in ADC was demonstrated at day 3 and day 5. Control data showed a significant decrease in ADC at day 5. No significant changes in Vv were demonstrated in any treatment group except a decrease in the cediranib group at day 5. Histology acquired after day 5 (Fig 2) indicated attenuated vasculature in the cediranib treatment group (1+ = reduced vascularity while 3+ = high vascularity) while no significant changes were shown in the AZD1480 or control groups (2a). Significant apoptotic activity (positive cParp staining in 2c) and significantly increased extracellular space (2c) was present in the AZD1480 group with no changes in the cediranib/control groups (2e).

DISCUSSION
The lack of significant group changes in Ktrans demonstrate that day 3 and day 5 of a 50 mg/kg q.d. dose of AZD1480 may be too early to see anti-angiogenic effects, compared to the VEGF signaling inhibitor cediranib. The more sensitive measure for AZD1480 treatment seemed to be ADC. Significant increases in ADC were indicated while histology indicated a significant increase in apoptosis and extracellular space in this treatment group. Thus, tumor cell death was evident in AZD1480-treated xenografts in the absence of anti-angiogenic activity while anti-angiogenic activity (decreased Ktrans) was detectable in xenografts treated with cediranib.

REFERENCES

ACKNOWLEDGEMENTS
AstraZeneca predoctoral training grant, NIH 1K25 EB005936, NCI U24 CA126588, and NCI P30 CA068485.