RESEARCH PAPER

Cabozantinib inhibits tumor growth and metastasis of a patient-derived xenograft model of papillary renal cell carcinoma with MET mutation

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ABSTRACT

MET plays an important role in the development and progression of papillary renal cell carcinoma (pRCC). Evaluation of efficacy of MET inhibitors against pRCC has been hampered by limited preclinical models depicting MET abnormalities. We established a new patient-derived xenograft (PDX) model of pRCC carrying an activating mutation of MET and tested the ability of cabozantinib, an inhibitor of receptor tyrosine kinases including MET, to inhibit tumor growth and metastasis. Precision-cut, thin tissue slices from a pRCC specimen obtained by nephrectomy were implanted under the renal capsule of Rag2\textsuperscript{\textminus}/\gamma-C\textsuperscript{\textminus}/mice to establish first generation TSG-RCC-030. Histologic and genetic fidelity and metastatic potential of this model were characterized by immunohistochemistry, direct DNA sequencing and quantitative polymerase chain reaction (qPCR). The effect of cabozantinib on tumor growth and metastasis was evaluated. Whether measurements of circulating tumor DNA (ctDNA) by allele-specific qPCR could be used as a biomarker of tumor growth and response to therapy was determined. Subrenal and subcutaneous tumor grafts showed high take rates and metastasized to the lung. Both primary tumors and metastases expressed typical markers of pRCC and carried the same activating MET mutation as the parental tumor. Cabozantinib treatment caused striking tumor regression and inhibited lung metastasis in TSG-RCC-030. Plasma ctDNA levels correlated with tumor volume in control mice and changed in response to cabozantinib treatment. TSG-RCC-030 provides a realistic preclinical model to better understand the development and progression of pRCC with MET mutation and accelerate the development of new therapies for pRCC.

Introduction

Papillary renal cell carcinoma (pRCC) is a histologically, genetically and clinically distinct subtype of renal cell carcinoma (RCC). Second in frequency to clear cell renal cell carcinoma (ccRCC) which accounts for approximately 70–80% of cases, pRCC represents up to 10–20% of all RCCs.\textsuperscript{1,2} Histologically, pRCC is characterized by a predominantly papillary growth pattern with tubular areas and fibrovascular cores.\textsuperscript{3} Genetically, pRCC is marked by high incidence of trisomy or tetrasomy of chromosome 7, where genes for the MET receptor and its ligand, hepatocyte growth factor (HGF), reside.\textsuperscript{4} Activating mutations in the kinase domain of MET are found in the majority of hereditary pRCC cases, as well as in 5–13% of sporadic pRCC cases.\textsuperscript{5,6} Elevated MET gene expression was observed in large molecular studies of pRCC and correlated with adverse clinical features,\textsuperscript{7–9} indicating a pivotal role of MET in pRCC.

Although pRCC overall has a better prognosis than ccRCC, currently available therapies provide only modest benefit for patients with advanced pRCC.\textsuperscript{1,2,10} In recent years, the treatment of ccRCC has undergone dramatic changes with the development of FDA-approved targeted therapies including mTOR inhibitors and tyrosine kinase inhibitors of VEGF and its relatives.\textsuperscript{11–13} However, there are few published clinical trials specifically evaluating the effects of these compounds in pRCC. Results from clinical trials enrolling patients with multiple RCC subtypes indicate that the clinical benefit of mTOR inhibitors is similar in ccRCC and pRCC patients,\textsuperscript{14} whereas agents targeting VEGF pathways are significantly inferior in pRCC compared to ccRCC patients.\textsuperscript{15,16}

The development of novel therapies to effectively treat pRCC has been hampered by a lack of realistic preclinical models. We previously developed tissue slice graft (TSG) models of RCC by implanting precision-cut, thin tissue slices from fresh tumor specimens either under the renal capsule or skin of immunodeficient mice.\textsuperscript{17,18} Here, we describe the establishment of a patient-derived xenograft (PDX) model of pRCC carrying an activating mutation of MET in its kinase domain using the same methodology. We characterized the histological and genetic fidelity and metastatic potential of this model (“TSG-RCC-030”) and evaluated the effect of cabozantinib, an
inhibitor of MET approved by FDA for the treatment of thyroid cancer, on tumor growth and metastasis. Finally, we investigated whether circulating tumor DNA (ctDNA) can be used as a biomarker for tumor growth and response to therapy.

Results

**TSG-RCC-030 from a pRCC specimen maintained histological fidelity**

First generation TSGs (TSG-RCC-030) were established by implanting precision-cut, thin slices of fresh tissue from a pRCC specimen of Fuhrman grade 4 with focal sarcomatoid pathology under the renal capsule of immunodeficient mice. Serial passages were similarly carried out by implanting precision-cut slices of fresh TSG tissues under the renal capsule of immunodeficient mice for up to 6 times. Subcutaneous TSGs were also successfully generated from cryopreserved precision-cut slices of fresh TSG tissues. The engraftment rate of each passage is listed in Table 1. After an initial take rate of 50% in the first generation, the take rate of all subsequent implantations was 100%. The immunohistological phenotype of the TSGs was similar to that of the parental tumor (Fig. 1). Specifically, both first generation (1°) subrenal and sixth generation (6°) subcutaneous TSGs, used in this study to evaluate activity of cabozantinib, showed similar histology to the parental tumor (Fig. 1A, B and D). High intensity of human-specific nuclear antigen Ku70, as in the parental tumor (Fig. 1F, G and I), confirmed the human origin of TSGs. In addition, both 1° subrenal and 6° subcutaneous TSGs showed strong staining of typical markers of pRCC, including AMACR and Pax-8, similar to the parental tumor (Fig. 1K, L, N, P, Q and S). Moreover, both subrenal and subcutaneous TSGs were negative for CD117, similar to the parental tumor (Fig. 1U, V and X). Overall, the staining patterns observed for AMACR, Pax-8 and CD117 were as expected for pRCC and demonstrated that both subrenal and subcutaneous TSGs from this pRCC specimen maintained immunohistological characteristics of the parental tumor.

**TSG-RCC-030 metastasized to clinically relevant sites**

One mouse bearing a first generation TSG was found to have gross lung metastases on autopsy (Table 1). These metastases expressed human-specific nuclear antigen Ku70 (Fig. 1H) that distinguished them from surrounding murine cells. Expression of AMACR, Pax-8 and CD117 in lung metastases was similar to that in the primary TSG and parental tumor (Fig. 1M, R and W). Subsequent passages of the subrenal TSG from this mouse also developed gross metastases to the lung (Table 1). In addition, lung metastases were also detected in mice carrying subcutaneous TSGs using immunohistochemistry (Table 1 and Fig. 1E, J, O, T and Y). Interestingly, the patient had pulmonary metastases at the time of diagnosis, demonstrating that tumor cells from TSGs metastasized to the same site as in the patient. Gross metastases were not visible in liver or spleen, and qRT-PCR with human-specific primers for GAPDH confirmed the absence of tumor cells in these organs (data not shown). These results show that TSG-RCC-030 has the capacity to produce metastatic disease to the lung from a primary tumor implanted either under the renal capsule or the skin.

**TSG-RCC-030 maintained the MET point mutation of the parental tumor**

To determine whether the parental tumor harbored any mutations in MET, the third most frequently mutated gene in pRCC (5–13% of pRCC patients), we sequenced exon 16–20 of the gene using human-specific primers because mutations were frequently found in this region.5,6 A missense mutation [T-3997C (M1268T)] that constitutively activates MET20,21 was detected in both parental tumor and its derived TSG-RCC-030 (Fig. 2A). In the parental tumor, both wild type and mutant alleles were detected (Fig. 2B), suggesting several mutually inclusive possibilities: 1) Tumor cells were heterozygous for this mutation; 2) Only a subset of tumor cells harbored a homozygous mutation; 3) MET was mutated in tumor cells but not in other cell types including stromal cells and infiltrating immune cells. In the 1° subrenal TSG, the same mutation was detected in 100% of the cells (Fig. 2C), ruling out the first possibility. If possibility 2 was correct, then the tumor cells carrying the mutation outgrew tumor cells that didn’t have the mutation. Finally, if possibility 3 was the case, then other cell types carrying wild type MET diminished over time. Moreover, in cfDNA isolated from plasma of mice carrying fourth generation (4°) subrenal TSGs, the same mutation was detected in 100% of the copies amplified using human-specific primers (Fig. 2D), pointing to the feasibility of using ctDNA as a biomarker in this PDX model. Altogether, these results demonstrated that TSG-RCC-030 maintained genetic fidelity to the parental tumor and released ctDNA with the same genetic mutation.

**Cabozantinib inhibited growth of TSG-RCC-030**

To determine whether cabozantinib, an FDA-approved MET inhibitor for treatment of medullary thyroid cancer,22 inhibited tumor growth in our PDX model, we generated subcutaneous TSGs in 10 mice and randomized them into control and treated groups when TSGs reached ~100–200 mm³. TSGs showed continuous growth in the control group with a 10-fold increase in tumor volume over 21 d (Fig. 3A). In contrast, TSG volume decreased by >14-fold in mice treated with 30 mg/kg of cabozantinib (once/day, 7 days/week) at the end of 21 d of treatment (Fig. 3A). No significant adverse effects associated with treatment were observed in treated mice, e.g. mice maintained weights similar to control group. Moreover, TSGs in the control group showed an average specific growth rate of 11–17%.

### Table 1. Engraftment rates of TSGs and metastasis rate.

| Number of generations | Number of mice used | Number of TSGs | Take rate | Implantation site | Lung metastasis rate by IHC |
|-----------------------|---------------------|----------------|-----------|------------------|---------------------------|
| 1°                    | 4                   | 2              | 50%       | subrenal         | 25%                       |
| 2°                    | 3                   | 3              | 100%      | subrenal         | ND*                       |
| 3°                    | 3                   | 3              | 100%      | subrenal         | ND                        |
| 4°                    | 6                   | 6              | 100%      | subrenal         | 60%                       |
| 5°                    | 3                   | 3              | 100%      | subrenal         | ND                        |
| 6°                    | 3                   | 3              | 100%      | subrenal         | ND                        |
| 6°                    | 5                   | 5              | 100%      | subcutaneous     | 100%                      |
| 7°                    | 3                   | 3              | 100%      | subrenal         | ND                        |

*ND = not determined.*
throughout the study, whereas TSGs in the treated group showed a negative specific growth rate of 13–23% (Fig. 3B), resulting in near complete tumor regression. Consistently, tumor cells in control mice showed strong nuclear Ki67 staining whereas tumor cells in treated mice were not proliferating (Fig. 3C). Moreover, a high level of phosphorylated MET, the active form of MET that mediates downstream response to MET activation, was observed in TSGs in control but not treated mice (Fig. 3C), suggesting that cabozantinib inhibited MET phosphorylation in pRCC as in other cancers. Finally, the level of phosphorylated ERK, a downstream target of phosphorylated MET, was significantly reduced in treated vs. control mice (Fig. 3C). These results demonstrated that cabozantinib was highly effective in inhibiting tumor growth of TSG-RCC-030 carrying an activating MET mutation and reduced MET activity.

Cabozantinib inhibited lung metastases

To determine whether cabozantinib inhibited lung metastases, we performed qRT-PCR using human-specific GAPDH primers, assuming that all human cells detected in mouse lung were metastatic tumor cells. As shown in Fig. 4A, the level of human GAPDH in the lung tissue of control mice was 735-fold higher than that in lungs of treated mice, demonstrating a high efficacy of cabozantinib in inhibiting lung metastases. Consistently, tumor cells expressing human-specific nuclear antigen Ku70 were detected only in lungs of control but not treated mice (Fig. 4B). These cells also expressed typical markers of pRCC such as Pax-8 (Fig. 4B), confirming the pRCC origin of these cells.

cDNA level correlated with tumor volume and responded to therapy

cDNA as a liquid biopsy has been shown to correlate with tumor volume and predict response to therapy. To determine whether cDNA levels in the TSG-RCC-030 model correlated with tumor volume, we compared cDNA levels in control and treated mice over the time course of 21 d. Using allele-specific primers that amplified the mutant allele of MET, cDNA levels in plasma from control and treated mice were quantified by qPCR. As shown in Fig. 5A, cDNA levels in control mice increased continuously over time to a 57-fold higher level at the end of the experiment. In addition, tumor volumes correlated with exponential increase in cDNA levels with a correlation coefficient of 0.97 (Fig. 5B). In treated mice, cDNA levels increased at 7 d after initiation of treatment, the first time point examined (Fig. 5C). The levels then decreased afterward to different extents ranging from 10–92% by the end of the experiment (Fig. 5C), demonstrating a more heterogeneous effect of treatment on cDNA level than on tumor volume. These results suggest that cDNA levels responded to therapy and can be used as a biomarker for tumor growth in this PDX model.

Discussion

Our study is the first to establish a metastatic PDX model of pRCC driven by an activating MET mutation in the kinase domain. Constitutive activation of MET as a result of activating mutations together with overexpression of MET caused by
DNA copy number gain contributes to oncogenesis in many cancer types including pRCC. A recent study by Schuller et al. identified two pRCC PDX models that do not carry MET mutations but contain regions of MET copy number gain, which is observed in a subset of pRCC. Together, these PDX models provide much-needed representative preclinical models not only for understanding the oncogenic role of MET in pRCC but also for the development of effective therapies for pRCC with deregulated MET signaling. Indeed, Schuller et al. showed that treatment with the selective MET inhibitor AZD6094 resulted in tumor regression in the 2 PDX models with MET copy number gain, supporting the clinical development of AZD6094 as effective therapy for pRCC. However, whether the primary tumors metastasized and whether AZD6094 inhibited metastases in those models was not clear. Our results demonstrated that our PDX model, TSG-RCC-030, can be cryopreserved and serially passaged and maintains histological fidelity as well as metastatic potential. Moreover, treatment with the MET inhibitor cabozantinib not only caused striking regression of primary tumors but also inhibited lung metastases, strongly supporting the testing of this FDA-approved agent in patients with pRCC expressing activating MET mutations.

One of the advantages of our pRCC PDX model is that tumor cells from TSGs metastasize to clinically relevant sites such as the lung, a phenomenon that we observed in our ccRCC PDX models in previous studies. This is particularly important in advancing our knowledge in understanding the biology and developing effective treatments of metastatic RCC since, overall, approximately 25–30% of RCC patients present with metastatic disease at the time of diagnosis. For example, it is not clear when metastatic cells from a primary tumor disseminate to distant organs and what effects removal of the primary tumor have on the proliferation of metastatic cells. Our PDX model may be useful in addressing these questions. Using highly sensitive molecular assays such as qPCR, one can determine the time course of spreading of MET mutant tumor cells to the lung in our PDX model. In addition, removing the primary tumor after cells have disseminated to the lung will allow us to study the effects of the primary tumor on the growth of metastases. Besides understanding the time course of metastasis, our model can also serve as a realistic platform to screen new therapeutic agents alone or in combination with existing therapies to better manage the treatment of metastatic pRCC.

Our results demonstrated that cabozantinib is highly effective in inhibiting pRCC carrying an activating mutation of MET, suggesting a role for cabozantinib in treating the subset of patients with activating MET mutations.
patients with this subtype of RCC. Unfortunately, despite the pivotal role of MET in pRCC, 2 recently completed clinical trials examining the safety and efficacy of cabozantinib in RCC only enrolled patients with clear cell histology. In a phase I study, Choueiri et al. reported that partial response was observed in 7 of 25 (28%) enrolled patients with metastatic ccRCC. Median progression-free survival (PFS) was 12.9 months, and median overall survival was 15.0 months. In a much larger Phase III study of 658 patients with advanced ccRCC, the same investigators observed an objective response rate (ORR) of 21% and median PFS of 7.4 months for cabozantinib, compared to ORR of 5% and PFS of 3.8 months for everolimus, a standard second-line treatment for advanced ccRCC. The high efficacy of cabozantinib in advanced ccRCC is not surprising since it inhibits multiple tyrosine kinases including MET, VEGF receptors and AXL, and MET and AXL are upregulated as a consequence of VHL inactivation commonly observed in ccRCC. We tested a soluble AXL inhibitor in our PDX model and it did not affect primary tumor growth or metastasis to the lung (data not shown), suggesting that the response to cabozantinib in our model was unlikely due to AXL inhibition. It will be interesting in future studies to test VEGF inhibitors such as sunitinib to determine whether the response to cabozantinib is entirely MET-dependent. Clinical trials to evaluate the efficacy of MET inhibitors in pRCC are now beginning to emerge. For example, a phase II study of 74 pRCC patients showed a 13.5% response rate to foretinib, a dual MET and VEGF receptor inhibitor, with a median PFS of 9.3 months. An on-going phase II study designed to evaluate the efficacy of tivantinib, a selective MET inhibitor with reported off-target effects, alone or in combination with erlotinib, an EGFR inhibitor, in pRCC is expected to be completed in 2016. Finally, a randomized, phase II efficacy assessment of multiple MET kinase inhibitors including cabozantinib in metastatic pRCC (PAPMET) was started in April 2016 and is expected to finish in 2019 (https://clinicaltrials.gov/ct2/show/NCT02761057?term=PAPMET&rank=1). Results from our study and others provide rationale for evaluating cabozantinib as a single agent or in combination with other agents in the treatment of pRCC. Resistance to a MET inhibitor in a patient carrying a MET mutation has just been reported. It will be interesting in future studies to determine whether resistance to cabozantinib will be observed with time in our PDX model. If so, TSG-RCC-030 will be useful for testing additional therapies after resistance to cabozantinib arises.

One interesting finding in our study was that tumor DNA carrying a MET mutation was detectable in mouse plasma and its level correlated with volume of primary tumor. Although ctDNA as a biomarker for detecting tumor presence, predicting prognosis and assessing therapeutic response has gained much attention in recent years, few publications describe the detection of ctDNA in mouse models. Thierry et al. demonstrated that ctDNA from mutated tumor (human) cells could be detected by using human-specific KRAS or PSAT1 primers and by assessing the presence of the BRAF V600E mutation, and the concentration of human ctDNA increased significantly with tumor growth. Gorges et al. also showed a correlation between ctDNA levels and tumor size in mice carrying xenografts derived from cultured breast cancer cells by qPCR targeting human Alu sequences. Interestingly, ctDNA levels in the

Figure 3. Regression of primary tumor induced by cabozantinib in TSG-RCC-030. Subcutaneous tumor growth was measured in control and cabozantinib-treated mice. Tumor volume significantly decreased over the time course of 21 d in treated mice while continuously increasing in control mice (A). The specific tumor growth rate was negative in treated mice and positive in control mice (B). IHC demonstrated strong expression of the proliferation marker Ki67 in a subset of cells (arrows) and phosphorylated MET as well as its target, phosphorylated ERK, in majority of tumor cells in control but not in treated mice (C). Data points represent mean +/- SD. *p < 0.05 by Student’s t-test. Magnification of all images is 200x.
breast cancer xenograft model showed consistent decreases at
day 3, 5 and 8 after treatment with a MEK inhibitor, whereas in
our model, the ctDNA level showed an initial increase 7 d (the
earliest time point monitored) after treatment with cabozanti-
nib. This may be explained by increased cell death and there-
fore increased ctDNA release in our model. Although little or
no expression of apoptotic marker caspase 3 was observed at
the end of the treatment (data not shown), we expect to see
high level of apoptosis at day 7 and prior in treated mice if it is
feasible to obtain tumor tissues. A similar early increase of
ctDNA has been observed in a melanoma patient with
V600EBRAF mutation 3 d after initiation of dabrafenib treat-
ment.40 Taken together, the characteristics of TSG-RCC-030
provide a unique opportunity to better understand the develop-
ment and progression of pRCC and accelerate the development
of new therapies for pRCC and to evaluate ctDNA as a bio-
marker in the detection and treatment of pRCC.

Materials and methods

Ethics statement

All animal studies were approved by the Stanford Administra-
tive Panel on Laboratory Animal Care (APLAC) and done in
compliance with the regulations for animal studies at Stanford
University. Patient-derived tissue was obtained immediately
after surgery under a protocol approved by the Stanford Insti-
tutional Review Board. The participant provided his written
informed consent to participate in this study.

Subrenal/subcutaneous implantation of precision-cut, thin
tissue slices

A core of tissue was obtained from a pRCC specimen immedi-
ately following nephrectomy. The specimen was from a
67 year-old male and had a clinical stage of pTa3pNxMx and
Fuhrman grade 4 with focal sarcomatoid pathology at the time
of diagnosis. Precision-cutting and subrenal and/or subcutane-
ous implantation of tissue slices were performed as previously
described.41 All animal work was done in accordance with
institutional regulations for laboratory animal studies. RAG2−/−γC−/− male and female mice, 6–8 weeks of age, were
engrafted with RCC tissue slices. Tumor tissues were cryopre-
served and thawed as previously described.30

Immunohistochemistry

Immunohistochemistry (IHC) was performed as previously
described.41 The sources and dilutions of the antibodies used in
this study are listed in Supporting Information Table 1.

Sequencing of MET

DNA was extracted from fresh patient tumor tissue or TSGs
preserved in Allprotect tissue reagent (Qiagen) using an All-
Prep DNA/RNA/Protein Mini Kit (Qiagen) according to the
manufacturer’s directions. The c-terminal exons (exon 16–20)
of MET were selected for polymerase chain reaction (PCR)
amplification and direct sequencing by Stanford Protein and
Nucleic Acid Facility. PCR primers for MET amplification were
forward: 5′-CGCTGGTGGTCTCCTACAC-3′ and reverse:
5′-GGTGCCAGCATTTAGCATT-3′.

Cabozantinib treatment of TSG-RCC-030

Subcutaneous TSGs were established in 10 mice and the mice
were randomized into control and treated groups when tumor
volume (length x width x width /2) reached ~100–200 mm³.
Cabozantinib (Exelixis, Inc.) was administered by oral gavage,
one a day, 7 days/week, at a dose of 30 mg/kg and vehicle
(water) was administered similarly as control. The cabozantinib
gavage solution was made fresh daily. Subcutaneous tumor

Figure 4. Inhibition of TSG-RCC-030 lung metastasis by cabozantinib. The level of human GAPDH in the lung tissue of control mice was significantly higher than in cabo-
zantinib-treated mice as assessed by qPCR using human-specific GAPDH primers (A). Tumor cells expressing human-specific nuclear antigen Ku70 were detected only in
control but not treated mice (B). These metastatic tumor cells also expressed Pax-8, a typical marker of pRCC (B). Data points represent mean+/− 5D. *p < 0.05 by
Student’s t-test. Magnification of images is 200×.
volume was determined by caliper measurements twice a week. Specific tumor growth rate was calculated as \( \ln(V_2-V_1)/(t_2-t_1) \) (\( V \) is tumor volume and \( t \) is time). All animals were sacrificed after 21 d of treatment. Tissues were collected and preserved either in Allprotect for DNA/RNA isolation or 10% formalin for IHC within 24 hr after the last treatment. The dose and schedule of cabozantinib used in this study were based on previous studies demonstrating effective tumor inhibition in preclinical trials.25,42

Isolation of circulating cell-free DNA (cfDNA)

One hundred microliters of blood were collected by retro-orbital bleeding every week from each mouse and centrifuged for 15 minutes at 2,000 x g. Plasma was transferred to a clean tube and EDTA was added to a final concentration of 10 mM to stabilize cfDNA.43 Plasma was stored at \(-80^\circ \text{C} \) before isolation of cfDNA. Fifty microliters of plasma were used for cfDNA isolation using a QIAamp DSP Virus Spin Kit (QiaGen) according to manufacturer’s instructions. Fifty microliters of nuclelease-free water were used to elute cfDNA. The quality of the cfDNA was examined by electrophoresis.

Quantitative polymerase chain reaction (qPCR)

For lung metastasis quantification: Tissues were preserved in Allprotect tissue reagent (Qiagen) at \(-20^\circ \text{C} \) before RNA extraction. RNA was isolated using Trizol reagent according to manufacturer’s instructions (Invitrogen). cDNA was synthesized from 2 \( \mu \text{g} \) of DNase-treated RNA using SuperScript First-Strand Synthesis System for RT-PCR (Invitrogen). One microliter of cDNA was subjected to PCR amplification using SYBR Green PCR Master Mix (Applied Biosystems). The level of 18S RNA was used as an internal control to normalize hGAPDH levels. For 18S RNA amplification, cDNA was diluted 1:50. PCR amplification was performed on the ABI Prism 7900 HT Sequence Detection System (Applied Biosystems). Relative quantitation of mRNA expression levels was determined using the relative standard curve method according to the manufacturer’s instructions (Applied Biosystems). The following primers were used to amplify specific target genes: hGAPDH, forward 5'-ATGGGGAAGGTGAAGGTCG-3' and reverse 5'-GGGGTCAATTGATGGCAACAAATA-3'; 18S RNA, forward 5'-GCCCGAAGCGTTTACTTTGA-3' and reverse: 5'-TCCATTATTCCTAGCTGCGGTATC-3'.

For ctDNA quantification: Five microliters of cfDNA were used in each qPCR reaction with human specific primers for the mutated MET (T-3997C(M1268T)): forward 5'-AAGCTGCCAGTGAAGTGGAT-3' and reverse 5'-GGGGTCATTGATGGCAACAAATA-3'.

Statistical analysis

Two-tailed Student’s t-test was used for 2-arm experiments. A \( p < 0.05 \) was considered significant. Statistical tests were performed with Excel Stats. Pearson correlation coefficients between ctDNA levels and tumor volumes were calculated in Excel.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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