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ORIGINAL ARTICLE

A novel anti-FGFR1 monoclonal antibody OM-RCA-01 exhibits potent antitumor activity and enhances the efficacy of immune checkpoint inhibitors in lung cancer models

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Background: Fibroblast growth factor receptor 1 (FGFR1) plays a crucial role in carcinogenesis. Exploring the combination of the novel humanized monoclonal anti-FGFR1 antibody OM-RCA-01 and immunotherapy was intriguing due to involvement of FGFR1 in mechanisms of resistance to checkpoint inhibitors.

Materials and methods: Lung cancer A549, exhibiting distinct levels of FGFR1 expression, were cultured in basic FGF medium with OM-RCA-01 supplementation. The efficacy of antibody monotherapy was validated in a lung cancer xenograft study. To investigate whether OM-RCA-01 could enhance the efficacy of immunotherapy *in vitro* and *in vivo*, mixed lymphocyte reaction/Staphylococcal enterotoxin B assays and FGFR1/programmed death-ligand 1-positive patient-derived xenograft model were established.

Results: The antibody effectively suppressed receptor phosphorylation, resulting in inhibited cell proliferation. OM-RCA-01 led to a substantial delay in tumor growth compared to non-specific immunoglobulin G in a xenograft study. The median tumor volume was 1048.5 mm^3 and 2174 mm^3 in the study and vehicle groups, respectively, representing a twofold difference in favor of the anti-FGFR1 antibody. *In vitro*, the combination of nivolumab and OM-RCA-01 resulted in higher levels of interferon gamma and interleukin-2 release compared with nivolumab alone. *In vivo*, pembrolizumab in combination with OM-RCA-01 produced a greater inhibitory effect on tumor growth compared with vehicle and pembrolizumab alone. The curve plateaued, indicating minimal tumor growth from day 16 onwards in the combination group. The OM-RCA-01 demonstrated no toxicity, even at therapeutic doses or higher doses.

Conclusions: Our preclinical studies demonstrate that OM-RCA-01 exhibits robust activity with minimal toxicity. Combining an anti-FGFR1 antibody with a checkpoint inhibitor may enhance the efficacy of both drugs. However, further studies are needed to elucidate the mechanism of this interaction.

Key words: fibroblast growth factor receptor 1, immune checkpoint inhibitors, anti-FGFR1 monoclonal antibody, immunotargeted therapy

INTRODUCTION

Cancer continues to be a formidable adversary on the global health front, necessitating innovative approaches to combat its relentless progression. Among the myriad molecular players driving oncogenesis, fibroblast growth factor receptor (FGFR) family stands out as a pivotal protagonist in the intricate tapestry of cancer biology.[1] As a member of the FGFR family, FGFR1 orchestrates key cellular processes, including proliferation, survival, and differentiation, rendering it a promising target for therapeutic intervention.[2] The FGFR1 pathway has been implicated in various cancer types.[3] For example, the prevalence of FGFR1 alterations is particularly notable in squamous cell lung carcinoma, where a significant proportion of cases exhibit amplifications of the FGFR1 gene.[4] The aberrant expression of the FGFR1 has been identified in renal cancer cells, and its presence is associated with a poorer prognosis for patients.[5,6] This genetic aberration not only serves as a potential diagnostic marker but also positions FGFR1 as a promising therapeutic target in different cancer types. Multiple preclinical and clinical studies have demonstrated that inhibiting the FGFR family results in the suppression of tumor growth.[7-9] The only one FGFR1 tyrosine kinase inhibitor has received approval for use in

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patients with relapsed or refractory myeloid/lymphoid neoplasms with FGFR1 rearrangement.[10]

FGFR1 not only plays a crucial role in the initiation and progression of cancers but has also emerged as a formidable factor in conferring tumor resistance to immunotherapy, including checkpoint inhibitors.[11] One facet of impact of FGFR1 on immunotherapy resistance lies in its ability to modulate the tumor microenvironment.[12] Activation of the FGFR1 pathway can contribute to the recruitment of immunosuppressive cells, such as regulatory T cells and myeloid-derived suppressor cells, creating an immune-permissive niche that fosters tumor immune evasion. Furthermore, FGFR1 signaling may lead to the up-regulation of immune checkpoint molecules expression, creating a shield against the cytotoxic effects of immune cells. In the context of immunotherapy resistance, understanding the interplay between FGFR1 and immune evasion mechanisms becomes crucial for developing strategies to overcome resistance and enhance the efficacy of immunotherapeutic interventions. Combinatorial therapeutic approaches using targeting of FGFR1 in conjunction with checkpoint inhibitors are extensively being investigated as potential strategies to disrupt the immunosuppressive environment stimulated by FGFR1 signaling.[13] This approach aims to fully leverage the potential of immune checkpoint inhibitors.

Monoclonal antibodies, with their precision and specificity, have emerged as promising therapeutic agents for FGFR1 blockade. Currently, there are no Food and Drug Administration-approved monoclonal antibodies targeting FGFR1. The humanized monoclonal antibody OM-RCA-01 takes center stage in our exploration of targeted FGFR1 inhibition.[14] This antibody has demonstrated the inhibition of *in vitro* kinase activity of FGFR1 with high affinity (Kd of 1.59 nM). Previously we demonstrated that OM-RCA-01 robustly suppressed FGF-mediated signaling and proliferation in Caki-1 human renal carcinoma cells over-expressing FGFR1. Importantly, OM-RCA-01 exhibited significant antitumor activity in the Caki-1 renal cell carcinoma nude mouse xenograft model. Recent findings highlight the significant impact of anti-FGFR1 therapy on FGF-induced angiogenesis.[15]

In this article, we present novel data concerning the preliminary efficacy and safety of the OM-RCA-01 antibody. Our primary investigation centered on assessing its activity in a lung cancer model. Additionally, we aimed to evaluate its efficacy in monotherapy as well as in combination with checkpoint inhibitors, with the objective of preventing the development of treatment resistance.

MATERIALS AND METHODS

In vitro functional assays

Growth inhibition assay. Lung cancer A549 cell line was purchased from the American Type Culture Collection (ATCC) (#CCL-185) and melanoma Mel Kor cell lines were obtained from the National Medical Research Center of Oncology (#2287578). Cell lines were cultured in Dulbecco's Modified Eagle Medium (ATCC), supplemented with fetal bovine serum (FBS) to a final concentration of 10% (ATCC). The cell line was cultured at 37°C/5% CO2 in a humidified incubator. To evaluate the effect of OM-RCA-01 on FGF-mediated signaling, cells were placed in a 96-well microculture plate (Costar white, flat bottom, Corning # 3917) in a total volume of 90 μl/well and incubated with OM-RCA-01 at 1-100 μg/ml. Control wells were left untreated. Three hours after dosing, basic FGF (#233-FB-010, R&D Systems, Minneapolis, MN) was added at a concentration of 50 ng/ml. Cell growth inhibition was determined using Promega's Cell Titer-Glo® assay. To assess the phosphorylation of FGFR1 in western blotting, cells were collected in lysis buffer (#9803, Cell Signaling Technology Danvers, MA). The specimens were loaded on to sodium dodecyl sulfate—polyacrylamide gel electrophoresis (SDS—PAGE), followed by separation and transfer on to polyvinylidene difluoride membranes. The membranes were blocked and probed overnight with anti-phospho-FGFR1 antibody (#sc-57132, Santa Cruz Biotechnology, Dallas, TX). β-actin (Sigma-Aldrich, St. Louis, MO) was measured as the control for equal loading.

Mixed lymphocyte reaction (MLR) and Staphylococcal enterotoxin B (SEB) stimulation. In the MLR assay, dendritic cells (DCs) were obtained by culturing monocytes isolated from peripheral blood mononuclear cells (PBMCs) using a monocyte purification kit (Miltenyi Biotec, Bergisch Gladbach, Germany) *in vitro* for 10 days with 300 U/ml interleukin-4 (IL-4) and 200 U/ml granulocyte-macrophage colony-stimulating factor (GM-CSF; STEMCELL Technologies, Vancouver, BC). Allogeneic DCs (1×10^4) and CD4+ T cells (1×10^5) were cultivated with nivolumab alone (0.5, 1, and 100 nmol/l), OM-RCA-01 alone (0.5, 1, and 10 nmol/l), or a combination thereof. Following a 5-day incubation period, the culture supernatants were collected and subjected to enzyme-linked immunosorbent assay (ELISA; BD Biosciences, Milpitas, CA) to measure interferon gamma (IFN-γ) release. Subsequently, cells were labeled with 3H-thymidine extended by 18 h to assess T-cell proliferation.

In the SEB assay, PBMCs from healthy human donors ($n = 10$) were cultured for 4 days with nivolumab (1 μg/ml), or nivolumab (1 μg/ml) and OM-RCA-01 (1 μg/ml) at the beginning of the assay together with serial dilutions of SEB (0.1-100 ng/ml). IL-2 levels were measured by ELISA analysis (BD Biosciences) in culture supernatants.

Efficacy in lung cancer xenograft study

Thirty-five female NCr nu/nu mice (6-12 weeks of age; Charles River, Boston, MA) were set up with 1 mm^3 A549 tumor fragments subcutaneously in flank. Twenty mice with established tumors (an average size of 80-120 mg) were randomly divided into vehicle [non-specific immunoglobulin G (IgG), 30 mg/kg] or OM-RCA-01 (30 mg/kg) groups with 10 animals per group. Tumor sizes were measured in a blind fashion twice a week with a Vernier caliper. Animals were monitored daily for signs of toxicity, and were humanely euthanized and deemed to have succumbed to disease progression if tumors reached $\geq$2000 mm^3 in size. In all animal experiments, tumor growth was monitored by caliper measurement every 3 days according to the formula: (length $\times$ width^2)/2 and tumor growth inhibition (TGI; %) was calculated as follows: TGI (%) = [1 − (mean of

treatment group tumor volume on evaluation day)/(mean of control group tumor volume on evaluation day)] × 100. Endpoint was significant differences in tumor growth delay. Differences were considered statistically significant at $P < 0.05$. All animal studies were approved by and carried out in accordance with the Institutional Animal Care and Use Committee protocols.

Efficacy of the combination of OM-RCA-01 and anti-PD-1 antibodies in the PDX model of lung cancer

To evaluate the efficacy of combined FGFR1 and programmed cell death protein 1 (PD-1) inhibition, a patient-derived xenograft (PDX) model of lung cancer was generated. Patient explants were obtained from 79 surgical specimens of primary non-small-cell lung cancer. An adenocarcinoma with programmed death-ligand 1 (PD-L1) expression of 60% measured by immunohistochemistry (IHC) with DAKO clone 22C3 (Agilent, Santa Clara, CA) and membranous FGFR1 IHC staining in 35% of cells (#63601, Abcam, Waltham, MA) was selected from these samples. PDX was implanted into humanized mice (Jackson Laboratory, Bar Harbor, ME) as described previously.[16] Briefly, 4- to 6-week-old mice were housed together in sterilized cages and maintained under required pathogen-free conditions. Each cell suspension or tumor fragment was inoculated subcutaneously into female mice. When the tumor had grown to 70 mm^3, 21 tumor-bearing mice were randomly assigned to treatment or control (saline) groups and dosing began. Antibodies were administered intravenously. Treatment groups included OM-RCA-01 (30 mg/kg) with pembrolizumab (10 mg/kg) and pembrolizumab alone (10 mg/kg).

The mice were euthanized by slow introduction with CO_2 gas when they reached endpoints (tumor volume exceeding 2000 mm^3, >10% reduction of body weight, or clinical signs indicating that mice should be euthanized for ethical reasons).

Impact of OM-RCA-01 on immune resistance in the cancer-associated fibroblasts (CAFs) in the in vivo model

FGFR1 in cancer cells has previously been shown to interact with cancer-associated fibroblasts (CAFs).[17,18] To investigate the potential contribution of CAFs to immune resistance and assess the impact of anti-FGFR1 antibody on this mechanism, we established an animal model with implanted cancer cells and CAFs. Immortalized human kidney CAFs were obtained from Neuromics (#CAF110-IM; Edina, MN) and incubated in CAF Growth Media (#CAFM03) at 37°C with 5% CO_2 in air atmosphere. Renca cells were purchased from the ATCC (#CRL-2947) and maintained in RPMI-1640 medium (ATCC) supplemented with 10% heat-inactivated FBS (ATCC) and cultured at 37°C and 5% CO_2 atmosphere.

Cancer cells (5×10^5) alone or premixed with CAFs (1.5×10^6) in Matrigel ($n = 20$) were implanted subcutaneously into mice (20 animals per group; Charles River). Treatment with anti-cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) mouse antibody (9H10-CP146; 200 mcl on days 7, 10, and 13) was initiated in both cohorts when tumor volumes reached 70 mm^3. Treatment with OM-RCA-01 (30 mg/kg; every 3 days) or IgG2a isotype control was added when tumor volumes reached 1500 mm^3. Mouse cytokine levels (IFN-γ and IL-2) were determined using multiplex immunodetection kits (Millipore, Darmstadt, Germany) in treatment groups.

Table 1. Toxicity studies

Study	Animals[a]	Doses, mg/kg	Route of administration
Acute toxicity	Mice, both sexes ($n = 84$) Rats, both sexes ($n = 84$)	0, 10, 60, 100, 150, 350, 500	Once, i.v. Once, i.p. Once, i.v. Once, i.p.
Subacute toxicity	Rats, both sexes ($n = 90$)	5, 55, 115	Once a week, 12 weeks, i.v.
Immunotoxicity	Mice, both sexes ($n = 48$)	0, 50, 250	Once, i.v.
Study of allergenic properties	Guinea pigs, both sexes ($n = 36$)	0, 50, 500	i.v.
Pyrogenicity	Rabbits, both sexes ($n = 6$)	0, 115	Once, i.v.
Local irritation	Rats, both sexes ($n = 60$)	0, 5, 55, 115	Once a week, 12 weeks, i.v.

i.v., intravenously; i.p., intraperitoneally.
[a]Animals were all purchased from Rappolovo: mice—CBA, rats—outbred white rats, Guinea pigs—albino, rabbits—New Zealand White.

Pharmacokinetics and toxicity

In a single-dose pharmacokinetic (PK) study (stage 1), 81 outbred white rats (Rappolovo, Rappolovo, Russia) were randomized to receive OM-RCA-01 intravenously 10 mg/kg, 30 mg/kg, or 100 mg/kg. In a repeated-dose PK study (stage 2), 24

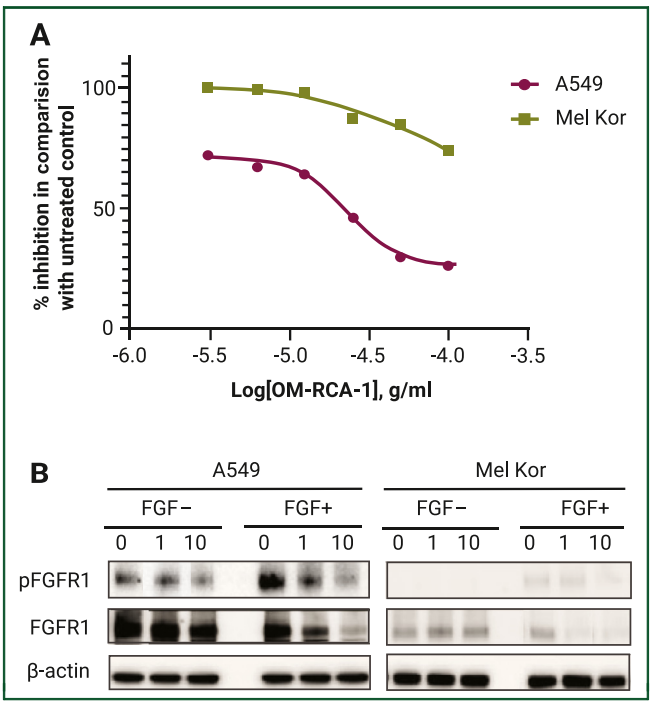

Figure 1. The mechanism of *in vitro* growth inhibition by OM-RCA-01 varies depending on the cell line and the level of FGFR1 expression. (A) Lung cancer A549 and melanoma Mel Kor cells were exposed to OM-RCA-01 and then calculated. The figure illustrates the percentage of cell growth inhibition relative to the untreated control. (B) Effect of OM-RCA-01 on bFGF/FGFR1 signaling. Western blot analysis for the phosphorylation of FGFR1.
FGFR1, fibroblast growth factor receptor 1.

rats received OM-RCA-01 intravenously at a dose of 30 mg/kg three times at a 3-day interval. In addition, 12 chinchilla rabbits (6 males and 6 females) were randomized into two groups to receive intravenous antibody at doses of 10 mg/kg and 30 mg/kg on day 1, day 15, and day 29. The optical densities (ODs) of a set of OM-RCA-01 concentration standards were detected and used to plot an OD versus concentration standard curve that was analyzed by a four-parameter curve fit. Antibody serum concentrations were identified from the standard curve using Origin Pro software (Northampton, MA). PK parameters included C0, Vd, Kel, Cl, area under the pharmacokinetic curves (AUC)0-t, AUC0-∞, T1/2, and mean residence time (MRT). Calculation of PK parameters was carried out using the validated Phoenix WinNonLin software (version 6.3; Radnor, PA).

Toxicology studies are summarized in Table 1. In particular, in a 3-month toxicity study, outbred white rats (15 males and 15 females per dose group) were injected intravenously with 5, 55, or 115 mg/kg OM-RCA-01 once a week for a total of 12 doses. Dosing levels were based on results from an acute toxicity study. Seventy-eight animals were euthanized 1 day following the last dose for primary necropsy. The remaining 12 rats (2/gender/group) were euthanatized 20 days after the last dose for recovery necropsy. Examinations included body weight, feed and water consumption, clinical pathology, and cardiovascular, respiratory, neurologic and ophthalmological evaluations. Organ weights and macroscopic and microscopic pathology were also assessed.

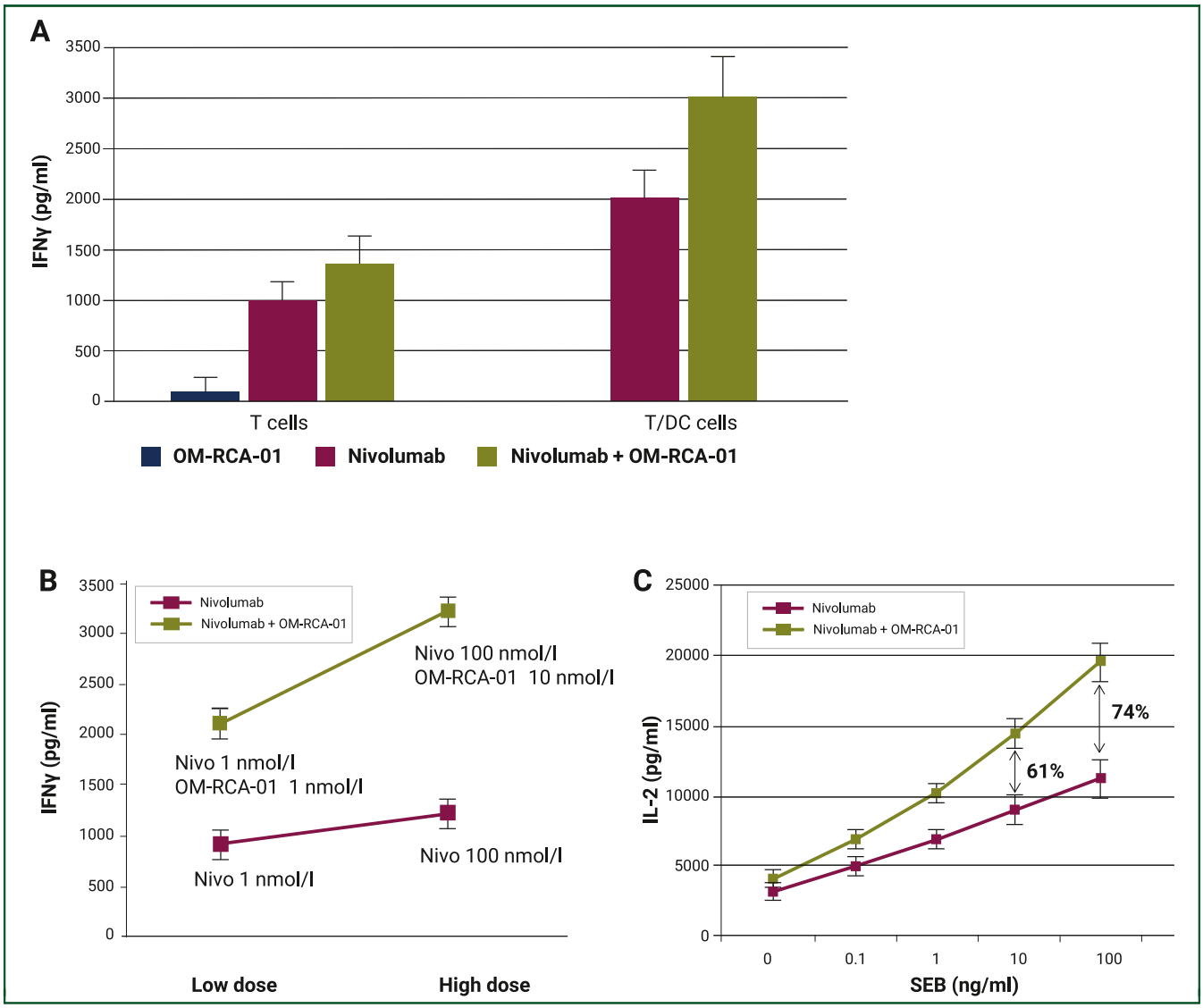

Figure 2. Co-cultivating of CD4+ T cells with nivolumab and the anti-FGFR1 antibody resulted in heightened levels of both IFN-γ and IL-2. (A) In an allogeneic mixed leukocyte reaction assay, allogeneic DCs and CD4+ T cells were cultured with nivolumab alone at concentrations of 0.5 nmol/l, OM-RCA-01 alone at concentrations of 0.5 nmol/l, or with a combination thereof. Following 5 days, IFN-γ release in culture supernatants was evaluated using ELISA, and cells were labeled with ^{3}H-thymidine for 18 h to assess T-cell proliferation. (B) Increasing the concentrations of both antibodies resulted in a corresponding increase in IFN release (T cells). (C) Combination of nivolumab and OM-RCA-01 demonstrated an augmentation in IL-2 secretion compared to the nivolumab alone in response to SEB using PBMCs.

DCs, dendritic cells; ELISA, enzyme-linked immunosorbent assay; FGFR1, fibroblast growth factor receptor 1; IFN, interferon; IL, interleukin; PBMCs, peripheral blood mononuclear cells; SEB, Staphylococcal enterotoxin B.

Statistical analysis

Means, medians, and proportions were used as descriptive statistics. When necessary, data were represented as means ± standard deviation of at least three independent experiments, and statistical analysis between groups was carried out using Student's *t*-test. The Mann—Whitney *U* test was used as a nonparametric test to analyze variables that were not normally distributed. All *P* values were two-sided, and values of <0.05 were considered statistically significant. All statistical analyses were carried out using IBM SPSS Statistics Base v22.0 software (SPSS, Inc., Chicago, IL).

RESULTS

OM-RCA-01 inhibits FGF-triggered cell proliferation

Antibodies targeting FGFR have demonstrated the ability to hinder cell growth and survival pathways mediated by FGF. To this end, the antiproliferative activity of OM-RCA-01 was evaluated using a model of FGF-mediated signaling against the human lung cancer and melanoma cell lines. The cells underwent treatment with escalating concentrations of the OM-RCA-01, ranging from 1 to 100 μg/ml. The anti-FGFR1 antibody demonstrated significant inhibition of cell growth in the lung cancer model, with a GI50 of 9.69 μg/ml (Figure 1A). However, no significant inhibition was observed in the melanoma model, where the GI50 exceeded 10 mmol/l. Variations in the inhibition of cell line proliferation may arise from differences in FGFR1 expression levels. Initially, both total and phosphorylated FGFR1 levels were notably higher in lung cancer cells and nearly undetectable in melanoma cells (Figure 1B). The inclusion of basic FGF led to a significant elevation in receptor activity. Culturing cells with a monoclonal antibody resulted in a dose-dependent suppression of FGFR1 phosphorylation.

The co-cultivation of OM-RCA-01 and nivolumab enhances T-cell function

The capacity of OM-RCA-01 to enhance T-cell responses to immunotherapy was assessed *in vitro* using human T cells. These assays encompassed an allogeneic MLR and stimulation of human PBMCs by the superantigen SEB. In the context of an MLR, the blockade of PD-1 with nivolumab systematically led to an augmentation of IFN-γ release (Figure 2A). Furthermore, the combination of nivolumab and OM-RCA-01 resulted in even higher levels of IFN-γ release, showing a 31% increase. There was also an observed enhancement in T-cell proliferation in some donor T-cell/DC pairs. Elevating the concentration of antibodies led to an increase in the release of IFN-γ, especially in the combination group (Figure 2B). Culturing cells with only anti-FGFR1 antibody caused significantly less IFN-γ production. Nivolumab and OM-RCA-01 also enhanced IL-2 secretion over nivolumab alone in response to SEB using PBMCs. The administration of OM-RCA-01 increased IL-2 secretion by a mean of 29%-74% over nivolumab (Figure 2C).

OM-RCA-01 has a potent direct antitumor activity in the lung cancer xenograft model

The antitumor activity of OM-RCA-01 was examined in lung cancer xenografts utilizing A549 cells expressing FGFR1. The monoclonal antibody OM-RCA-01 was administered intravenously twice a week at a dose of 30 mg/kg. Administration of OM-RCA-01 resulted in significant suppression of tumor growth compared to non-specific IgG ($P < 0.0001$; Figure 3). The median tumor volume was 1048.5 mm^3 in the study group and 2174 mm^3 in the vehicle group, representing a twofold difference in favor of the anti-FGFR1 antibody. In the vehicle group, all tumors reached the volume endpoint by day 31, indicating aggressive growth of the lung cancer xenografts. Conversely, in the antibody group, not a single tumor reached a size of 2000 mm^3 during the same period. Importantly, during the study no mean weight loss, clinical toxicity, or animal death was observed in either group.

The combination of OM-RCA-01 with pembrolizumab demonstrated an enhanced efficacy in the lung cancer PDX model

To investigate whether OM-RCA-01 could enhance the efficacy of immunotherapy *in vivo*, we established the FGFR1/PD-L1-positive PDX model in humanized mice. PDX was derived from a primary lung adenocarcinoma. The combination of OM-RCA-01 and pembrolizumab produced a greater inhibitory effect on tumor growth compared with vehicle and pembrolizumab alone ($P < 0.001$; Figure 4). The curve reached a plateau and there was almost no tumor growth from day 16 in the combination group. Median tumor volume was 97.1, 417.1, and 863.5 mm^3 in the combination, pembrolizumab, and vehicle groups, respectively. Consequently, the inhibition rates for the

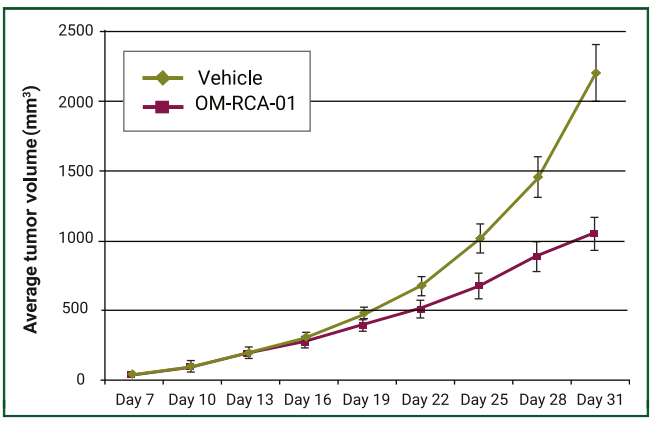

Figure 3. The use of OM-RCA-01 as a monotherapy yields a statistically significant delay in the growth of lung cancer xenografts. The capacity of monoclonal antibody administered intravenously at 30 mg/kg every 3 days, starting on day 7 after tumor inoculation, was evaluated in subcutaneous A549 xenografts during 31 days after tumor inoculation (day 0). The study comprised group 1 (non-specific IgG as a vehicle; $n = 10$) and group 2 (OM-RCA-01; $n = 10$). The administration of OM-RCA-01 resulted in a significant suppression of tumor growth compared to non-specific IgG ($P < 0.0001$). The median tumor volume was 1048.5 mm^3 in the study group and 2174 mm^3 in the vehicle group, representing a twofold difference in favor of the anti-FGFR1 antibody.
FGFR1, fibroblast growth factor receptor 1.

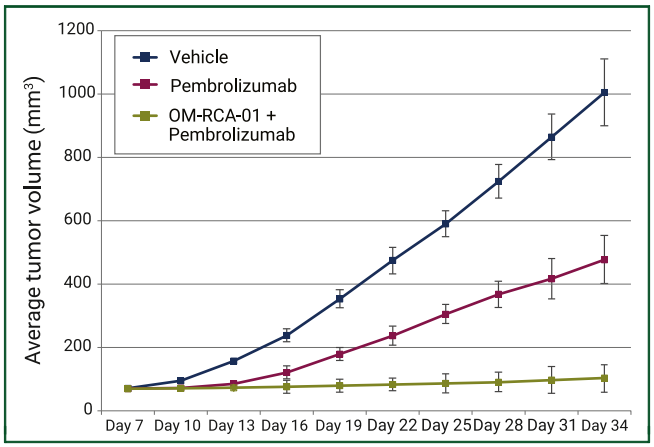

Figure 4. Co-treatment of OM-RCA-01 and pembrolizumab demonstrated significant tumor growth delay phenotype compared to vehicle and pembrolizumab alone in a lung cancer PDX model. FGFR1-positive, PD-L1-positive lung adenocarcinoma PDX model was generated by implanting PDX into humanized mice. Twenty-one tumor-bearing mice were randomly assigned to receive OM-RCA-01 (30 mg/kg) in combination with pembrolizumab (10 mg/kg), pembrolizumab alone (10 mg/kg), or saline (vehicle) intravenously. The coadministration of OM-RCA-01 and pembrolizumab yielded a more pronounced inhibitory impact on tumor growth when contrasted with both the vehicle and pembrolizumab alone ($P < 0.001$). The median tumor volumes were 97.1 mm^3, 417.1 mm^3, and 863.5 mm^3 in the combination, pembrolizumab, and vehicle groups, respectively.
FGFR1, fibroblast growth factor receptor 1; PD-L1, programmed death-ligand 1; PDX, patient-derived xenograft.

combination of pembrolizumab with OM-RCA-01 and pembrolizumab alone were 89% and 52%, respectively. Moreover, the mean weight of tumors in the combination group was 47% lower compared with the pembrolizumab group and less than nine-fold compared with the vehicle group (all $P < 0.05$). Tumor necrosis and cell degeneration were more prevalent in the combination group compared to the pembrolizumab alone group. Nevertheless, tumor infiltration by lymphocytes remained comparable between the two groups. Importantly, no instances of death or decrease in body weight were observed in all groups, indicating that the combination regimen did not result in increased toxicity.

FGFR1 inhibition could potentially impact immune resistance associated with CAFs

Next, we evaluated the influence of CAFs on the efficacy of the checkpoint inhibitor and explored the potential of overcoming resistance by incorporating an anti-FGFR1 antibody. To achieve this, we carried out an animal model wherein premixed renal cancer cells, with or without CAFs, were implanted subcutaneously. Both cohorts received mouse anti-CTLA-4 antibody. Upon reaching a tumor volume of 1500 mm^3, mice were allocated to either the OM-RCA-01 antibody or non-specific IgG supplementation groups. Significant differences were identified between the CAF-positive and CAF-negative groups treated solely with the anti-CTLA-4 antibody. The median tumor size in the CAF-positive group was 44% larger ($P = 0.027$; Figure 5A). The administration of the OM-RCA-01 antibody was linked to a slowdown in tumor growth compared to the control group, observed in both the CAF-positive (tumor growth delay [TGD] 96%, $P < 0.001$) and CAF-negative (TGD 75%, $P < 0.001$) cohorts. Moreover, treatment with OM-RCA-01 resulted in a 43% increase in IFN-γ release and a 65% increase in IL-2 secretion compared to the control (Figure 5B).

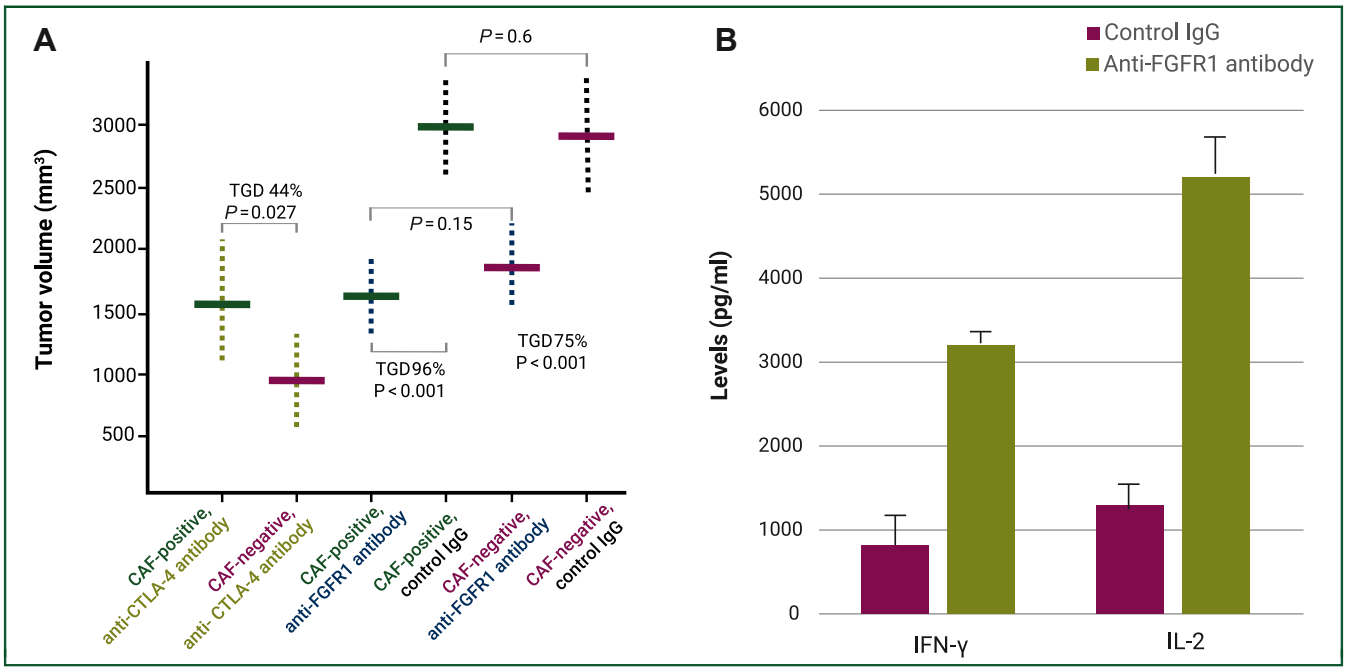

Figure 5. Evaluating the effect of OM-RCA-01 on immune resistance in CAFs model. (A) The CAF-positive group treated with the anti-CTLA-4 antibody exhibited a higher median tumor size. OM-RCA-01 targets CAF-dependent and CAF-independent resistance to immunotherapy in renal cancer *in vivo*. (B) Elevated levels of plasma cytokines were observed in the group treated with OM-RCA-01.
CAF, cancer-associated fibroblast; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; FGFR1, fibroblast growth factor receptor 1; IFN, interferon; Ig, immunoglobulin; IL, interleukin; TGD, tumor growth delay.

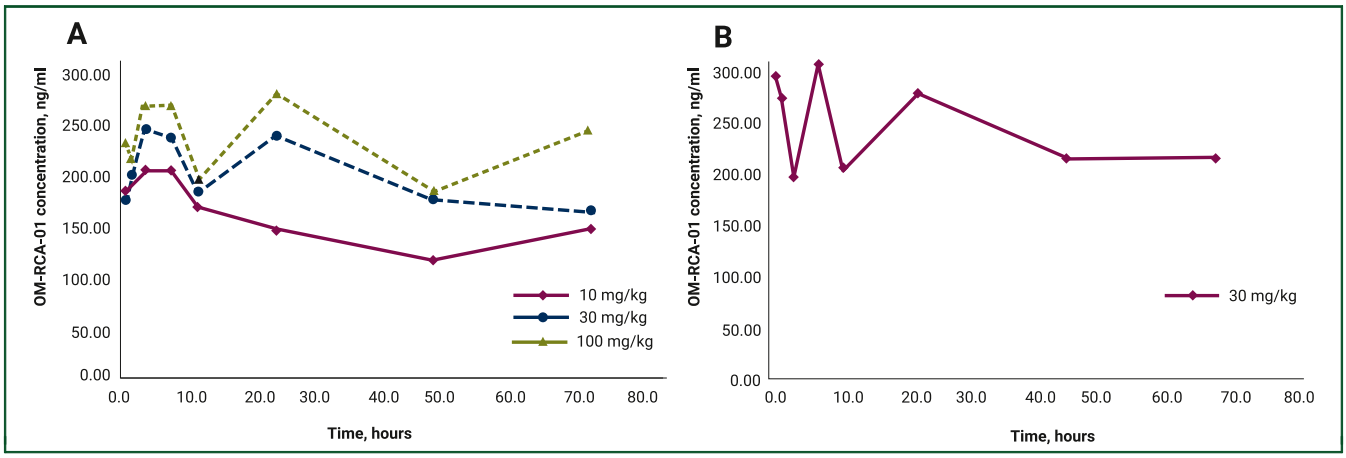

Figure 6. Pharmacokinetic curves (in linear coordinates) describing changes in the concentration of OM-RCA-01 in the blood of rats after a single (A) and multiple (B) intravenous administration.

OM-RCA-01 demonstrates long-term elimination

For the investigation of PK parameters, we established and validated a method using ELISA with photometric detection to quantitatively determine OM-RCA-01 in rat and rabbit blood serum. The sensitivity of the analytical method was determined to be 31 250 ng/ml. Reproducibility, accuracy, and reliability of the analysis results met the acceptance criteria across the entire range of analyzed concentrations (31 250-2 000 000 ng/ml).

Using this validated method, 108 rat serum samples were analyzed following a single intravenous administration of OM-RCA-01 at three different doses (10, 30, or 100 mg/kg) or a dose of 30 mg/kg administered three times at 3-day intervals. All analytical batches met the acceptance criteria for calibration standards and quality control samples. Based on the obtained concentrations of OM-RCA-01, averaged PK profiles were constructed (Figure 6A), and the PK parameters were calculated (Table 2).

The initial concentrations of the OM-RCA-01 in the blood serum following a single intravenous administration at doses of 10, 30, and 100 mg/kg were nearly identical, measuring 1859, 1769, and 244 814 ng/ml, respectively. The values of the AUC0-∞ rose with the escalating administration of OM-RCA-01. Nevertheless, it is important to highlight that the augmentation in the AUC0-∞ happened disproportionately in relation to the increase in the administered dose. High values of the volume of distribution, exceeding the actual volume of rat body fluids, indicate the active penetration of OM-RCA-01 into biological fluids and tissues.

Upon repeated dosing, there was a slight accumulation of the antibody observed (Figure 6B). In comparison with a single intravenous administration at the same dose (30 mg/kg), after three intravenous administrations, an almost twofold increase in the initial concentration of the OM-RCA-01 in the blood serum was noted. Simultaneously, the AUC0-∞ increased by only 58%. A marginal increase in the half-life of the drug (∼30%) was also recorded. The total volume of distribution and total clearance exhibited slight decreases, while the MRT of the drug in the bloodstream remained unchanged.

A similar trend in PK parameters was observed when the antibody was intravenously administered to rabbits. The study involved 12 rabbits (6 males and 6 females) with an approximate weight of 1.5 kg each, divided into two dosage groups (10 mg/kg and 30 mg/kg) with 6 rabbits in each group. After single and multiple administrations of OM-RCA-01, a total of 252 blood samples were collected at various time points, including 0.5, 1, 2, 6, 12, 24, 48, 72, 96, and 168 h at 1-8 and 29-36 days of the study. The PK values are summarized in Table 3.

Table 2. PK values of OM-RCA-01 in the blood serum of rats after a single and multiple intravenous administration

Single i.v. administration									
Dose, mg/kg	C0, ng/ml	Kel, h	$t_{½}$, h	Vd, l	Cl, l/h	AUC0-t, ng-h/ml	AUC0-∞, ng-h/ml	AUC0-t/AUC0-∞, %	MRT, h
10	186	0.0057	121	532	3.05	10 665	32 818	33	34
30	177	0.0051	136	2533	12.88	14 237	46 602	31	34
100	245	0.0030	234	7569	22.44	16 383	89 131	18	35
Multiple i.v. administration									
Dose, mg/kg, three times	C0, ng/ml	Kel, h	$t_{½}$, h	Vd, l	Cl, l/h	AUC0-t, ng-h/ml	AUC0-∞, ng-h/ml	AUC0-t/AUC0-∞, %	MRT, h
30	347	0.0039	179	2096	8.11	17 849	74 011	24	35

AUC, area under the pharmacokinetic curves; MRT, mean residence time; PK, pharmacokinetic.

Table 3. PK values of OM-RCA-01 in the blood serum of rabbits after a single and multiple intravenous administration

Single i.v. administration									
Dose, mg/kg	C0, ng/ml	Kel, h	$t_{½}$, h	Vd, l	Cl, l/h	AUC0-t, ng-h/ml	AUC0-∞, ng-h/ml	AUC0-t/AUC0-∞, %	MRT, h
10	200	0.0064	108	75	0.48	17 916	27 061	66	68
30	250	0.0032	216	180	0.57	27 553	65 571	42	75
Multiple i.v. administration									
Dose, mg/kg, three times	C0, ng/ml	Kel, h	$t_{½}$, h	Vd, l	Cl, l/h	AUC0-t, ng-h/ml	AUC0-∞, ng-h/ml	AUC0-t/AUC0-∞, %	MRT, h
10	280	0.0042	165	54	0.23	28 662	56 205	51	73
30	340	0.0041	170	132	0.54	39 177	78 758	50	75

AUC, area under the pharmacokinetic curves.

Toxicity of OM-RCA-01

The acute toxicity study was conducted on two animal species, involving 144 mice (72 males and 72 females) and 144 rats (72 males and 72 females). Single intravenous and intraperitoneal administrations were found to be non-fatal. Administration of the OM-RCA-01 at all doses (10, 60, 100, 150, and 350 mg/kg) did not induce visible signs of toxicity. When a maximum dose of 500 mg/kg was intravenously administered to mice, a temporary decrease in motor activity and shortness of breath was observed. However, these symptoms resolved within 15 min and did not impact the subsequent condition of the animals during the 14-day observation period. Biochemical and hematological values of animals receiving OM-RCA-01 were comparable to those of control animals. Results from a pathological autopsy carried out after 14 days indicated that a single injection of the antibody, even at a dose of 500 mg/kg, did not lead to morphometric and morphological changes in internal organs, except for a significant increase (60% compared to the control) in the mass coefficient of the spleen. Histological analysis revealed no cytomorphological changes in the tissues of the internal organs.

For the assessment of subacute toxicity, the study involved 90 rats (45 males and 45 females) allocated to the control group or to receive intravenous doses of OM-RCA-01 at 5, 55, and 115 mg/kg for 12 weeks. No instances of animal deaths were found throughout the study. Parameters such as body weight, food and water consumption, and physical activity exhibited no significant differences across different dose levels, genders, and in comparison with the control group. OM-RCA-01 delivery did not elicit signs of pain or local inflammatory reactions. Ophthalmological examinations were conducted, evaluating the condition of mucous membranes, the presence of correct corneal reflexes, and measurements of pupil size and palpebral fissure width for both eyes. Over the 60-day observation period, no discernible differences were noted between animals in the control and experimental groups. Pathomorphological and histological analyses also revealed no evidence of damage to internal organs and tissues at any of the dose levels.

The immunotoxicity study included 48 mice (24 males and 24 females) that were assigned to receive OM-RCA-01 at doses of 50 and 250 mg/kg. OM-RCA-01 did not adversely impact the functional activity of neutrophils, induce a delayed-type hypersensitivity reaction, or result in an increase in antibody titers (IgG and IgM) in the blood. The allergenic properties of the antibody were studied in guinea pigs ($n = 36$, 18 males and 18 females; 50 and 500 mg/kg). General anaphylaxis reaction, immune complex reaction, conjunctival test, and mast cell degranulation reaction were comparable to the control group. Moreover, the drug was found to be pyrogen free and did not induce a local irritant effect upon repeated administration in studies conducted on rabbits ($n = 6$, 3 males and 3 females; 115 mg/kg) and rats ($n = 60$, 30 males and 30 females; 5, 55, and 115 mg/kg), respectively.

DISCUSSION

In our study, we evaluated the impact of blocking FGFR1 with the monoclonal humanized anti-FGFR1 antibody OM-RCA-01 on tumor cell proliferation *in vitro*, as well as on tumor growth inhibition *in vivo*. Additionally, we hypothesized that supplementing checkpoint inhibitors with the anti-FGFR1 antibody could yield improved efficacy outcomes, particularly in addressing evolving resistance linked with the tumor microenvironment.

In vitro study showed that OM-RCA-01 antibody significantly inhibited FGF-triggered lung cancer cell proliferation. The suppression of proliferation correlated with a dose-dependent inhibition of FGFR1 phosphorylation. Conversely, the initial low phosphorylation of the receptor on melanoma cells might have contributed to the limited antiproliferative effect of OM-RCA-01. Several authors have presented similar data highlighting the association between FGFR1 phosphorylation and cancer cell proliferation.[19-21] As a result, binding to the active site of the extracellular domain of the receptor can lead to inhibition of intracellular tyrosine kinase phosphorylation, consequently suppressing tumor cell activity. It is intriguing to examine the phosphorylation of downstream kinases when culturing cells with OM-RCA-01. Studies on other members of the FGFR family have demonstrated that blocking the extracellular domain of the receptor also results in the inhibition of downstream proteins in this signaling pathway.[22,23]

These *in vitro* findings were confirmed in a lung cancer xenograft model. *In vivo*, tumors in mice treated with non-specific IgG continued their aggressive growth, reaching a size of 2000 cm^3. On the other hand, monotherapy with OM-RCA-01 significantly arrested further tumor growth and demonstrated a twofold difference in tumor volume

compared to the vehicle group on day 31. This effect may arise from both a direct impact on tumor cell proliferation and angiogenesis. As shown previously, OM-RCA-01 strongly inhibited FGF-induced angiogenesis compared with bevacizumab in the Matrigel assay.[14]

In the subsequent phase of the study, it was compelling to assess the initial efficacy of a combined immunotargeted therapy comprising a checkpoint inhibitor and an anti-FGFR1 antibody. Checkpoint inhibitors have revolutionized anticancer therapy and represent the standard of care for various cancers. However, resistance inevitably develops in some cases, resulting in patient mortality. For instance, only 30% and 48% of patients with metastatic lung cancer and kidney cancer treated with pembrolizumab or nivolumab combined with ipilimumab as first-line therapy survive for 5 years, respectively.[24,25] Therefore, novel approaches are imperative to enhance the effectiveness of immunotherapy and modulate resistance mechanisms. Thus far, efforts to combine small-molecule FGFR2/3 tyrosine kinase inhibitors with PD-1 inhibitors showed preliminary efficacy and tolerability.[26,27] Nevertheless, there are no completed studies investigating the efficacy of combinations involving FGFR-blocking monoclonal antibodies and immunotherapy, which presents a matter of scientific interest. A phase III FORTITUDE-102 clinical trial assessing the efficacy of a combination of anti-FGFR2 antibody bemarituzumab with nivolumab and chemotherapy in patients with metastatic gastric cancer is currently underway and enrolling participants.[28]

In our *in vitro* study, we illustrated that nivolumab increases T-cell activity. Our findings closely align with prior research, which also reported elevated levels of IFN-γ and IL-2 when lymphocytes and DCs were cultured with nivolumab alone or in combination with ipilimumab.[29,30] When the anti-FGFR1 antibody was introduced alongside nivolumab, IFN release increased by a third, while IL-2 secretion surged to 74%. It remains challenging to delineate the precise mechanism behind this reciprocal effect. We have previously shown that FGFR expression occurs on human lymphocytes.[31] It is possible that the anti-FGFR1 antibody blocks these receptors on lymphocytes, which increases their activity. Another explanation may be a non-specific effect of immunoglobulin on lymphocyte activity. However, the OM-RCA-01 antibody alone did not trigger an active release of IFN-γ. Additionally, significant activation of the immune system was not observed in studies assessing the immunotoxicity of the OM-RCA-01 in animals.

The results of the *in vivo* study also indicated that the combination of the anti-FGFR1 antibody and checkpoint inhibitor improves the efficacy of conventional immunotherapy in the FGFR1-overexpressing lung cancer PDX model. Currently, pembrolizumab monotherapy is administered in patients with metastatic non-small-cell lung cancer with PD-L1 expression of $\geq$50%. The prevalence of PD-L1 and FGFR1 co-expression remains uncertain, and it is unclear whether FGFR1 expression affects the efficacy of immunotherapy for PD-L1-positive lung cancer. A phase II NIVOFGFR2 clinical study showed that the efficacy of first-line nivolumab therapy was reduced when PD-L1 and FGFR2 were co-expressed in metastatic gastric cancer.[32] In our PDX model, we used lung adenocarcinoma with strong expression of PD-L1 and FGFR1. In the control group, exponential tumor growth was observed, indicating its aggressiveness. The combination of OM-RCA-01 and pembrolizumab resulted in a twofold inhibition of tumor growth compared to pembrolizumab monotherapy. A limitation of this experiment is the lack of a cohort that received only OM-RCA-01 antibody. Nevertheless, indirect comparisons across various models also indicate that the efficacy of OM-RCA-01 is augmented when combined with pembrolizumab.

The tumor microenvironment plays an important role in the development of resistance to checkpoint inhibitors.[33] CAFs represent one of the primary components of the microenvironment. These cells influence cancer metastasis by synthesizing and remodeling the extracellular matrix, producing growth factors, and impacting angiogenesis, tumor mechanics, drug access, and therapy responses.[34] Exploring methods to target CAFs, including altering their numbers, subtype, or functionality, is being investigated as a strategy to enhance cancer therapies. In our study, as expected, the tumor growth rate was higher in the CAF environment, correlating with a lower efficacy of nivolumab. At the same time, in the case of the development of immune resistance, the initiation of anti-FGFR1 antibody therapy was accompanied by a deceleration in growth of tumors, the median size of which never reached 2000 mm^3 in the CAF-positive group, in contrast to the control group, in which non-specific immunoglobulin was administered. The use of OM-RCA-01 during immunotherapy again led to an increase in the release of IFN-γ and IL-2. Certainly, this model only permits us to speculate about the impact of anti-FGFR1 antibody on the tumor microenvironment; nevertheless, it cannot be discounted that the antibody directly influenced the tumor itself. Consequently, further investigations into the mechanism of action are warranted.

The OM-RCA-01 antibody exhibited no toxicity, either at therapeutic doses or at higher doses. PK parameters indicate prolonged elimination and sufficient plasma concentration.

In conclusion, our preclinical studies indicate that the OM-RCA-01 exhibits robust activity with minimal toxicity. The antitumor mechanism of action of the antibody may be associated with a direct effect on tumor cells expressing FGFR1 and angiogenesis. Combining an anti-FGFR1 antibody and a checkpoint inhibitor may result in increased activity of both drugs. Although the study highlights the antitumor effects of OM-RCA-01, it falls short of providing in-depth mechanistic insights into how OM-RCA-01 augments the efficacy of checkpoint inhibitors such as nivolumab and pembrolizumab. A detailed understanding of the specific biological interactions and pathways involved would significantly reinforce the final conclusions. A phase Ib/II clinical trial is currently planned to evaluate the safety and preliminary efficacy of the OM-RCA-01 monotherapy in patients with FGFR1-expressing lung cancer who have exhausted standard therapy.

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DISCLOSURE

IT reports patent No. US10358499 for the invention. All other authors have declared no conflicts of interest.

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