Neutralizing anti-FGFR1 antibody as a combined partner of anti-PD-1 antibodies in tumor models

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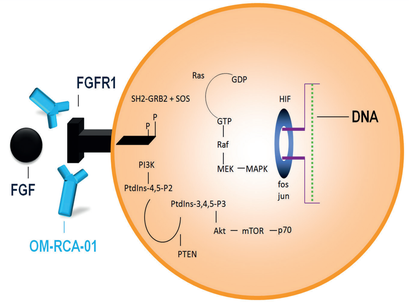
FGFR1 AS POTENTIAL ANTI-CANCER TARGET

- FGFR1 and PD-1 - mediated signals are involved in the growth and survival of cancer and should be considered as targets for therapeutic approaches.
- Several studies showed the key role of FGFR inhibition on remodeling the immune microenvironment of tumors, especially inducing new T-cell responses, which in turn acts in concert with anti-PD-1 to promote antitumor immunity.
- OM-RCA-01, a novel humanized anti-FGFR1 antibody (Bureau of Cancer Research) with high affinity (Kd of 1,59 nM) has a potent antitumor activity in renal cell carcinoma model *in vivo*. (Tsimafeyeu et al. Investigational New Drugs, 2013)
- Here, we report the potential activity of an OM-RCA-01 antibody, either alone or in combination with an anti-PD-1 antibody.

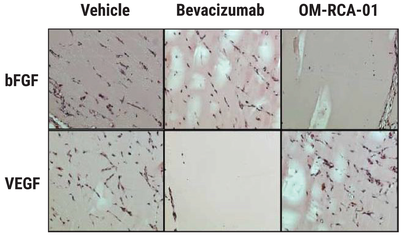
OM-RCA-01: TARGETING FGF/FGFR1 PATHWAY



OM-RCA-01 EFFECT ON ANGIOGENESIS: *IN VITRO* STUDY

- Matrigel plugs containing 100 ng bFGF or VEGF were subcutaneously implanted to mice.
- Mice were treated with 10 mg/kg OM-RCA-01 or bevacizumab.
- After treatment number of endothelial cells/vessels was calculated.
- Control groups were left untreated.

OM-RCA-01 EFFECT ON ANGIOGENESIS: RESULTS

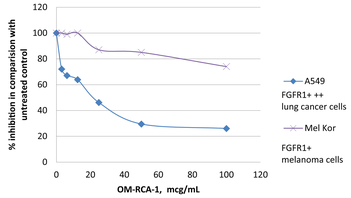


- bFGR and VEGF strongly induced angiogenesis.
- bFGF-induced angiogenesis was significantly inhibited by OM-RCA-01.
- Bevacizumab has no effect on bFGF-induced angiogenesis.

OM-RCA-01 EFFECT ON NSCLC: *IN VITRO* STUDY

- Human NSCLC A549 and human melanoma Mel Cor FGFR1-expressing cells were incubated (0.5% FBS) and dosed with OM-RCA-01.
- Six hours after dosing, basic FGF was added at a concentration of 100 ng/ml.
- Control cells were left untreated.
- Cell growth was determined using Cell Titer-Glo assay (Promega).

RESULTS

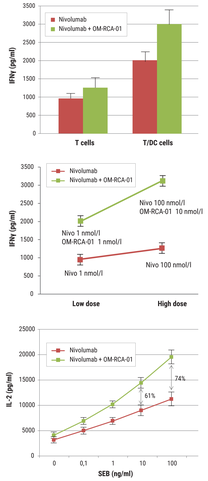


- Dose dependent inhibition of FGF-induced NSCLC cell proliferation in comparison with control (P=0.01).
- OM-RCA-01 IC50 for A549 cell line = 9.69 μg/mL.

LYMPHOCYTE RESPONSES TO STIMULATION WITH OM-RCA-01 AND ANTI-PD-1 ANTIBODIES: *IN VITRO* STUDY

- Assays included an allogeneic mixed lymphocyte reaction (MLR) and stimulation of human peripheral blood mononuclear cell (PBMC) by the superantigen Staphylococcal enterotoxin B (SEB)
- In MLR assay, dendritic cells (DC) were generated by culturing monocytes isolated from PBMCs using a monocyte purification kit (Miltenyi Biotec) *in vitro* for 10 days with 300 U/mL IL-4 (IL-4) and 200 U/mL GM-CSF (STEMCELL Technologies).
- CD4+ T cells (1 × 105) and allogeneic DCs (1 × 104) were cocultured with or without dose titrations of nivolumab (1 nmol/l and 100 nmol/l) +/- OM-RCA-01 (1 nmol/l and 10 nmol/l) added at the initiation of the assay.
- After 5 days, IFNγ secretion in culture supernatants was assessed by ELISA (BD Biosciences), and cells were labeled with 3H-thymidine for an additional 18 hours to measure T-cell proliferation.
- In SEB assay, PBMCs with PD-1 and FGFR1 expression 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 initiation of the assay together with serial dilutions of SEB (0.1-100 ng/ml). IL-2 levels in culture supernatants were measured by ELISA analysis (BD Biosciences).

RESULTS



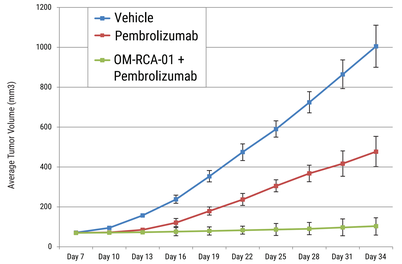
- In an allogeneic MLR, PD-1 blockade with nivolumab systematically resulted in an enhancement of IFNγ release and combination of nivolumab and OM-RCA-01 resulted in higher levels of IFNγ release by 31%.
- In some donor T-cell/DC pairs, enhanced T-cell proliferation was also observed. An increase in the concentration of antibodies led to an increase in the release of IFNγ, especially in the combination group.
- Nivolumab and OM-RCA-01 also enhanced IL-2 secretion over nivolumab alone in response to SEB using PBMCs. Addition of OM-RCA-01 increased IL-2 secretion by a mean of 29% to 74% over nivolumab.

OM-RCA-01 AND ANTI-PD-1 ANTIBODIES EFFECT ON NSCLC *IN VIVO*: PDX MODEL

- To evaluate the efficacy of FGFR1 and PD-1 co-inhibition, patient explants were obtained from surgical lung specimens.
- Adenocarcinoma with PD-L1 expression >50% measured with DAKO 22C3 antibody was used.
- Patient-derived xenograft (PDX) models were generated by implantation of PDX into humanized NSG mice (Jackson Laboratory, Sacramento, CA, USA) as previously described (Wang, FASEB J. et al, 2018).
- Treatment with
  - OM-RCA-01 (30 mg/kg) with pembrolizumab (10 mg/kg), N=7 or
  - pembrolizumab alone (10 mg/kg), N=7 or
  - saline (vehicle), N=7

  every 3 days was started when the tumors reached 70 mm^3 in volume.
- Measurements of tumor volume were performed by digital calipers every 3 days.

RESULTS



Group	Tumor volume (day 31)	Significance*
OM-RCA-01 and Pembrolizumab	97.1 mm^3	P<0.001
Pembrolizumab	417.1 mm^3	P<0.001
Vehicle	863.5 mm^3	

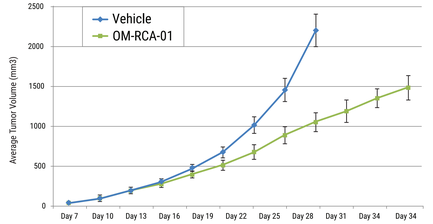
*comparison with vehicle

- In the PDX study, the differences between groups were statistically significant (all P<0.001).
- No group mean body weight losses or clinical manifestations of toxicity were observed in treatment groups

OM-RCA-01 EFFFECT ON NSCLC *IN VIVO*: XENOGRAFT MODEL

- Thirty-five Charles River female NCr nu/nu mice (6-12 weeks of age) were set up with 1 mm3 A549 tumor fragments sc in flank.
- Twenty mice with established tumors (an average size of 80 - 120 mg) were randomly divided into vehicle and treatment groups 10 animals each.
- Animals were treated with 30 mg/kg of antibody.
- Endpoint was significant differences in tumor growth delay

RESULTS



Group	Tumor volume (day 31)	Significance
Vehicle	1048.5 mm^3	–
OM-RCA-01	2174 mm^3	p<0.0001

CONCLUSION

- High-affinity humanized anti-FGFR1 antibody OM-RCA-01 inhibited bFGF-induced angiogenesis in Matrigel grafts *in vivo*.
- OM-RCA-01 suppressed ligand-induced proliferation of human lung carcinoma cells *in vitro*.
- OM-RCA-01 has a potent antitumor activity in mouse PDX and xenograft models of human non-small cell lung cancer.
- **Combination of anti-FGFR1 and anti-PD-1 antibodies drives activation of T-cell clones to support enhanced antitumor activity.**