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A comparative assessment of preclinical chemotherapeutic response of tumors using quantitative non-Gaussian diffusion MRI

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Abstract

Background—Diffusion-weighted MRI (DWI) signal attenuation is often not mono-exponential (i.e. non-Gaussian diffusion) with stronger diffusion weighting. Several non-Gaussian diffusion models have been developed and may provide new information or higher sensitivity compared with the conventional apparent diffusion coefficient (ADC) method. However the relative merits of these models to detect tumor therapeutic response is not fully clear.

Methods—Conventional ADC, and three widely-used non-Gaussian models, (bi-exponential, stretched exponential, and statistical model), were implemented and compared for assessing SW620 human colon cancer xenografts responding to barasertib, an agent known to induce apoptosis via polyploidy. Bayesian Information Criterion (BIC) was used for model selection among all three non-Gaussian models.

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Results—All of tumor volume, histology, conventional ADC, and three non-Gaussian DWI models could show significant differences between control and treatment groups after four days of treatment. However, only the non-Gaussian models detected significant changes after two days of treatment. For any treatment or control group, over 65.7% of tumor voxels indicate the bi-exponential model is strongly or very strongly preferred.

Conclusion—Non-Gaussian DWI model-derived biomarkers are capable of detecting tumor *earlier* chemotherapeutic response of tumors compared with conventional ADC and tumor volume. The bi-exponential model provides better fitting compared with statistical and stretched exponential models for the tumor and treatment models used in the current work.

Keywords

high b; diffusion; MRI; chemotherapeutic response; SW620; barasertib; ploidy; BIC

INTRODUCTION

Diffusion-weighted MRI (DWI) is well-established imaging method that can probe tissue microstructure non-invasively. It has been widely used to provide potential biomarkers, notably the apparent diffusion coefficient (ADC) which may better characterize tissues in e.g. stroke (1) and cancer (2). Due to the relatively long diffusion times used in pulse sequences, ADC is typically sensitive to cellularity (3) or cell density (4) of tumors, but not to subcellular structures (5). ADC may be used as a biomarker of tumor response to treatment (6–9). With typical b values (diffusion weighting factors) of e.g. less than 1000 sec/mm² in the brain, the diffusion signal attenuations show a mono-exponential behavior, and hence a minimum of two b values are then needed to calculate an ADC which represents the overall averaged diffusion properties of water protons in each voxel.

In addition to conventional ADC measurements in the mono-exponential range, the development of high-performance gradient coils enables DWI measurements with stronger diffusion weighting using higher b values > e.g. 1500 sec/mm² and increased diffusion contrast. Under these conditions the signal attenuation is often non-mono-exponential. This is a consequence of restricted diffusion as the mean-squared displacements of diffusing protons are no longer Gaussian distributed. Several quantitative non-Gaussian diffusion models have been developed to fit diffusion signals with high b values. The bi-exponential model assumes two (i.e. “fast” and “slow”) diffusion components without water exchange, although attempts to correlate these components to any specific microstructural compartments have failed, and the no-exchange assumption is inherently implausible. Even more (three or four) components are necessary to fit data with even higher b values (10). Diffusional kurtosis imaging measures the excess kurtosis as a biomarker of deviation from Gaussian diffusion (11). The statistical diffusion model assumes a large number of “spin packets” whose individual diffusion coefficients obey a non-zero Gaussian-type distribution, and the corresponding position of the distribution maximum (peak) D_{cen} and the width of the distribution σ can be fit from DWI measurements (12). The stretched exponential diffusion model introduces a heterogeneity index α and distributed diffusion coefficient DDC (13), and has shown interesting results to detect brain tumor infiltration (14). Other non-Gaussian

models include those established based on anomalous diffusion (15), fractal models (16), and fractional order calculus (17,18).

Several of these non-Gaussian diffusion models have been implemented in cancer imaging and appear to show new information or higher sensitivity compared with conventional ADC measurements (19). For example, the heterogeneity index α obtained using the stretched exponential model has been suggested as a marker of brain tumor invasion (14), while a higher sensitivity was found to detect brain tumor early response to radiation therapy using DWI with high b values. Other studies failed to show that non-Gaussian DWI models with high b values provide an earlier or a more sensitive detection of brain tumor therapeutic response (20–22) compared with ADC measurements. Since different cancers have different molecular drivers, and different therapies have different microstructural effects, previous results obtained in one cancer setting may not necessarily apply to other cancer treatment models. Therefore, it is worthwhile comparing non-Gaussian DWI and conventional ADC measurements in different cancers and treatment models to investigate the usefulness of high-b DWI in other applications. In addition, it is also informative to compare different non-Gaussian diffusion models using the same high-b diffusion data sets. A comprehensive comparison between different non-Gaussian diffusion models has been performed in healthy subjects (23) and cancer patients (24) previously.

In the current work, both conventional ADC and non-Gaussian model measurements and analyses were performed and compared to assess the early therapeutic response of human colon cancer (cell line: SW620) to barasertib. Barasertib is a selective inhibitor of Aurora B kinase that induces polyploidy in treated cells, ultimately leading to cell death via apoptosis (25). Previous studies have suggested such a treatment model can cause tumor microstructural changes at both subcellular (polyploidy and increased nuclear size) and cellular (increased cell size and cell death) levels (26). This provides an opportunity to investigate the pathological factors that affect non-Gaussian DWI measurements. In addition, Bayesian information criterion (BIC) was used to evaluate which non-Gaussian diffusion model provides the best fits based on the same DWI data.

MATERIALS AND METHODS

Diffusion models to be tested

In the current work, the Gaussian (mono-exponential) diffusion model and three widely used non-Gaussian diffusion models (i.e. bi-exponential model (10), stretched exponential model (13) and statistical model (12)) were implemented and compared.

Mono-exponential model (two fitting parameters: S_0 and ADC)—When the diffusion weighting (b values) is relatively small (e.g. $< \sim 1000 \text{ sec/mm}^2$), the Gaussian diffusion approximation is usually valid, and the diffusion signal attenuation can be written as a single mono-exponential function, i.e.

$$S = S_0 e^{-b \cdot ADC}, \quad [1]$$

where b is the diffusion weighting factor. A minimum of two b values are required to obtain ADC. Note that the use of e.g., $b < 150 \text{ sec/mm}^2$ may be contaminated with blood flow/perfusion in highly vascular tissue such as tumor (2). However, the use of $b = 0 \text{ sec/mm}^2$ is still recommended in clinical cancer studies presumably for better sensitivity (27). Therefore, two b values (0 and 1000 sec/mm^2) were used in the current work to follow recommended clinical guidelines.

Stretched exponential model (three fitting parameters: S_0 , DDC and α)—The stretched exponential model assumes a collection of water molecules with a distribution of apparent diffusion coefficients, and thereby describes the diffusion signals as (13)

$$S = S_0 e^{-(b \cdot \text{DDC})^\alpha}, \quad [2]$$

where DDC is the distributed diffusion coefficient and α is the heterogeneity index which varies between 0 and 1. Note that $\alpha = 1$ indicates a Gaussian diffusion and Eq.[2] becomes Eq.[1].

Statistical model (three fitting parameters: S_0 , D_{cen} and σ)—The statistical model assumes a non-zero Gaussian distribution of a large number of individual diffusion coefficients and hence provides

$$S = S_0 \frac{1 + \text{erf}\left(\frac{D_{cen}}{\sqrt{2}\sigma} - \frac{b\sigma}{\sqrt{2}}\right)}{1 + \text{erf}\left(\frac{D_{cen}}{\sqrt{2}\sigma}\right)} \exp\left(-bD_{cen} + \frac{1}{2}b^2\sigma^2\right), \quad [3]$$

where $\text{erf}()$ is the error function, D_{cen} is the position of the distribution maximum (peak), and σ is the width of the distribution.

Bi-exponential model (four parameters: S_0 , V_f , D_f , and D_s)—The bi-exponential model assumes two diffusion components without exchange, i.e.

$$S = S_0 \left[V_f \cdot e^{-bD_f} + (1 - V_f) \cdot e^{-bD_s} \right], \quad [4]$$

where V_f is the water fraction of “fast” component, D_f and D_s are diffusion coefficients of “fast” and “slow” components, respectively.

Animal and tumor model

All procedures were approved by our Institutional Animal Care and Use Committee at Vanderbilt University. Human colon cancer cell line SW620 was obtained from American Type Culture Collection (ATCC #CCL-227) and cultured according to vendor’s guidance. Thirty-seven female Athymic nude mice (Harlan Laboratories, Inc., Indianapolis, IN) were each injected with 1×10^7 SW620 cancer cells subcutaneously into right hind limb. The xenografts reached approximately $200\text{--}300 \text{ mm}^3$ after ~ 14 days after injections (26), and

nine mice were then sacrificed for the baseline histology. All mice survived the study procedures without adverse effect. All mice gave analyzable data and none were excluded from analysis.

Therapy

28 mice were randomized into 4 groups (N = 7 for each), with two receiving daily treatments of barasertib (treatment groups) and two of drug vehicle (dimethyl sulfoxide (DMSO)) only (control groups). For each treatment, 25 mg/kg of barasertib was dissolved in 30 ml of DMSO and administered by a single intraperitoneal injection. One treatment group were treated for 2 (TX2) and the other one for 4 (TX4) successive days. The same treatment strategy was performed to control groups to obtain CT2 and CT4 groups with injections of vehicle for 2 and 4 successive days, respectively. All mice were scanned with diffusion MRI before and 2 (for CT2 and TX2 groups) or 4 (for CT4 and TX4 groups) days after initial treatments.

Diffusion MRI *in vivo*

Mice were anesthetized with a 2%/98% isoflurane/oxygen mixture before and throughout the whole MRI measurements. The magnet bore temperature was kept at ~ 32°C using a warm-air feedback system. Stretchable tapes were used to restrain any respiration-induced movement, as well as motion-induced artifacts in DWI data. Respiratory gating (SA Instruments, Stony Brook, NY) was applied to further reduce motion artifacts. The ADC value of a doped water phantom (5mM CuSO₄, beneath the animal) was measured to monitor the consistency and accuracy of ADC measurements.

All MRI measurements were acquired on a 4.7T Varian horizontal small animal scanner equipped with a self-shielded SGRAD 115/60/S gradient system with a strong maximum gradient strength up to 100 G/cm (Magnex Scientific Limited, Yarnton, UK) and a 40mm inner diameter “Millipede” volume coil for both transmission and reception. A fast spin echo (FSE) readout was used for all diffusion measurements. The imaging parameters were: diffusion gradient duration $\delta = 20$ ms, separation = 26.2 ms, echo train length = 8, echo spacing = 9.2 ms. Axial slices with 1 mm thickness were acquired to cover the whole tumors, with in-plane matrix size 128×64 and field-of-view = 40×20 mm yielding an isotropic in-plane resolution of 312.5 μm . The number of excitations (NEX) = 4. Six b values including the influences from other (e.g. imaging) gradients were: 1118, 2162, 2963, 3962, 4968, and 5972 s/mm². Two non-phase encoded navigator echoes were acquired at the end of each echo train, and a twin-echo navigation correction was performed to reduce residual motion-induced artifacts (28). Note that fat suppression was not used in the current study, while lipid content might change after treatment and hence might contribute to signal changes. Diffusion MRI typically suffers low SNRs at high b values. The micro-gradient coil used in the current enables the opportunity to achieve high SNRs in diffusion images with high b values. For all experiments across all animals and time points, the SNR of tumor regions with the highest b value ($b = 5972$ s/mm²) was estimated as 21.8 ± 3.2 (mean $\pm$ STD). Therefore, no correction for Rician noise was necessary in the current work (for example, see Figure 4).

Tumor volume

Tumor volume has been widely used as an indicator of tumor response to treatment. Typically, tumor volume is measured using calipers manually. This oversimplified approach has been shown to be inaccurate compared to the imaging-based direct measurements of tumor volume (29). Therefore, the whole volume of each tumor in this work was estimated using the corresponding T2-weighted $b = 0$ anatomical images. All tumors showed in hyperintensity compared with surrounding muscles in hypointensity (for example, see Figure 3) on $b = 0$ images, and all tumor regions-of-interest were manually segmented.

Histology

Animals were sacrificed by cervical dislocation under deep anesthesia after the second MRI scans (either 2 or 4 days after initial treatments), and tumor tissues were then collected immediately, fixed in 5% formalin for at least 24 hours and then transferred to 70% ethanol. Tumor tissues were paraffin embedded, cut into 4- μ m slices, and then stained using haematoxylin and eosin (H&E). All histological images were obtained at a magnification of 40X using light microscopy. A mean nuclear size of each tumor was estimated from corresponding histological images using ImageJ (Rasband, W.S., ImageJ, U.S. National Institute of Mental Health, Bethesda, Maryland, USA, <http://rsb.info.nih.gov/ij/>).

Data analysis

Analyses were performed blinded as to treatment and time-point. All data analyses were performed using Matlab (Mathworks, Natick, Massachusetts). ADC were obtained using a two-point ($b = 0$ and 1000 s/mm²) method, and non-Gaussian diffusion model parameters were obtained by fitting DWI signals using non-linear curve-fitting in least-squares sense (Matlab function *lsqcurvefit*). The squared 2-norm of the fitting residuals was minimized in the fittings,

$$\epsilon(\mathbf{p}) = \sum_{k=1}^{N_b} \left[S_k(b_k) - \tilde{S}_k(b_k, \mathbf{p}) \right]^2 \quad [5]$$

where $\mathbf{p}$ is the model parameter vector, b_k is the k^{th} b value, $S_k(b_k)$ is the measured diffusion signal with b_k , and $\tilde{S}_k(b_k, \mathbf{p})$ is the model fitted value with b_k and $\mathbf{p}$.

The one-way analysis of variance (ANOVA) was used to evaluate if percentages of all parameters in each group detected any significant differences after treatments of drug or vehicle. The non-parametric Wilcoxon signed-rank test was used to evaluate whether there is significant percentage change of each specific model parameter responding treatment. For conducting multiple comparisons, the false discovery rate (FDR) adjusted p values were calculated based on the linear-step-up procedure introduced by Benjamini and Hochberg (30).

For analysis of model selection with consideration of model complexity, the Bayesian Information Criterion (BIC) (31) has used to compare the performance of different non-Gaussian diffusion models (32), i.e.

$$BIC = -2\log(L) + N_p \log(N_b), \quad [6]$$

where N_p is the number of free parameters to be estimated, and $\log(L)$ is the log likelihood of the measurements given the model estimates, namely, (33)

$$\log(L) = \sum_{k=1}^{N_b} \left[\log \tilde{S}_k - 2 \log \sigma_n + \log I_0 \left(\frac{S_k \tilde{S}_k}{\sigma_n^2} \right) - \frac{S_k^2 + \tilde{S}_k^2}{2\sigma_n^2} \right] \quad [7]$$

where σ_n is the standard deviation of the noise, and I_0 is the zero-order modified Bessel function of the first kind. For each voxel, if BIC of one model is at least 6 smaller than any BIC value of the other two models, this model is considered as the “best fit”, i.e., strongly (BIC = 6-10) or very strongly (BIC > 10) preferred compared with the other two models.

RESULTS

Changes in tumor volume

Figure 1 is a box-and-whisker plot of the percentage changes of tumor volume in control and treatment groups. The tumor volume increase is $88.7 \pm 44.3\%$ in the CT4 group, significantly higher than $40.9 \pm 25.0\%$ in the TX4 group ($p = 0.04$ using the Wilcoxon rank-sum test). This is consistent with previous report that barasertib significantly reduces the rapid growth rate of SW620 xenografts (25), which means SW620 tumors responded to barasertib treatment in the current work. Note that an average of 30.2% inhibition of tumor volume is achieved in this work after 4 days of treatment, consistent with the maximum 65% inhibition of tumor volume reported previously using the same dosage (25).

Changes in histology

Representative histological images from five animal groups are shown in Figure 2. There are no significant changes in cellularity in CT2, CT4 and TX2 groups compared with the pre-treatment group. There are increases in the nuclear sizes and cell sizes in the TX4 group, consistent with the known effect that barasertib treated SW620 cells develop polyploidy with swelled nuclei (25,26). Specifically, the group mean nuclear sizes $\pm$ group STDs are: 7.17 ± 0.83 mm (pre-treatment), 7.93 ± 0.53 mm (CT2), 7.21 ± 0.48 mm (CT4), 9.24 ± 1.33 mm (TX2), and 13.34 ± 0.65 mm (TX4). Because DWI is sensitive to microstructural variations at such a cellular level, ADC is expected to be capable of detecting these changes at least in the TX4 group as shown in (26).

Non-Gaussian diffusion MRI

Figure 3 shows the representative diffusion-weighted MR images with seven different b values. Note there was an NMR tube filled with doped water beneath the animal to monitor the accuracy of ADC measurements. The water ADC was measured as $2.77 \pm 0.39 \mu\text{m}^2/\text{ms}$ across all experiments of different animals and time points, consistent with previously

reported pure water diffusion coefficient $2.72 \mu\text{m}^2/\text{ms}$ at 32°C (34), which validates the accuracy of gradient performance in our DWI measurements.

Figure 4 shows DWI signals from a representative voxel in a representative SW620 tumor. The corresponding fitting results using non-Gaussian diffusion models are also provided. All three non-Gaussian diffusion models are able to fit the signals well, although the statistical model appear to yield a relatively larger deviation from data points. The standard deviations of signals in a 20×10 pixel background region at the lower right corner of images are also shown in Figure 4 to indicate the noise floor. It should be emphasized that the DWI signals, even with the highest b value ($b = 5972 \text{ s/mm}^2$), is significantly larger than the noise level in the current work. This provides an opportunity to investigate and compare different non-Gaussian diffusion models with markedly reduced influence of noise.

Treatment response evaluated using MRI

Percentage changes of non-Gaussian diffusion model derived parameters of all treatment and control groups are summarized in Figure 6. One-way analysis of variance provides $p = 0.080$ and 0.844 for CT2 and CT4 groups, indicating no parameters detected any statistically significant changes in any control group. Consistently, FDR-adjusted p values of all parameters are > 0.067 . By contrast, one-way analysis of variance provides < 0.001 in both TX2 and TX4 groups, suggesting at least one parameter detected significant changes after treatment. Specifically, DDC, D_{cen} and D_f all detect significant changes in the TX2 group with FDR-adjusted p values of 0.045 , 0.045 and 0.009 . It should be emphasized that the conventional ADC does not show any significant change in the TX2 group with FDR-adjusted $p = 0.240$. In the TX4 group, except α and D_s ($p = 0.206$ and 0.690), all non-Gaussian diffusion model derived parameters show significant changes (see Figure 6), while ADC also increase significantly ($p = 0.001$). It is interesting that although marked changes have occurred, neither the tissue heterogeneity index α nor the “slow” component diffusion coefficient D_s show any significant change in any of the four groups. This suggests α and D_s may be insensitive to the microstructural changes of the tumor and treatment models used in the current work.

Model selection

It is of interest to evaluate which non-Gaussian diffusion model provides the best fits based on the same DWI data sets. Note that there are four free parameters to be fitted in the bi-exponential model, one more than those in the statistical and stretched exponential models. Typically, more complex models (with more parameters) would yield “better” fittings with less fitting residuals. However, the model complexity (number of parameters) needs to be evaluated to indicate fitting performance. The Bayesian information criterion used here penalizes the models with high number of parameters, and hence provides more appropriate evaluation of model selection. The Table 1 lists the percentages of all tumor voxels that are best fit by a specific non-Gaussian model evaluated by BIC. The bi-exponential model always provides the highest fraction of best fits larger than 65.7% in any of the groups (pre-treatment, CT2, CT4, TX2 and TX4). Because the threshold is defined as $\text{BIC} \geq 6$, this indicates that the bi-exponential is strongly ($\text{BIC} = 6 - 10$) or very strongly ($\text{BIC} > 10$) preferred to fit most voxels with the non-Gaussian DWI data obtained in the current work.

DISCUSSION

The assessment of tumor therapeutic response at an early stage of therapy provides an opportunity to assist clinicians to better evaluate tumor status, which could enable necessary therapeutic adjustments in a timely manner to enhance treatment efficacy and avoid unnecessary treatment delays, toxicity, and expense. Conventional ADC measurements have been widely used to assess tumor early therapeutic response before any significant changes are detectable in tumor volume (2,6,8). Using a broader range of b values, non-Gaussian DWI is expected to glean more microstructural information than conventional ADC, and may provide an earlier assessment of tumor therapeutic response. However, previous studies usually indicated non-Gaussian DWI measurements did not necessarily provide an earlier assessment of tumor response to treatment. For example, Hoff et al. found that, compared with conventional ADC, the stretched exponential model and bi-exponential model did not provide additional sensitivity to treatment response of 9L rodent brain tumors treated with BCNU (21). Pre-treatment non-Gaussian DWI was suggested as a predictive indicator of human brain tumor response to radiation therapy (22), but ADC showed a similar sensitivity in the same study. Some other studies also reported non-Gaussian DWI with high b values was capable of predicting treatment outcome and/or early monitoring of tumor response to therapy, but no comprehensive comparison with conventional ADC results were provided (35). One exception is that Mardor et al. reported non-Gaussian DWI with high b values provided earlier detection of tumor response before ADC, but changes in ADC and high-b DWI both showed significant correlations with changes in tumor size in that study (36). All of the above studies raise concerns whether non-Gaussian DWI measurements may provide more useful information (e.g. earlier detection) about tumor response to treatment. The current study demonstrates that non-Gaussian DWI derived parameters are capable of detecting changes in SW620 human colon cancer xenografts very early, even before changes are apparent in conventional ADC and tumor volume. This is encouraging because it indicates that non-Gaussian DWI with high b values may have clinical potential to provide earlier assessment of tumor therapeutic response *before* currently used ADC.

The bi-exponential model has been widely adopted due to its easy-to-understand assumptions (i.e. “fast” and “slow” diffusion components) and ability to fit most high-b diffusion signals well. However, attempts to correlate these components pathologically have not been successful. Some other conceptual models have been proposed to try to explain the experimental observations (37), but other studies have demonstrated that any restriction to water molecules even in a single and uniform compartment can cause a bi-exponential diffusion behavior (38,39). This may indicate that restrictions and hindrances dominate the non-Gaussian diffusion behavior in biological tissues (40), and it has also been confirmed that non-Gaussian diffusion is independent on e.g. tissue compartmentalization (41) and inhomogeneous relaxation (42). This may explain the experimental observation in the current study. In the TX2 group, histology confirmed there were no significant microstructural changes in cell density, and hence conventional ADC did not detect any significant change. However, the non-Gaussian model derived parameters DDC , D_{cen} and D_f exhibited significant changes. This may be due to the relatively higher sensitivity of high b DWI to variations of cell membrane permeability (43,44). Diffusion measurements with

very short diffusion times are relatively insensitive to the influences of membrane permeability (45), and our previously reported studies suggested ADC with short diffusion times did not detect significant changes after 2 days of treatment in the same cancer model (26). In the TX4 group, there were significant microstructural changes at both the subcellular and cellular levels. The treatment-induced polyploidy caused markedly increased DNA and intracellular organelles that increase restriction/hindrance to intracellular water molecules, i.e. caused larger fractions of water molecules to be more restricted. This was confirmed as a decrease in ADC measurements at short diffusion times previously (26). If D_s is attributed to reflect water molecules with slow-mobility and $(1 - V_f)$ is the corresponding proton fraction, D_s may be expected to decrease and/or $(1 - V_f)$ is expected to increase. However, interestingly, D_s did not show any significant changes in either of the two treatment groups, and V_f increased, which was contradictory to expectation. Meanwhile, the swelled cell size and decreased cell density in the TX4 group may explain the significant increases in ADC, DDC and D_{cen} , but seems contradictory to the significant decrease of D_f if it truly represents the “fast” diffusion component. This may imply that the “fast” and “slow” components obtained in the bi-exponential model may not be attributed to any specific compartment or spin populations.

Treatment responses of cancer cells are usually inhomogeneous. In the current cancer and treatment model, this may lead to a combination of normal looking cells, cells with polyploidy, and those undergoing apoptosis in the TX4 group (see Figure 2). Therefore, the tissue heterogeneity index α obtained using the stretched model and the distribution width σ obtained using the statistical model are both expected to increase after effective treatments. However, although σ did increase as expected in the TX4 group, α did not show significant changes in either of the two treatment groups. This may raise concerns if α truly detects the tumor cell heterogeneity. In addition, a possible further study could be performed to quantitatively measure the cell membrane permeability (46,47) and compare with the non-Gaussian DWI measurements.

It is also of interest to compare non-Gaussian DWI measurements with ADC measurement with varying different diffusion times e.g. using OGSE methods (48,49). According to Einstein’s relationship, the root mean square displacement of water molecules is dependent on diffusion time, and hence tuning diffusion time provides a powerful approach to selectively probe different length scales in biological tissues (5,50). A recent study has suggested non-Gaussian DWI might distinguish different diffusion restriction scales (51), by which the specificity of non-Gaussian DWI can be significantly enhanced. However, this is contradictory to previous studies that suggested non-Gaussian DWI even with b values up to 10,000 s/mm² could not distinguish intracellular microstructural variations when there were no differences in cell size and density (52), and DWI with long diffusion time is incapable of fitting intracellular diffusion coefficient accurately (53,54). Therefore, it is of interest to investigate whether non-Gaussian DWI has the capability to probe restrictions at different length scales selectively in tumors.

There are numerous models to analyze non-Gaussian DWI with high b values. Some model-free parameters have been defined trying to capture the non-mono-exponential behavior, such as the normalized summation over the diffusion signal curves (22,35) and entropy (55).

In addition to the three non-Gaussian DWI models used in the current study, kurtosis diffusion model is also widely used. However, kurtosis diffusion model may not be suitable to fit DWI data with very high b values (e.g. 6000 s/mm²) used in the current study (56). Comparing the three non-Gaussian models used in the current work, the bi-exponential model provided the best fits in most tumor voxels compared with the other two models based on the Bayesian information criterion. Although performed in tumors, these results are consistent with a previous study that compared these non-Gaussian models performed in healthy human brain (23). This indicates that the bi-exponential model may be a better choice to fit non-Gaussian DWI data at least for the tumor and treatment model used in the current work, although the precise underlying mechanisms of bi-exponential derived parameters remain unclear. Note that different types of cancers are very heterogeneous so the conclusion obtained in the current work might not be directly translated to other cancer and treatment models. It is of great interest to compare different non-Gaussian diffusion models in other cancer and treatment models.

CONCLUSIONS

The conventional ADC and three non-Gaussian DWI models, i.e. bi-exponential model, stretched exponential model and statistical model, were implemented and compared to assess preclinical chemotherapeutic responses of SW620 human colon cancer xenografts to barasertib. The results suggest that the non-Gaussian DWI model derived parameters are capable of detecting tumor *earlier* changes to treatment compared with conventional ADC. Although more studies should be performed to elucidate the factors that affect non-Gaussian model derived parameters in tumors, the current work suggests non-Gaussian DWI may have potential to provide an opportunity to assist better evaluation of tumor status earlier than ADC and tumor volume that are currently widely used in clinical cancer research.

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Figure 1. Percentage changes of tumor volume of the control (CT2 and CT4) and treatment groups (TX2 and TX4). (*: $p = 0.04$. **: $p = 0.02$)

Figure 2. Representative histological images (40X) of SW620 tumor tissues without any treatments (Pre-treat), on day-2 (CT2) and day-4 (CT4) in the control groups, and on day-2 (TX2) and day-4 (TX4) in the treatment groups.

Figure 3.

Representative diffusion-weighted MR images with different b values. Note the tumor was in the upper left, and there was an NMR tube filled with doped water beneath the animal to monitor the accuracy of ADC measurements. All b values are in units of s/mm^2 .

Figure 4. Diffusion MRI signals from a representative voxel in a SW620 tumor and corresponding fitting results using non-Gaussian diffusion models. The noise was calculated as the standard deviations of signals in a 20×10 pixel region at the lower right corner of images without the presence of any objects.

Figure 5.
Multi-parametric maps of a central slice from a representative mouse.

Figure 6. Percentage changes of multiple diffusion-MRI derived parameters after treatment with vehicle and drug (barasertib). CT2 and CT4 are control groups treated with vehicle for 2 and 4 days, and TX2 and TX4 are treatment groups treated with barasertib for 2 and 4 days.

Table 1

Percentages of all tumor voxels that were best fit by a specific non-Gaussian model evaluated by the Bayesian information criterion (BIC). The best fit of a model in a voxel is defined as its BIC is at least 6 smaller than any BIC value of the other two models, indicating this model is strongly ($\text{BIC} = 6-10$) or very strongly ($\text{BIC} > 10$) preferred compared with the other two models.

Model	Pre-treatment	CT2	CT4	TX2	TX4
statistical model	2.2%	1.5%	1.5%	2.0%	5.1%
stretched model	9.0%	6.8%	5.5%	2.2%	6.2%
bi-exponential model	81.2%	82.6%	84.6%	66.5%	65.7%