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Co-clinical quantitative tumor volume imaging in ALK-rearranged NSCLC treated with crizotinib

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Abstract

Purpose—To evaluate and compare the volumetric tumor burden changes during crizotinib therapy in mice and human cohorts with ALK-rearranged non-small-cell lung cancer (NSCLC).

Methods—Volumetric tumor burden was quantified on serial imaging studies in 8 bitransgenic mice with *ALK*-rearranged adenocarcinoma treated with crizotinib, and in 33 human subjects with *ALK*-rearranged NSCLC treated with crizotinib. The volumetric tumor burden changes and the time to maximal response were compared between mice and humans.

Results—The median tumor volume decrease (%) at the maximal response was –40.4% (range: –79.5% to +11.7%) in mice, and –72.9% (range: –100% to +72%) in humans (Wilcoxon $p=0.03$). The median time from the initiation of therapy to maximal response was 6 weeks in mice, and 15.7 weeks in humans. Overall volumetric response rate was 50% in mice and 97% in humans. Spider plots of tumor volume changes during therapy demonstrated durable responses in the human cohort, with a median time on therapy of 13.1 months.

Conclusion—The present study described an initial attempt to evaluate quantitative tumor burden changes in co-clinical imaging studies of genomically-matched mice and human cohorts with ALK-rearranged NSCLC treated with crizotinib. Differences are noted in the degree of maximal volume response between the two cohorts in this well-established paradigm of targeted therapy, indicating a need for further studies to optimize co-clinical trial design and interpretation.

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Introduction

Recent advances in the development of genetically engineered mouse models have enabled co-clinical studies of lung cancer in which lung tumors harboring specific oncogenic drivers are treated with selective targeting agents in mice, mirroring the human lung cancer trials of the agents.¹ Co-clinical studies of murine lung cancer models have provided valuable data for identifying genetic modifiers of treatment response, validating markers of response and resistance, and predicting strategies to overcome resistance. The information helps to rapidly generate new clinically relevant data that can inform the interpretation of completed trials and the design of future clinical studies.¹ The role of co-clinical studies in the efforts to advance precision medicine for cancer has been increasingly recognized.²

Assessment of tumor burden using quantitative imaging has been a key component in oncology trials in mice and humans, as it characterizes tumor response and progression and defines trial endpoints.^{1, 3-8} Among different quantitative imaging methods, tumor volume has been the primary quantitative measure for therapeutic monitoring in mouse models for lung cancer during therapy^{1, 3, 4, 9-11}. Tumor volume has been applied in human trials as an addition to conventional diameter measurements using RECIST, and has been shown to more accurately characterize tumor burden changes compared to diameters¹²⁻¹⁵.

In spite of the growing interest and increasing number of reports describing co-clinical investigations in cancer, there is a lack of studies focusing on quantitative imaging data in mice with detailed comparisons with humans. For example, the optimal time points for serial follow-up scans in mice that best mirror the landmark time points in humans remain to be determined. It also remains unknown if the degree of maximal tumor shrinkage (or the depth of response) demonstrates similar distributions in human and mice cohorts when both cohorts have tumors driven by the same genomic alterations and are treated with same anti-cancer agents. Further investigations of the comparison of co-clinical quantitative imaging data is needed to maximize the utility of information obtained from co-clinical oncology studies.

The anaplastic lymphoma kinase (ALK)-rearranged non-small-cell lung cancer (NSCLC) is one of the representative genomically-defined subsets of NSCLC that can be effectively treated with ALK inhibitors. Crizotinib is an ALK inhibitor that was granted FDA approval for the treatment of ALK-rearranged NSCLC in 2011, and has been widely used for the treatment of this subset of patients in the clinical setting.¹⁶ Genetically-engineered mouse models with ALK-rearranged adenocarcinoma have been previously generated and studied in a co-clinical trial demonstrating higher response rates and survival benefits in crizotinib-treated mice compared to chemotherapies using pemetrexed or docetaxel,³ mirroring a phase 3 trial of crizotinib in ALK-positive human NSCLC patients.¹⁷ The quantitative imaging data obtained during the co-clinical murine trial of ALK- rearranged adenocarcinomas have been made available, providing a unique opportunity to investigate the quantitative tumor burden changes and response in the mice cohort and perform detailed comparisons with a human cohort of ALK-positive NSCLC treated with crizotinib.

The purpose of the present study is to compare the volumetric tumor burden changes on quantitative imaging during therapy in mice and human cohorts, using ALK-rearranged NSCLC treated with crizotinib as a well-established paradigm, and obtain insights for further advances of co-clinical imaging studies.

Materials And Methods

Bitransgenic mice and imaging

Mice cohort consisted of 8 bitransgenic mice harboring *EML4-ALK*-positive adenocarcinoma that were treated with crizotinib monotherapy and underwent serial magnetic resonance imaging (MRI) as a part of the previously published co-clinical trial.³ Generation of bitransgenic mice with lung-specific doxycycline-inducible *EML4-ALK* expression was described previously.¹⁸ Tumor-bearing mice were treated with crizotinib at the dose of 50 mg/kg or 100 mg/kg by daily oral feeding in water, as described previously.³

All mice had baseline MRI prior to the initiation of crizotinib therapy, and serial follow-up scans at 2, 6, 9, and 13 weeks of therapy. MRI scans were performed using a previously described protocol on a 4.7 Tesla scanner (BioSpec 47/40; Bruker BioSpin). Lung tumor volume measurement on serial MRI scans by 2 operators using 3D Slicer software performed as a part of the previously published study was analyzed in the present study.^{1, 3}

Human Subjects and Imaging

Human subjects included 33 advanced ALK-positive NSCLC patients (13 males, 20 females; Median age: 52 years; Age range: 28-82 years) treated with crizotinib monotherapy at Dana-Farber Cancer Institute, who had a baseline chest CT performed prior to initiating therapy demonstrating at least one measurable lung lesion, and had at least one follow-up chest CT. All patients had histologically or cytologically confirmed NSCLC with ALK rearrangement determined by FISH assay. Clinical characteristics of human subjects are listed in Table 1.

Chest CT scans were performed at baseline prior to crizotinib therapy to assess response to therapy. Scans were performed every 6 or 8 weeks according to trial protocols in those treated in trials, and per clinical provider's discretion for those treated as a standard clinical care. Tumor volume of the dominant lung lesion (one lesion per patient) was measured on baseline and serial follow-up CT scans until the end of crizotinib therapy or last follow-up using the previously validated technique on the volume analysis software (Vitrea 2; Vital Images, Minnetonka, MN)^{14,19-21}.

Volumetric Tumor Response Analysis

Proportional tumor volume change (%) at each time point was calculated in reference to the baseline volume for both mice and human subjects. The proportional volume change at the maximal volumetric response was calculated, and the maximal response timepoint was identified for each subject. Volumetric response was assessed according to a previously described cutpoint of at least 30% decrease of tumor volume comparing to baseline for

response.^{1, 3} The distributions of the proportional volume change at the maximal volumetric response were compared between two cohorts using a Wilcoxon rank sum test.

Results

The waterfall plots of the tumor volume decrease (%) were generated at the maximal response in the mice cohort and in the human cohort (Fig. 1A, B). Tumor volume decrease (%) at the maximal response ranged from -79.5% to +11.7% in mice, and from -100% to +72% in human (median: -40.4%, -72.9%, respectively; Wilcoxon $p=0.03$) (Fig. 2). The median time from the initiation of therapy to maximal response was 6 weeks in mice, and 15.7 weeks in humans. Volumetric response rate was 97% in human and 50% in mice at the maximal response.

Spider plots of tumor volume changes during therapy demonstrate tumor volume dynamics in mice and humans (Fig. 3A, B). Median time on therapy was 13.1 months in the human cohorts, and durable responses were maintained with the majority of patients with tumor burden below the baseline burden during therapy (Fig. 3B). In the mice cohort, 4 mice (50%) experienced tumor growth beyond the baseline while tumor burden stayed below the baseline in others, during the duration of crizotinib therapy (13 weeks for 6 mice and 9 weeks for 2 mice.)

Discussion

The present study evaluated the volumetric tumor burden changes in ALK-rearranged NSCLC during crizotinib therapy in transgenic mice and human subjects, and reported comparisons of the quantitative tumor volume data between the two cohorts. Similarities and differences of quantitative imaging data noted in the well-established paradigm of targeted therapy for NSCLC provide insights for further investigations to optimize quantitative imaging approach for co-clinical studies.

The degree of the proportional tumor volume shrinkage in reference to baseline at the maximal response was greater in human subjects than in mice, and the response rate was higher in humans than in mice. The observation is notable as it indicates that a certain degree of difference is expected between mice and human cohorts, even when they harbor tumors driven by the same genomic alterations and are treated with the same anti-cancer agent. Moreover, crizotinib for ALK-rearranged NSCLC is an FDA approved treatment that has shown superiority compared to platinum-based chemotherapies in a human phase 3 trial,¹⁷ and the previously published murine co-clinical trial mirroring the human trial has demonstrated the concordant observations.³ Quantitative imaging data may not completely match between humans and mice in co-clinical trials, even in a well-established paradigm of molecular targeted therapy using crizotinib for ALK-rearranged NSCLC. The difference could be due to the differences of epigenetic factors in mice and humans, drug doses and pharmacokinetics, and technical differences in image acquisition and tumor burden quantification. The absolute volume of baseline tumor burden may also influence the results, because tumor shrinkage is obtained as a proportional change comparing to the baseline.

The allowable degree of differences of quantitative tumor imaging data between mice and humans for concordant co-clinical trial results remain to be further investigated.

The choice of optimal time point to assess response that can predict clinical outcome is another important issue when designing co-clinical trials of mice and humans. In humans, a certain landmark time point can be chosen based on the trial or clinical follow-up scheme (for example 8 weeks of therapy) to test early predictive or prognostic markers during therapy. Selecting a corresponding time point in mice that can adequately mirror a human study design remains a challenge given the differences in the length of overall survival and progression-free survival in mice and humans. The time to maximal tumor response was about 2.5 times longer in humans than in mice (15.7 weeks vs. 6.0 weeks, respectively), which provides some insights about the choice of optimal time points, at least for the specific cohorts with ALK-rearranged NSCLC. Future co-clinical trials should be designed to allow identification and testing of corresponding landmark time points in mice and humans, which may be specific to tumor types and therapies.

Duration of therapy was much longer in the human cohort, and overall more durable responses were observed in humans than in mice. While it is somewhat expected given a different survival time of the two cohorts and the mice study design of a predetermined follow-up length, the observation indicates challenges in co-clinical studies for the evaluations of longer term effects such as occurrence of acquired resistance during therapy and subsequent tumor growth. Current designs of co-clinical trials may be better suited to test early markers of efficacy and outcome, while further investigations may be needed to generate quantitative data in mice that mirrors humans during the later course of therapy.

Limitations of the present study include small numbers of subjects in mice and human cohorts with ALK-rearranged tumors from a single institution experience. It would have been ideal to analyze the large prospective phase 3 human cohort from the published trial; however, the logistic barriers do exist to obtain serial imaging studies from multi-center international trials. The clinical cohort of ALK-positive NSCLC patients treated at a single institution was chosen due to the availability of serial scans performed using a standard imaging protocol at the institution. The imaging modalities for mice and human cohorts were different, and MRI was used for mice and CT was used in humans. This is because the choice of the modality was based on the most frequently used method specific to each cohort. Likewise, the tumor volume measurement technique was chosen based on the prior data of reproducibility testing, which identified the most optimal algorithm to quantify tumor burden in each cohort. Although the modalities were different, the quantitative imaging metrics used the same metrics, tumor volume, to assess tumor burden changes, and the modality chosen in each cohort has been previously validated as a reliable measure of tumor burden. Mice and human tumors in the present study were not individually matched, as the mouse lung cancer model was genetically-engineered to develop lung tumors and not developed from xenografts. Nevertheless, the mice tumors shared the same genomic driver with the human tumors, and developed de novo in the mice lungs thus best simulating human lung cancers. We acknowledge that the present study is an initial investigation of the small cohorts, and further studies are needed to address issues in co-clinical quantitative imaging.

In conclusion, the present study described an initial attempt to compare quantitative tumor burden changes during co-clinical trials of genomically-matched mice and human cohorts with ALK-rearranged NSCLC treated with crizotinib, and described similarities and differences, providing insights for further studies to optimize murine co-clinical trial designs.

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Highlights

Role of co-clinical studies in precision cancer medicine is increasingly recognized.

This study compared tumor volume in co-clinical trials of ALK-rearranged NSCLC.

Similarities and differences of tumor volume changes in mice and humans were noted.

The study provides insights to optimize murine co-clinical trial designs.

Fig. 1. Waterfall plots of the proportional tumor volume changes at the maximal response in mice (A) and human (B) cohorts.

Fig. 2.
The distribution of the proportional tumor volume changes at the maximal response in mice (A) and human (B) cohorts.

Fig. 3. Spider plots of tumor volume changes during therapy in mice (A) and human (B) cohorts.

Table 1
Demographics and clinical characteristics of 33 human subjects

Variables		Number of patients (%)
Sex	Male	13
	Female	20
Age	Median	52 years
	Range	28 - 82 years
Race	White	28
	Asian	2
	Black	1
	Hispanic or Latino	2
Histology	Adenocarcinoma	31
	Adenosquamous carcinoma	1
	Poorly differentiated NSCLC	1
Smoking	Non-smoker	20
	Former smoker	13
Prior systemic therapy	None (1 st line crizotinib)	8
	1 (2 nd line)	17
	2 (3 rd line)	5
	3 (4 th line)	2
	4 (5 th line)	1