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FULL PAPER

Assessment of early therapeutic response to sorafenib in renal cell carcinoma xenografts by dynamic contrast-enhanced and diffusion-weighted MR imaging

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Objective: To investigate the feasibility of dynamic contrast-enhanced MRI (DCE-MRI) and diffusion-weighted MRI (DWI) in monitoring early therapeutic response to sorafenib in renal cell carcinoma (RCC) xenograft models.

Methods: Sorafenib (40 mg kg⁻¹) was administered orally to BALB/c nude mice ($n=9$) bearing subcutaneous tumours of human RCC ACHN xenografts. DCE-MRI and DWI were obtained 0, 1, 3 and 7 days after therapy, and DCE-MRI parameters (K^{trans} and v_e) and apparent diffusion coefficient (ADC) values were calculated. Tumour size and volume changes were correlated with changes in DCE-MRI parameters or ADC values after therapy.

Results: Following therapy, K^{trans} showed a significant decrease over time ($p=0.005$), whereas v_e did not demonstrate

significant changes between time points ($p=0.97$). ADC values showed a progressive increase over time ($p=0.004$). Compared with pre-therapy, K^{trans} showed a significant decrease after 3 days of therapy ($p=0.039$), and ADC values increased significantly after 7 days ($p=0.039$). Tumour size and volume did not show significant changes during 7 days. Tumour size and volume changes were not associated with changes in DCE-MRI parameters or ADC values.

Conclusion: DCE-MRI and DWI may show early physiological changes within 1 week after initiating sorafenib treatment on human RCC xenografts.

Advances in knowledge: The quantitative parameters of DCE-MRI and DWI may offer the potential for assessing early therapeutic response to sorafenib in clinical trials.

INTRODUCTION

The treatment of advanced renal cell carcinoma (RCC) has undergone a radical change by the development of drugs targeting vascular endothelial growth factor (VEGF) and its receptor.^{1,2} Sorafenib, a multikinase inhibitor with potent antiangiogenic effects, has shown substantial antitumour activity and prolonged median progression-free survival in patients with advanced RCC.^{3,4}

Response Evaluation Criteria in Solid Tumors has traditionally been used to grade tumour responses.⁵ This standard objective response criterion which is based on morphological change in tumour dimension to evaluate and monitor the therapeutic response was primarily designed for cytotoxic drugs, which may not be an appropriate indicator for activity of new targeted agents because the morphological changes in gross tumour size significantly lag behind biological and molecular changes that happen early in treatment responders.⁶ New therapeutics such as antiangiogenic agents

may induce alterations in vascular supply, rather than morphological changes, and imaging studies incorporating tumour physiology may be more appropriate. Recent MRI studies in RCC treated with antiangiogenic agents have focused on assessments of functional alterations attributed to therapeutic response. In patients with metastatic RCC treated with sorafenib, novel response criteria combining early MRI changes in both tumour enhancement and size were helpful to identify the lack of responsiveness to antiangiogenic therapy.⁷ Furthermore, perfusion measurements using arterial spin labelling MRI have shown utility as surrogate markers for the effect of sorafenib in the RCC xenograft models.^{8,9} Other potential imaging strategies to assess functional therapeutic response are dynamic contrast-enhanced MRI (DCE-MRI) and diffusion-weighted MRI (DWI).

DCE-MRI can assess tissue perfusion and permeability by monitoring the uptake and clearance of injected contrast agents in specific tissues over time. Prior studies have

supported a negative correlation between perfusion parameter (K^{trans}) and microvessel density after antiangiogenic therapy.^{10,11} DCE-MRI has been widely investigated to measure early tumour responses after treatment with angiogenesis inhibitors, as reported in clinical^{12,13} and pre-clinical studies.^{10,14} DWI can provide microstructural information on tissue cellularity, cell membrane integrity and extracellular space tortuosity based on water diffusivity measurements. Any therapeutic agents that cause necrosis and cell lysis will lead to increases in water diffusion and corresponding increases in ADC values.¹⁵ DWI has been also used to monitor the effects of treatment in patients with cancer,^{16–18} and its use in assessing acute effects induced by antiangiogenic agents has been evaluated.^{10,19}

The ability to assess early therapeutic effects of antiangiogenic agents may help to determine whether treatment is effective and should be continued or not. However, to the best of our knowledge, no published studies have evaluated the potential of DCE-MRI and DWI as imaging biomarkers for monitoring early therapeutic response to antiangiogenic agents in RCC. Therefore, the purpose of our study was to investigate the feasibility of DCE-MRI and DWI in monitoring early therapeutic response to sorafenib in RCC xenograft models.

METHODS AND MATERIALS

This study was reviewed and approved by the Institutional Animal Care and Use Committee of Samsung Medical Center. All procedures were performed in the Medical Center Animal Research Facility.

Tumour model and experimental protocol

The human RCC ACHN cell line was cultured in RPMI 1640 medium supplemented with 10% foetal bovine serum, mixed with 1% antibiotics in a standard humidified incubator at 37°C in an atmosphere of 5% CO₂.

12 male BALB/c nude mice (mean weight, 30 g; age range 6–8 weeks) were used. BALB/c nude mice were purchased from Orient Bio (Sunnam, Republic of Korea) and housed in specific pathogen-free conditions. The xenograft tumours were established by subcutaneous injection of 5×10^6 ACHN cells in a total volume of 0.1 ml of serum-free medium containing 50% BD Matrigel™ (BD Bioscience, San-Jose, CA) into the right thigh under isoflurane mixture (70% N₂O/30% O₂ and 1.5% isoflurane)-induced anaesthesia. Tumours developed in 92% (11 of 12) of the mice and 2 mice succumbed to disease prior to treatment initiation. Thus, nine mice were included for the study. Small tumours were usually visible within 7 days of implantation. Tumours were measured daily with callipers to ensure a consistent size for the outset of treatment. All mice ($n = 9$) were treated with sorafenib (40 mg kg⁻¹ per day) by oral gavage when tumours reached 6 mm in diameter. Sorafenib treatment was continued daily for 7 days during the experiment. MRI was performed at baseline (before initiating sorafenib treatment) and at scheduled intervals after treatment.

MRI acquisition

All images were obtained using a 7.0-T MRI System (Bruker Biospin, Fällanden, Switzerland) equipped with a 20-cm gradient

set capable of supplying up to 400 mTm⁻¹ with a rise time of 100 ms. A birdcage coil (inside diameter, 72 mm; Bruker Biospin) was used for excitation, and an actively decoupled surface coil (inside diameter, 20 mm; Bruker Biospin) was used for signal reception. The mice were anaesthetized with isoflurane (5% for induction and 1–1.5% for maintenance) in a mixture of O₂ and air gases (3:7) delivered to a nose cone for spontaneous respiration throughout the experiment. All mice were used to fix the position of the thigh tumour before placement in the magnet. Temperature and respiration were monitored during scanning. The rectal temperature was maintained at $37 \pm 1^\circ\text{C}$ using an overhead radiant heater and a warming matrix during the scanning. The respiration sensor was placed under the chest of the mouse. The respiration rate was monitored using a MRI-compatible small-animal respiratory monitoring device. The targeted respiration rate was 60–70 breaths min⁻¹. The maintenance dose of isoflurane (1–1.5%) and heated air flow were adjusted continuously to ensure stable and reproducible depth of anaesthesia.

DCE-MRI and DWI were obtained 0, 1, 3 and 7 days after sorafenib treatment to assess the change in intratumoral vascularity and apparent diffusion coefficient (ADC) values. DCE-MRI was obtained using a T_1 weighted, two-dimensional, fast low-angle 4-shot sequence (matrix size = 128×128 ; resolution = $234 \times 234 \mu\text{m}^2$; flip angle = $5^\circ, 15^\circ, 35^\circ, 60^\circ, 70^\circ$; repetition time (TR)/echo time (TE) = 60/3 ms; slice thickness = 2 mm, without any interslice gap, number of slices between four and seven and a sampling interval of 5.7 s). Baseline images were acquired for 57 s, followed by an automatic injection (Harvard PHD 2000 infusion pump; Harvard Apparatus, Inc., Holliston, MA) of 0.1 mmol kg⁻¹ of gadoterate meglumine (Dotarem®; Guerbet, Paris, France) over 5 s, followed by further acquisitions, over a total time of 11 min 31 s (repetitions = 120). A catheter for lateral tail vein access was made with insulin syringe and polyethylene tube. One side of the catheter is 31 G needle of insulin syringe (Becton, Dickinson and Company, Franklin Lakes, NJ). The other side of insulin syringe was thinned with heat application and then connected with polyethylene tube (inside diameter = 0.288 mm; Becton, Dickinson and Company, MD). The total length of the in-house developed catheter was 60 cm. Via this tail vein catheter, contrast was injected using 1 cc syringe with 30 G needle.

DWI was obtained using an echoplanar imaging sequence (b -values = 0/100/200/400/600/800/1000 s mm⁻², number of excitations = 4; field of view = 25.6×25.6 mm, matrix = 128×128 , TR/TE = 3000/100 ms, slice thickness = 2 mm, slice number = 4, duration = 5 min 36 s).

Anatomic MRI was performed on the same time with DCE-MRI and DWI to monitor tumour volume and size change during therapy. Two-dimensional T_2 weighted spin echo images (matrix size = 256×256 , resolution = $117 \times 117 \mu\text{m}^2$, flip angle = 180° , TR/TE = 3000/60 ms, slice thickness = 2 mm, without any interslice gap) were obtained.

Data analysis

All data obtained at DCE-MRI and DWI were transferred to a personal computer workstation and analysed using previously

validated in-house software written using MATLAB® v. 7.6 (Mathworks®, Natick, MA). For quantification of DCE-MRI data, the concentration of contrast agent $C(t)$ at time point in a tissue voxel was estimated using the variable flip angle method.²⁰ The concentration of the contrast agent was estimated by determining the difference in longitudinal relaxation rates: $C(t) = (1/T_1(t) - 1/T_{10})/r1$, where $r1$ is the longitudinal relaxivity in plasma ($2.92 \text{ s}^{-1} \text{ mM}^{-1}$).²¹ DCE pharmacokinetic modelling employs Tofts' two-compartment model: $C_t(t) = K^{\text{trans}} C_p(t) \otimes \exp(-K^{\text{trans}} t/v_e)$, where C_t is the concentration of contrast agent in the observed tissue, C_p is the concentration in the blood plasma, K^{trans} is the volume transfer contrast and v_e is the fractional extravascular extracellular space per unit volume of tissue. The arterial input function (AIF) was manually determined near the femoral artery before treatment based on the shape of concentration, and the determined AIF was used for analysis of DCE-MRI data after treatment for each mouse. If the determination of AIF from DCE-MRI data before treatment was failed, the average AIF was used for estimation of pharmacokinetic parameters before and after treatment. Non-linear least squares fitting was implemented using *lsqnonlin* function from the MATLAB® Optimization Toolbox™. ADC values were computed by exponential fitting of the local signal intensity vs b -values on a voxel-wise basis. At each time points, DCE-MRI parameters (K^{trans} and v_e) and ADC values were calculated in the tumour. For measuring the values of pharmacokinetic DCE-MRI parameters and ADC in the tumour, the regions of interest (ROIs) were manually drawn in the pre- and post-treatment images. In each tumour, the ROIs in the tumours were drawn to include as much of the tumour as possible in a single image that had the greatest amount of visible tumour and mean value of the voxels in the ROIs were used (Figure 1). Moreover, the ROIs were selected to avoid necrotic areas of non-enhancement. The mean ROIs of the tumour were 3.8 mm^2 (range $1.8\text{--}6.3 \text{ mm}^2$).

The tumour size and volume were also measured on the basis of T_2 weighted images. Tumour size was defined as the maximum diameter measured with a calliper tool on axial and coronal T_2

weighted images. Tumour volume was calculated as the summation of all of the areas of tumour on axial T_2 weighted images multiplied by the slice interval. Final tumour size and volume response (in percentage) was calculated according to the following equation: $[\text{pre-treatment size (volume)} - \text{post-treatment size (volume)}] / \text{pre-treatment size (volume)} \times 100$. Tumour size and volume changes on 7 days after therapy were correlated with changes in DCE-MRI parameters or ADC values during therapy. A single investigator (TYJ) made all measurements to maintain consistency. Measurement of the tumour size and volume or drawings of ROIs were taken twice, and the results were then averaged.

Statistical analysis

Data were analysed using PASW® statistical software v. 20.0 (IBM Corp., New York, NY; formerly SPSS Inc., Chicago, IL). Analysis using mixed model was performed to evaluate changes in pharmacokinetic DCE-MRI parameters and ADC values in the tumour and to assess changes in tumour size and volume at each time point. The pairwise comparisons between two time points were performed using paired Student's t -test with Bonferroni's correction. Spearman correlation analysis was used to evaluate the relations between changes of MR parameters during sorafenib treatment and final tumour size and volume at 7 days after treatment. A p -value < 0.05 was considered statistically significant.

RESULTS

Dynamic contrast-enhanced MRI parameters and apparent diffusion coefficient values

DCE-MRI and DWI in all of the RCC xenografts ($n = 9$) were successfully obtained. Results of pharmacokinetic parameters of DCE-MRI and ADC values are summarized in Table 1.

The K^{trans} changes showed a significant difference between certain time points after sorafenib treatment ($p = 0.005$). Compared with pre-therapy, K^{trans} showed a significant decrease at 3 days after therapy ($p = 0.039$) and 7 days after therapy ($p = 0.033$), but K^{trans} between pre-therapy and 1 day after therapy was not

Figure 1. Dynamic contrast-enhanced MR image of male BALB/c nude mouse with subcutaneously injected xenograft tumour model of human renal cell carcinoma. (a) The dotted region of interest (ROI) was placed on the enhancing portion of tumour. (b) The enhancing portion shows high signal intensity of viable peripheral tumour compared with adjacent muscle on T_2 weighted image (arrows). (c) Concentration-time curve of the ROI shows a decrease of contrast enhancement (open circles: observed data; blue: baseline; red: 7 days after sorafenib treatment; dotted line: fitting data using pharmacokinetic modelling method). TR, repetition time. For colour image see online.

Table 1. Results of pharmacokinetic parameters of dynamic contrast-enhanced MRI and apparent diffusion coefficient (ADC) values in tumours at each time point

Parameter	Baseline	1 day	3 days	7 days	<i>p</i> -values
K^{trans} (min^{-1})	0.055 ± 0.013	0.025 ± 0.010	0.011 ± 0.006	0.020 ± 0.015	0.005
v_e	0.677 ± 0.109	0.693 ± 0.107	0.659 ± 0.149	0.649 ± 0.113	0.970
ADC ($\times 10^{-3} \text{ mm}^2 \text{ s}^{-1}$)	0.243 ± 0.191	0.258 ± 0.156	0.452 ± 0.173	0.550 ± 0.164	0.004

Data are expressed in mean $\pm$ standard deviation. *p*-values are determined with analysis using mixed model.

significantly different ($p = 0.072$). Although v_e showed a gradual decrease from 1 to 7 days after therapy, it did not show a statistical difference at any time points ($p = 0.97$). The ADC changes showed a significant difference between certain time points ($p = 0.004$) (Figure 2). Compared with pre-therapy, ADC showed a significant increase at 7 days after therapy ($p = 0.039$), but ADC between pre-therapy and 1 day and ADC between pre-therapy and 3 days after therapy were not significantly different ($p = 1.0$ and $p = 0.468$, respectively).

Tumour size and volume changes

The size and volume of the tumours at each time point are summarized in Table 2. The changes of mean tumour size and volume did not show statistical differences at any time point ($p = 0.093$ and $p = 0.273$, respectively). The mean reduction rates of tumour size were $3.2\% \pm 5.0\%$ at 1 day, $10.3\% \pm 3.7\%$ at 3 days and $9.9\% \pm 2.8\%$ at 7 days after therapy initiation. The mean reduction rates of tumour volume were $2.51\% \pm 6.4\%$ at 1 day, $8.9\% \pm 11.4\%$ at 3 days and $13.4\% \pm 13.9\%$ at 7 days

Figure 2. The change in tumour apparent diffusion coefficient (ADC) values (dotted region of interest) at each time point, showing that mean ADC values increased over time: (a) baseline colour-coded ADC map overlaid on T_2 weighted image before treatment with sorafenib ($0.082 \times 10^{-3} \text{ mm}^2 \text{ s}^{-1}$), (b) colour-coded ADC maps overlaid on T_2 weighted images after 1 day of treatment ($0.125 \times 10^{-3} \text{ mm}^2 \text{ s}^{-1}$), (c) after 3 days of treatment ($0.524 \times 10^{-3} \text{ mm}^2 \text{ s}^{-1}$) and (d) after 7 days of treatment ($0.622 \times 10^{-3} \text{ mm}^2 \text{ s}^{-1}$).

Table 2. Results of tumour size and volume at each time point

Parameter	Baseline	1 day	3 days	7 days	<i>p</i> -values
Size (mm)	5.9 ± 0.8	5.8 ± 0.8	5.4 ± 0.9	5.3 ± 0.6	0.093
Volume (mm ³)	78.1 ± 2.4	76.4 ± 2.5	70.3 ± 1.9	66.2 ± 1.7	0.273

Data are expressed in mean ± standard deviation. *p*-values are determined with analysis using mixed model.

after therapy. Final tumour size and volume changes during 7 days were not associated with changes in K^{trans} ($p = 0.104$ and $p = 0.391$, respectively), v_e ($p = 0.505$ and $p = 0.285$, respectively) and ADC values ($p = 0.873$ and $p = 0.873$, respectively) at 1 day, K^{trans} ($p = 0.624$ and $p = 0.007$, respectively), v_e ($p = 0.391$ and $p = 0.873$, respectively) and ADC values ($p = 0.391$ and $p = 0.873$, respectively) at 3 days, and K^{trans} ($p = 0.624$ and $p = 0.188$, respectively), v_e ($p = 0.624$ and $p = 0.188$, respectively) and ADC values ($p = 0.505$ and $p = 0.873$, respectively) at 7 days.

DISCUSSION

Angiogenesis is essential for the progression of all solid tumours, and angiogenesis inhibition remains a promising approach in cancer therapy. The antiangiogenic agent we used, sorafenib, is an orally active multikinase inhibitor with effects on VEGF receptors, platelet-derived growth factor receptors and tyrosine kinase.¹ Sorafenib has proven antitumour activity by inhibiting angiogenesis as well as promoting tumour cell apoptosis and necrosis in animal models.^{1,22} It is currently approved for advanced hepatocellular carcinoma and RCC in humans and has been shown to have significant clinical activity as evidenced by prolonged progression-free survival in patients with metastatic RCC in whom previous systemic therapy had failed.^{3,4} However, a significant portion of patients with advanced RCC develop resistance to sorafenib.³ The mechanism of resistance is conjectural but likely comprises a component of non-VEGF-mediated angiogenic escape.²³ The ability to determine the development of sorafenib resistance at an early time point for treatment decisions is important. Therefore, we investigated the feasibility of DCE-MRI and DWI in monitoring early tumour response to sorafenib in animal models. The application of these MR techniques to RCC after sorafenib treatment, however, has not been often attempted.^{12,13}

In our study, the quantitative parameters such as K^{trans} and v_e derived from DCE-MRI and ADC values in human RCC xenograft models showed temporal changes following treatment with sorafenib. As early as 3 days after the start of sorafenib, K^{trans} was significantly decreased compared with baseline values. In addition, a significant increase in ADC values was observed after 7 days of sorafenib. These findings indicate that DCE-MRI and DWI as imaging biomarkers might be a useful tool for evaluating early changes of tumour physiology induced by sorafenib in RCC. The final tumour size and volume changes were not associated with changes of perfusion parameters (K^{trans} and v_e) and ADC values within 7 days after sorafenib treatment. However, our study did not analyse the correlation of the perfusion parameters/morphological changes with the long-term outcome. Further research may be warranted to define this issue.

DCE-MRI has been currently used in the field of oncologic imaging because it can evaluate the microenvironment occurring within tumours by quantitatively measuring perfusion parameters. DCE-MRI has already been shown to be able to predict responses and monitor the effects of tumour treatment with neoadjuvant chemotherapy or radiation therapy in several anatomic sites, including brain,²⁴ breast²⁵ and pelvic organ tumours,^{26,27} and possibly androgen-ablative treatment in prostate cancer.²⁸ Recent investigations tried to assess early therapeutic response in tumour vascular parameters after treatment with angiogenesis inhibitors in human studies^{12,13} and pre-clinical animal models.^{10,14} Clinical studies have shown the significant parameter changes derived from DCE-MRI after 4–12 weeks of single-agent sorafenib in patients with advanced RCC.^{12,13} Pre-clinical xenograft studies using DCE-MRI demonstrated that VEGF receptor inhibitor decreased vascular permeability in prostate cancer xenografts.¹⁴ The volume transfer constant K^{trans} was the only quantitative DCE-MRI parameter that showed significant reduction in our results, which was in concordant with previous studies.^{12,13} The K^{trans} describes the transport of the contrast agent from vascular space to the extravascular extracellular space. It also approximates the tissue blood flow and permeability surface area product per unit volume. Thus, reduction of K^{trans} may suggest either decreased tissue blood flow or decreased microvessel surface area. In our study, the rapid and marked decrease in K^{trans} values is more likely explained by the antiangiogenic effects of sorafenib than tumour cell death.

DWI provides a quantitative measure for the diffusivity of water molecules in tissue. In oncologic imaging, DWI has been applied for tissue characterization and treatment response. The low values of diffusion found in a variety of cancers have been attributed to their increased cellular density, tissue disorganization and increased extracellular space tortuosity; successful treatment is reflected by rising ADC values according to a decrease in these factors.²⁹ Because tumour cell death and vascular changes in response to treatment can precede a quantifiable morphological tumour change, parameter changes derived from DWI could be a useful treatment response biomarker for therapies that induce apoptosis or inhibit angiogenesis. Increases in ADC values have been noted in a wide variety of cancer types, including primary and metastatic cancers to the liver^{17,18} after chemotherapy and brain tumour³⁰ after radiation therapy. More recently, its use in assessing therapeutic effects of angiogenesis inhibitor has been evaluated. Our study showed a significant increase of ADC values at 7 days after sorafenib treatment. Similar results were reported by Moestue et al¹⁰ who investigated pre-clinical testing using DWI in breast cancer xenograft model after treatment with bevacizumab and showed rapid increase of ADC values at

3 days. On the contrary, it has been noted that sorafenib treatment in patients with hepatocellular carcinoma leads to cellular swelling in the early phases of apoptosis and intralesional haemorrhage that lowers ADC values.¹⁹

Our study has the following limitations. First, the ROI analysis concentrated on the peripheral tumour regions to avoid the potential impact of necrosis; therefore, it may not reflect the inherent heterogeneity of the entire tumour. However, when the viable peripheral tumour regions were evaluated, measurement accuracy using DCE-MRI improved.³¹ In addition, necrosis had little influence on the evaluation of treatment response using DWI.¹⁰ Nonetheless, in future studies, histogram analysis may be recommended to quantify heterogeneous changes of the entire tumour during treatment. Second, as noted previously, while quantitative DCE-MRI parameter of K^{trans} may be more physiologically meaningful than semi-quantitative data, its calculation requires accurate determination of the AIF. This approach may be limited by inflow effects and inaccurate T1 measurements caused by B_1 field inhomogeneity.³² Third, we only assessed early alterations of perfusion and diffusion parameters induced by sorafenib. It is unclear, however, whether acute effects can predict final treatment outcome. As tumours can regrow despite early positive response, the dynamic turnover of angiogenesis cannot be anticipated based on early changes alone. Longitudinal evaluations of these parameters are needed to monitor tumour response to antiangiogenic therapy. Fourth,

our study did not evaluate histopathological changes in RCC xenografts at the same time points with MRI acquisition after treatment. However, it is impossible to obtain histological specimen in a longitudinal evaluation of quantitative MRI parameters in the same subject after treatment. Fifth, our study was not randomized into the control and treatment groups. The purpose of our study was to investigate the feasibility of DCE-MRI and DWI in detecting early therapeutic change of tumour biology. Finally, internal validation for perfusion parameters from DCE-MRI was not made. The perfusion parameters can be influenced by variables related to heart rate, blood pressure and respiration rate. We attempted to maintain the targeted respiration rate between 60 and 70 breaths min^{-1} , but other factors were not controlled.

In conclusion, our results support the potential for DCE-MRI and DWI to function as surrogate markers for early therapeutic response in cancer patients receiving antiangiogenic agents such as sorafenib. One direct clinical benefit of this approach would be to identify non-responders to sorafenib early in the course of therapy and preclude toxicity and other negative side effects. It might allow individualizing treatment strategies and hopefully increase long-term survival in clinical trials.

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